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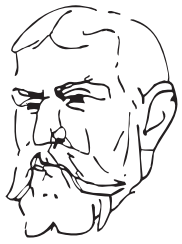

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ORIGINAL ARTICLE

MODERN MORPHOLOGICAL DIAGNOSTICS OF ULCERATIVE COLITIS AND IRRITABLE BOWEL SYNDROME: HISTOLOGICAL, HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL CRITERIA

DOI: 10.36740/WLek202103101

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SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

ABSTRACT

The aim is to establish diagnostic and differential-diagnostic criteria for UC and IBS based on a complex morphological (histological, histochemical, immunohistochemical) study.
Materials and methods: In this study, it was used autopsy and biopsy material – fragments of the mucous membrane of the large intestine. The material was divided into 5 groups. The first group (G 1) included autopsy material from 6 cases, in which, during autopsies and microscopic examination, we found no general pathological processes in the gastrointestinal tract. The second group (G 2) included biopsy material from 34 patients with diagnosed UC of the 1st activity degree. The third group (G 3) included the biopsy material of 27 patients with UC of the 2nd degree of activity. The fourth group (G 4) included biopsy material from 19 patients, diagnosed with UC of the 3rd degrees of activity. The fifth group (G 5) included biopsy material from 82 patients with clinically diagnosed IBS. Histological, histochemical, immunohistochemical, statistical research methods were used.
Results: There are characteristic morphological changes in the mucous membrane of the large intestine in UC of varying degrees of activity, such as changes in the architectonics of crypts of varying severity; presence of erosive and ulcerative defects. Inflammatory and desquamative-dystrophic changes take place in the epithelial layer adjacent to erosive and ulcerative defects. The number of goblet cells of crypts decreases and the size of vacuoles in goblet cells reduces. The intensity of mucin secretion contained in the vacuoles of the goblet cells lowers; there is a partial or complete loss of pericryptal myofibroblasts; the proliferative potential of the intestinal crypts epithelium activates.
Conclusions: Differential diagnostic criteria, revealed by the author, improve the morphological diagnosis of UC and IBS, optimizing the tactics of managing patients with this colorectal pathology.

KEY WORDS: ulcerative colitis, irritable bowel syndrome, morphological differential-diagnostic criteria

Wiad Lek. 2021;74(3 p.I):381-387

INTRODUCTION

The number of people diagnosed with diseases of the gastrointestinal tract is increasing every year all over the world [1]. UC in terms of prevalence is significantly inferior to other gastroenterological diseases. However, due to severity of the course, frequency of complications and mortality, it is a leader in the structure of diseases of the gastrointestinal tract [2].

The endoscopic method of research (colonoscopy) plays a decisive role in UC diagnosis [3]. Despite its effectiveness and diagnostic accuracy in identifying intestinal pathology, colonoscopy has a number of disadvantages (invasiveness, risk of complications, pain and discomfort) [4]. Often during a colonoscopy, we take a targeted biopsy from various parts of the large intestine, followed by its examination by a pathologist [5].

In UC, colonobioptate examination has not only diagnostic, but also prognostic value [6]. The morphological diagnosis of UC sometimes causes certain difficulties even for the most experienced pathologists. Despite the available arsenal of modern morphological research methods, certain diagnostic difficulties also arise in the differential diagnosis of UC with various pathological conditions including IBS.

Differential diagnosis of UC and IBS is difficult due to the common clinical manifestations, pathophysiological mechanisms, etc. The existing diagnostic difficulties have caused disagreements among scientists regarding the relationship between UC and IBS. Most scientists believe that UC and IBS are different pathological conditions of the large intestine. Some scientists defend the concept of recognizing UC and IBS as different forms of the same disease. There is also a high incidence of UC against the background of IBS, frequent persistence of IBS-like symptoms after achieving remission of UC, possible «superimposing» of one disease on the course of another [7].

All of the above actualizes the need for a continuous search for morphological criteria that would allow accurate and correct UC and IBS diagnostics, including a differential one.

THE AIM

The aim is to establish diagnostic and differential-diagnostic criteria for UC and IBS based on a complex morphological (histological, histochemical, immunohistochemical) study.

MATERIALS AND METHODS

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

The resulting material, fixed in a 10% solution of neutral formalin (pH 7.4) for 24-48 hours, was treated according to the generally accepted technique and embedded in paraffin. From paraffin blocks on a rotary microtome HM 325 (Thermo Scientific, USA), serial sections with a thickness of 2-3 μm were made, stained with hematoxylin and eosin. To determine the morpho-functional state of the goblet cells, we performed the PAS reaction.

Immunohistochemical study was performed using rabbit monoclonal antibodies (MCA) to Ki-67 (clone SP6) and murine MCA to alpha-smooth muscle actin (clone 1A4). An Ultra Vision Quanto HRP detection system was used for visualization. The MCA and the imaging system 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.

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

In G 1, while studying microspecimens stained with hematoxylin and eosin, it was found focal desquamative changes in the epithelium of the colon mucosa in 1 case (16.7%). The revealed desquamation of the epithelial layer is due, from our point of view, to its artifactual change, occurring during the histological processing of the material.

Observational microscopy of the slides in G 2 in the epithelium of the mucous membrane of the colon in all cases revealed moderate inflammatory, degenerative and desquamative changes. In 29 cases (85.3%) they were combined with formation of multiple superficial erosions, the depth of which reached the surface parts of the lamina propria of the mucous membrane (fig. 1).

In G 3, in 22 cases (81.5%), we found multiple erosions in the mucous membrane, superficial in places, deep in some of the visual fields. Deep erosions led to defects that spread to the entire thickness of the lamina propria of the mucous membrane. In 5 cases (18.5%), erosive and ulcerative changes were detected in the mucous membrane. The depth of the ulcers reached the muscle plate of the mucous membrane or the submucosa (fig. 2). It was determined pronounced inflammatory, dystrophic and desquamative changes at the edges and at some distance from the erosive and ulcerative defects in the epithelium of the mucous membrane in all cases.

Observational microscopy in G 4 in the mucous membrane showed erosive changes in 10 cases (52.6%), erosive and ulcerative in 9 cases (47.4%). In the epithelial layer, which was adjacent to these defects, in all cases, pronounced inflammatory and desquamative-dystrophic changes were determined.

Thus, with UC of varying degrees of activity, characteristic morphological changes in the mucous membrane of the colon are erosive and ulcerative defects, as well as inflammatory and desquamative-dystrophic changes in the epithelial layer. Other scientists have also noted similar morpho-functional changes [9].

In the study, it was also revealed an increase in erosive-ulcerative, inflammatory, desquamative-dystrophic changes from G 2 to G 4 in the epithelial layer of the colon mucosa.

In UC, the processes of apoptosis and necrosis are the leading in the formation of erosive and ulcerative defects in the mucous membrane of the large intestine. Scientists have shown an increase in the processes of apoptosis as the course of UC increases with the subsequent dominance of necroinflammatory changes in colonocytes [10].

In all cases in G 5, it was revealed moderate inflammatory and degenerative changes in the epithelial layer in the mucous membrane of the colon. In 20 cases (24.4%), a combination of inflammatory, dystrophic and desquamative changes was found in the epithelium of the mucous membrane (fig. 3).

Comparing the results, obtained in G 2-5, we noted that in IBS compared with UC of varying degrees of activity in the mucous membrane of the colon, firstly, erosive and ulcerative changes were not detected; secondly, in all cases less pronounced inflammatory and dystrophic changes were found in the epithelium, which in some cases were combined with desquamative changes.

Complex epithelial barrier of the colon, as is known, allows you to maintain tolerance to commensal microflora and food agents, effectively protecting the body from pathogens [11]. Damage of the epithelial barrier, revealed in the study in the wall of the large intestine in IBS and UC, upsets this balance and leads to the development of inflammation.

The first barrier of the large intestine, protecting the internal medium of the body from bacteria and damaging factors, is the mucus, produced by the goblet cells. As we know, goblet exocrinocytes are abundant in crypts. The mucus, produced by these cells, prevents the adhesion and invasion of microorganisms, not interfering with the

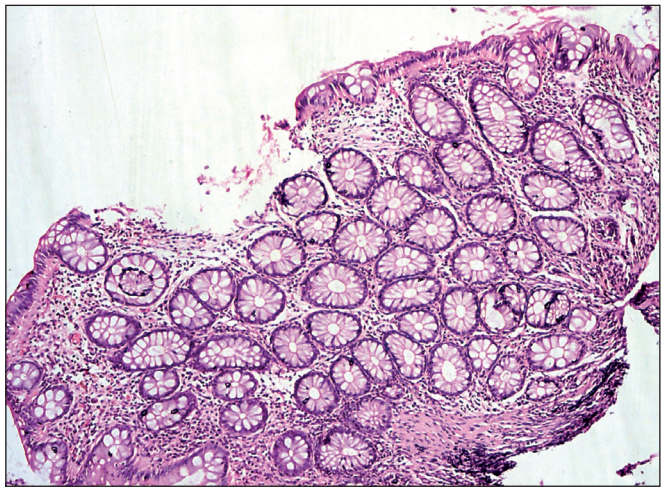

Fig. 1. G 2. Superficial erosive defect in the mucous membrane of the colon. Stained with hematoxylin and eosin, × 50.

transport of nutrients, serves as a substrate for the attachment and nutrition of the commensal microflora, acts as a lubricant, facilitating the passage of the chyme through the intestines [11].

We used the PAS reaction in this research to study morpho-functional features of goblet cells.

In G 1 in all cases when staging the PAS reaction the goblet cells contained a large vacuole with mucin. The nuclei of the goblet cells were small, hyperchromic and basally pushed back. In all cases, mucin had pronounced staining.

In G 2, compared with G 1, the number of cases with unchanged sizes of vacuoles in goblet cells decreased ($p<0.05$) (22, 64.7%) and cases with focal decrease in the size of vacuoles appeared (12, 35.3%) (fig. 4). The intensity of mucin staining in this group decreased compared to G 1, as evidenced by a decrease ($p<0.05$) in the number of cases with pronounced staining (8, 23.6%) and the appearance of cases with weak (13, 38.2%) and moderate (13, 38.2%) staining. In the intestinal glands, the intensity of mucin

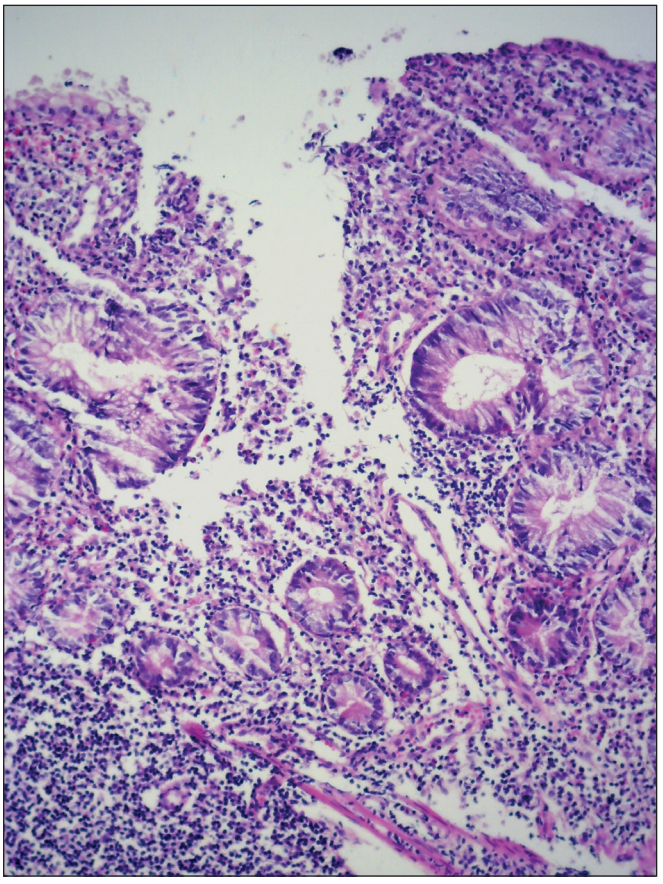

Fig. 2. G 3. Ulcerative defect in the mucous membrane of the colon. Stained with hematoxylin and eosin, × 100.

staining changed in 8 cases (23.5%) in their superficial parts, in 12 cases (35.3%) in 2/3 of their thickness, in 6 cases (17.7%) in the entire their thickness.

In the G 3, compared with G 1, the number of cases with unchanged in size vacuoles (9, 33.4%) decreased ($p<0.05$), and also there were cases in which vacuoles decreased in size focal (10, 37.0%) or diffusely (8, 29.6%).

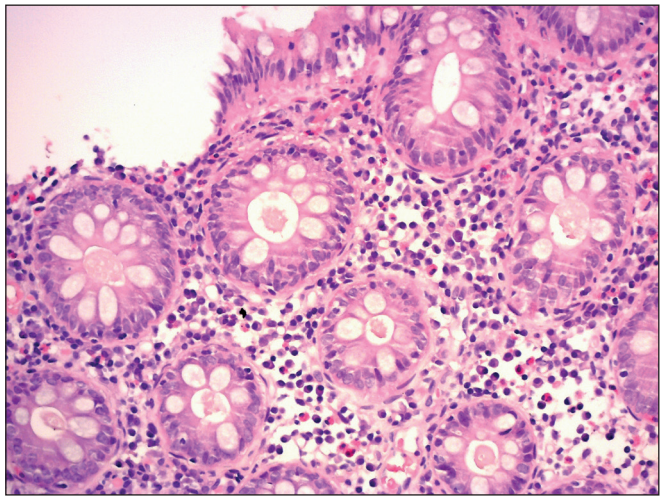

Fig. 3. G 5. Moderate inflammatory, dystrophic and desquamative changes in the epithelium of the colon mucosa. Stained with hematoxylin and eosin, × 200.

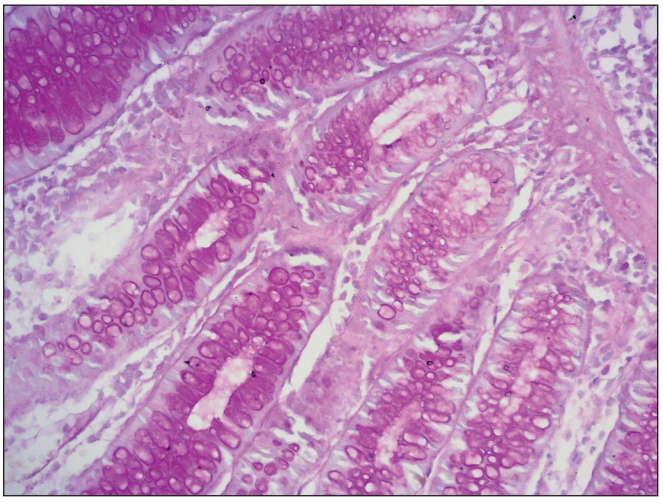

Fig. 4. G 2. Focal decrease in the size of vacuoles with mucin in goblet cells, unevenly pronounced decrease in the intensity of mucin staining. PAS reaction, × 200.

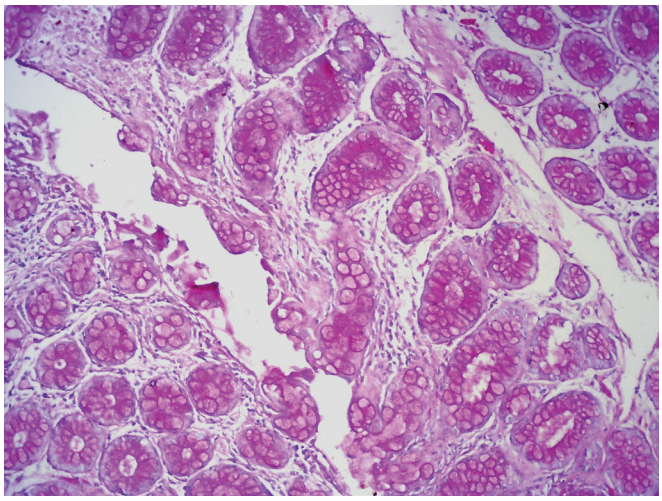

Fig. 5. G 5. Decreased intensity of mucin staining in goblet cell vacuoles. PAS reaction, × 100.

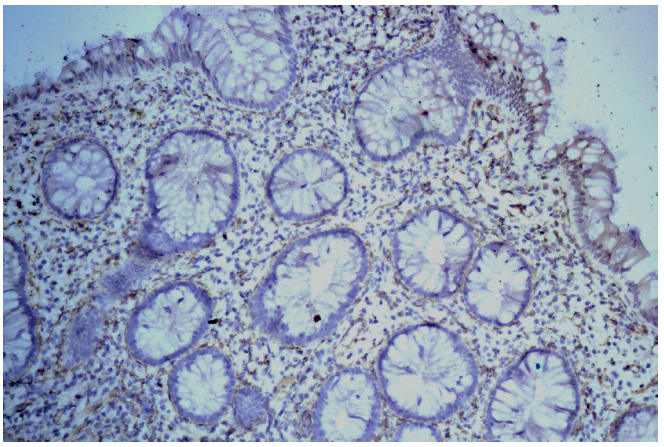

Fig. 6. G 3. Focal or diffuse decrease in the number of pericryptal myofibroblasts. Immunohistochemical reaction with MCA to alpha-smooth muscle actin, × 100.

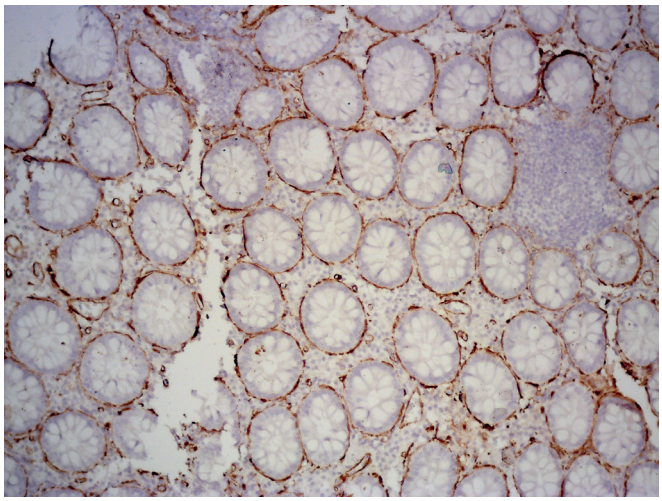

Fig. 7. G 5. Evenly spaced pericriptal myofibroblasts. Immunohistochemical reaction with MCA to alpha-smooth muscle actin, × 100.

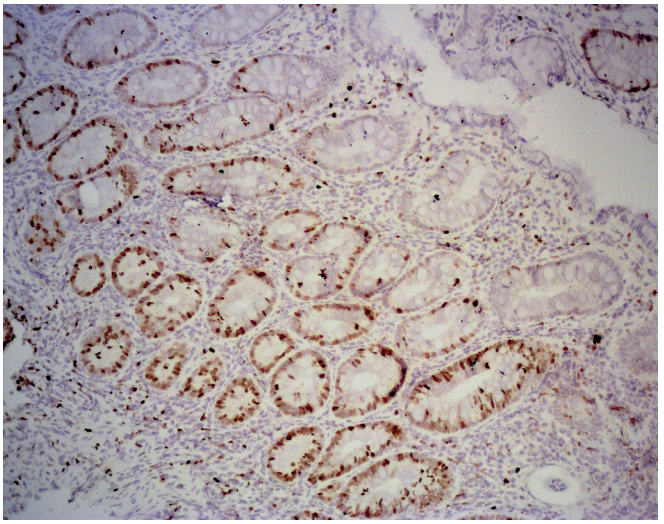

Fig. 8. G 3. Expression of the Ki-67 proliferation marker by the epithelium of the intestinal glands. Immunohistochemical reaction with MCA to Ki-67, × 100.

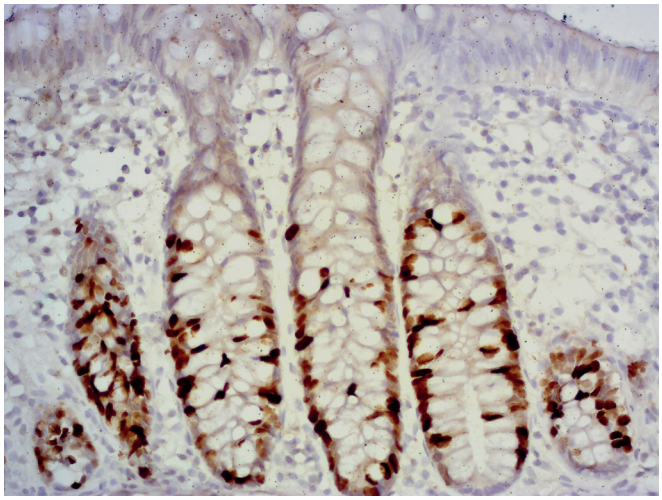

Fig. 9. G 5. Expression of the proliferation marker Ki-67 by the epithelium of the intestinal glands. Immunohistochemical reaction with MCA to Ki-67, × 400.

In this group, compared with G 1, the intensity of mucin staining changed (the number of cases with its pronounced staining decreased ($p<0.05$) (7, 25.9%), there were cases with no staining (2, 7.4%), weak (14, 51.9%) or moderate (4, 14.8%) staining). The intensity of mucin staining in 1 case (3.7%) changed in the surface parts of the crypts, in 6 cases (22.2%) – in their 2/3 of the thickness, in 13 cases (48.2%) – in their entire thickness.

In G 4, in 4 (21.1%) and 8 (42.1%) cases, vacuoles of goblet cells of crypts, respectively, focal and diffusely decreased in size, compared with G 1 decreased the number of cases with unchanged sizes of vacuoles (7, 36.8%). In 4 cases (21.1%) a negative PAS reaction was detected, and weak and moderate staining of mucin in 12 (63.2%) and 3 (15.7%) cases. Changing intensity of mucin staining was determined in 1 case (5.3%) in the superficial parts of the intestinal glands, in 3 cases (15.8%) – in 2/3 of the thickness of the glands, and in 15 cases (78.9%) – in the entire their thickness.

In G 5 the sizes of goblet cell vacuoles in all cases were similar to G 1. The intensity of mucin staining in this group was weak, moderate and pronounced, respectively, in 15 (18.3%), 21 (25.6%) and 46 (56, 1%) cases (fig. 5). There were fewer cases with a pronounced intensity of mucin staining in this group ($p<0.05$), compared with G 1. A change in the intensity of mucin staining in the intestinal glands in 18 cases (22.0%) was noted in their superficial parts, in 10 cases (12.2%) – in 2/3 of the thickness of the glands, in 8 cases (9.7%) – in the entire their thickness.

Summarizing the results of the given PAS reaction, it becomes obvious that a decrease in the size of vacuoles in the goblet cells of crypts, as well as their number, and a decrease in the intensity of mucin staining, contained in the vacuoles of goblet cells, are morphological changes in the mucous membrane of the large intestine characteristic of UC. With an increase in UC activity (from G 2 to G 4), a decrease (focal or diffuse) in the size of vacuoles as well as in the number of goblet cells was noted. The intensity of mucin staining decreased up to its complete disappearance, as evidenced by a negative PAS reaction. It was also noted that there was a decrease in the intensity of mucin staining in UC of the 1st degree of activity, mainly in the superficial parts of the intestinal glands and in their two-thirds of the thickness; UC of the 2nd degree of activity – in 2/3 of the thickness or throughout the entire thickness of crypts, in UC of the 3rd degree of activity – throughout the entire thickness of the crypts.

In our earlier studies, in the works of other scientists, a decreasing number of goblet cells and inhibition of mucus production was also revealed in the mucous membrane of the colon in active UC [12, 13].

In IBS, the size of the vacuoles containing mucin in the goblet cells, as well as the number of goblet cells, did not change in the mucous membrane of the colon. The mucin-forming function of the goblet cells suppressed, when staging a PAS reaction, leading to lower intensity of mucin staining, more pronounced in the superficial parts of the intestinal glands.

Thus, in IBS, compared with UC, the number of goblet cells and the size of vacuoles with mucin, containing in these cells, do not change in the mucous membrane of the colon; there is a less pronounced decrease in the intensity of mucin staining.

When setting an immunohistochemical reaction with MCA to alpha-smooth muscle actin in the mucous membrane of the large intestine in G 1, it was revealed evenly spaced pericryptal myofibroblasts. These cells were clear, uniform and had pronounced cytoplasmic brown staining. Myofibroblasts formed a kind of coupling around the intestinal glands. The latter, as we know, provides control over the self-maintenance of crypts [14]. In G 2-4, there was focal or diffuse absence of pericryptal myofibroblasts (fig. 6). In G 5 it was determined features of alpha-smooth muscle actin expression similar to G 1 (fig. 7).

When calculating the relative number of pericryptal myofibroblasts, a decrease ($p<0.05$) of this indicator was revealed in G 2-4 (G 2 – (74.4±2.74)%, G 3 – (61.6±2.67)%, G 4 – (50.8±3.81)%) compared with G 1 ((95.3±2.74)%). From G 2 to G 4, the indicator of the relative number of pericryptal myofibroblasts decreased ($p<0.05$). In G 5, the indicator of the relative number of pericryptal myofibroblasts did not differ significantly ($p>0.05$) from the control indicator.

Thus, in UC, in contrast to IBS, there is a partial or complete loss of pericryptal myofibroblasts, and the intensity of their expression decreases. With an increase in the degree of UC activity, there is a decrease ($p<0.05$) in the number of pericryptal myofibroblasts in the mucous membrane of the large intestine.

The deficiency of pericryptal myofibroblasts leads to the fact that the epithelium in crypts proliferates and grows without their participation. As a result, the architectonics of the crypts disrupts, which is a pathognomonic histological characteristic of the UC. Our survey microscopy revealed a violation of the crypt architectonics in all cases of G 2-4. The severity of this histological finding increased from G 2 to G 4. In G 5, the intestinal glands had histological features similar to G 1.

In our study in G 2-4, we found accumulations of myofibroblasts/fibroblasts in the lamina propria or in the muscle lamina of the mucous membrane, as well as in the submucosa against the background of partial or complete disappearance of pericryptal myofibroblasts. Groups of myofibroblasts/fibroblasts often localized at sclerosis loci. As is known, excessive activation of the morpho-functional state of myofibroblasts leads to the overproduction of connective tissue fibers, which contributes to the shortening, thickening of the intestinal wall and, in the future, can lead to disruption of the anatomical structure [15].

An immunohistochemical study with the Ki-67 proliferation marker in all groups revealed its expression by the epithelium of the intestinal glands. In G 1 the expression of this marker was observed mainly in the basal areas of the crypts, with the relative number of Ki-67-positive cells being (35.6±0.95)%. In G 2-4, the reaction with the proliferation marker increased as compared with G 1. This is evidenced by its predominant expression in 2/3 of the thickness or in the entire crypt thickness (fig. 8), as well as an increase ($p<0.05$) in the relative number of Ki-67-positive cells (G 2 – (51.9±0.96)%, G 3 – (51.2±1.38)%, G 4 – (53.0±1.31)%).

In G 5, the expression of the proliferation marker was determined mainly in 2/3 of the thickness of the intestinal glands (fig. 9). The indicator of the relative number of Ki-67-positive cells increased ($p<0.05$), compared with G 1 and amounted to (52.7±0.56)%.

It is interesting to note that the expression of the proliferation marker Ki-67 in crypt epithelial cells did not depend on the degree of UC activity and it was not different in IBS and UC of various degrees of activity. Previously identified chronic inflammation in varying degrees of severity in the mucous membrane of the colon in UC and IBS, as well as an increase in the proliferative potential of crypt epithelial cells, from our point of view, can serve as a background for colorectal cancer. The starting point for the realization of

oncological changes, as we know, are the epithelial cells of the intestinal mucosa [16, 17].

At present, there are at least four main pathways of colon cancer carcinogenesis – transformation of adenoma into carcinoma, Lynch syndrome, development of cancer «de novo», transformation of chronic colitis. Carcinogenesis of the «inflammatory» type is as follows: inflammatory changes progress to latent dysplasia, then to mild and severe dysplasia, which transforms into cancer [18].

Equally pronounced features of crypt epithelium proliferative activity in UC and IBS, identified by author, should develop oncological alertness both in doctors and in patients. The authors of Rome IV Criteria recommend colonoscopy for patients with IBS over 50 years of age with «anxiety symptoms» and a family history of colorectal cancer [19]. Our results indicate the need to revise the above recommendations for colonoscopy in patients with IBS.

CONCLUSIONS

1. There are characteristic morphological changes in the mucous membrane of the large intestine in UC of varying degrees of activity, such as changes in the architectonics of crypts of varying severity; presence of erosive and ulcerative defects. Inflammatory and desquamative-dystrophic changes take place in the epithelial layer adjacent to erosive and ulcerative defects. The number of goblet cells of crypts decreases and the size of vacuoles in goblet cells reduces. The intensity of mucin secretion contained in the vacuoles of the goblet cells lowers; there is a partial or complete loss of pericryptal myofibroblasts; the proliferative potential of the intestinal crypts epithelium activates.
2. Severity of erosive-ulcerative changes in epithelium increases with rising UC activity; changes in the architectonics of crypts become more noticeable. Inflammation and desquamative-dystrophic changes in the epithelial layer intensifies. The number of goblet cells and the size of vacuoles in these cells decrease. The intensity of mucin staining in the vacuoles of the goblet cells decreases until its complete disappearance as well as the number of pericryptal myofibroblasts, while the proliferative activity of the crypt epithelium remains unchanged.
3. In IBS, there are inflammatory and dystrophic changes in the epithelium in the mucous membrane of the colon, sometimes combined with desquamative changes; the intensity of mucin staining in the goblet cell vacuoles decreases; the proliferative potential of crypt epithelial cells grows.
4. In the mucous membrane of the large intestine in IBS, compared with UC, the architectonics of crypts is not disturbed, erosive and ulcerative changes are not determined. Less pronounced inflammatory and dystrophic changes in the epithelium sometimes combine with desquamative changes. The number of goblet cells and the size of the vacuoles with mucin that contain these cells do not change. There is a less pronounced decrease in the intensity of mucin staining; the number of pericryptal myofibroblasts corresponds to the physiological norm. The proliferative activity of crypt epithelium does not differ in IBS and UC of varying degrees of activity.
5. In the mucous membrane of the large intestine in UC of varying degrees of activity and IBS, it was established an activation of the proliferative potential of the epithelial cells of the intestinal glands, which should generate equally pronounced oncological alertness in doctors and patients. The revealed features of the proliferative activity of the crypt epithelium in patients with IBS actualize the need to revise the recommendations of the Rome IV criteria regarding colonoscopy in these patients.
6. Differential diagnostic criteria, revealed by the author, improve the morphological diagnosis of UC and IBS, optimizing the tactics of managing patients with this colorectal pathology.

REFERENCES

1. Stepanov YuM, Skirda IYu, Petishko OP. Hvoroby organiv travlennja – aktualna problema klinichnoi medycyny. Digestive system diseases: the actual problem of clinical medicine. Gastroenterologìa. 2019;53(1):1-6. (Ua)
2. Ivashkin VT, Shelygin YuA, Abdulganiyeva DI, Abdulkhakov RA, Alekseyeva OP, Achkasov SI et al. Rekomendacii Rossijskoj gastrojenterologicheskoj associacii i Associacii koloproktologov Rossii po diagnostike i lecheniju vzroslyh bolnyh jazvennym kolitom. Guidelines of the Russian gastroenterological association and Russian Association of Coloproctology on diagnostics and treatment of ulcerative colitis in adult. Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2015;1:48-65. (Ru)
3. Rastogi A, Wani S. Colonoscopy. Gastrointestinal endoscopy. 2017;85(1):59-66.
4. Ivanova EV, Fedorov ED, Tikhomirova EV, Avakimyan AV, Marenich NS. Kolonoskopija s pomoshhju videokapsuly: vozmozhnosti neinvazivnoj diagnostiki zabolevanij tolstoj kishki. Colon capsule endoscopy: possibilities of non-invasive diagnostics of colon diseases. Research'n Practical Medicine Journal. 2017;4(1):13-22. (Ru)
5. Feakins RM. Inflammatory bowel disease biopsies: updated British Society of Gastroenterology reporting guidelines. Journal of Clinical Pathology. 2013; 66(12):1005-1026.
6. Geboes K, Nemolato S, Leo M, Faa G, eds. Colitis. A practical approach to colon biopsy interpretation. Springer, 2014.200 p.
7. Sheptulin AA, Vinogradskaya KE. Vospalitelnye zabolevanija kishechnika i sindrom razdrazhennogo kishechnika: sochetanie dvuh nozologicheskih form ili raznye varianty odnogo zabolevanija? Inflammatory bowel diseases and irritable bowel syndrome: overlap of two nosological forms or two variants of the same disease? Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2019;29(5):43-48. (Ru)
8. Kobzar AI. Prikladnaja matematicheskaja statistika. Applied mathematical statistics. M.: Fizmatlit, 2012. 816 p. (Ru)
9. DeRoche TC, Xiao S-Y, Liu X. Histological evaluation in ulcerative colitis. Gastroenterology Report. 2014;2:178-192.
10. Osadchuk AM, Ivashkin VT. Diffuznaja jendokrinnaja sistema i narushenie processov kletochnogo obnovlenija kolonocitov pri razlichnyh stepenjah tjazhesti nespecificheskogo jazvennogo kolita. Diffuse endocrine system and disorders of cellular regeneration of colonocytes in nonspecific ulcerative colitis. Vestnik of Volgograd State Medical University. 2005;3(15):58-61. (Ru)

11. Zolotova NA, Akhrieva KhM, Zayratyants OV. Jepitelialnyj barer tolstoj kishki v norme i pri jazvennom kolite. Epithelial barrier of the colon in health and patients with ulcerative colitis. Clinical Gastroenterology.2019;162(2):4-13. (Ru).
12. Snisarevskyi PP, Dyadyk OO, Dorofeyev AE, Snisarevska PP. Rol morfologichnogo doslidzhennja v diagnostyci nespecyfichnogo vyrazkovogo kolitu. The significance of morphological research in the diagnosis of ulcerative colitis. Art of medicine. 2018; 3(7):127-132. (Ua)
13. Stange EF, Schroeder BO. Microbiota and mucosal defense in IBD: an update. Expert Review of Gastroenterology and Hepatology. 2019;13(10):963-976.
14. Barinov EF, Sulayeva ON. Gastrointestinalnye miofibroblasty – rol v reguljacii fiziologicheskoj aktivnosti i reparacii zheludochno-kishechnogo trakta. Gastrointestinal myofibroblasts: role in regulation of physiological activity and reparation of gastro-intestinal tract. Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2010;3:9-18.(Ru)
15. Fedulova EN, Zhukova EA, Tutina OA, Kuznetsova TA, Shumilova OV, Fedorova OV. Nekotorye morfologicheskie kriterii prognozirovanija stepeni tjazhesti techenija bolezni Krona u detej. Morphological criteria for prediction of severity of Crohn's disease clinical course in children. Current Pediatrics. 2013;12(5):108-111. (Ru)
16. Nikipelova EA, Kit OI, Shaposhnikov AV, Zlatnik EY, Novikova IA. Kolokancerogenez: onkoimmunologija lokalnyh izmenenij. Colocarcinogenesis: oncoimmunology of local changes. Malignant tumours. 2016;4(1):81-86. (Ru)
17. Tamm TI, Tsodikov VV, Sedak VV, Pliten ON, Miroshnichenko MS. Izmenenie gistostruktury hronicheskoj analnoj treshhiny v zavisimosti ot dlitelnosti zabolevanija. Changes of histostructure of chronic anal fissure, depending on duration of the disease. Klinichna khirurhiia. 2016;(1):54-57. (Ru)
18. Raskin GA, Petrov SV, Orlova RV. Osobennosti kancerogeneza adenokarcinomy tolstoj kishki. Special features of carcinogenesis of colon adenocarcinoma. Siberian Journal of Oncology. 2015;4:73-79. (Ru)
19. Schmulson MJ, Drossman DA. What is new in Rome IV. Journal of Neurogastroenterology and Motility. 2017;23(2):151-163.

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ORIGINAL ARTICLE

MORPHOLOGICAL CHANGES OF THE ENDOMETRIUM IN HYPERPLASTIC PROCESS IN WOMEN OF REPRODUCTIVE AGE

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ABSTRACT

The aim: To conduct a morphological study of endometrial tissue to identify changes characteristic of viral lesions to develop improved antirelapse treatment of HPE in women of reproductive age.

Materials and methods: We surveyed 90 patients of the gynecological department who sought medical for hyperplastic processes of the endometrium in reproductive age. All women underwent hysteroscopy, the resulting material was subjected to morphological examination.

Results: It became known that the virus is involved in the pathogenesis of endometrial hyperplasia. It is likely that it exists in epitheliocytes not only as a "passenger", but also as an etiological factor. It became known that it was in complex hyperplasia with atypia that the percentage reached the highest level, which is a precancerous condition.

Conclusions: Typical morphological change of the endometrium – multinucleation, multinuclearity and koilocytotic atypia in women of childbearing age with HPE – was revealed. The presence of infectious pathogens in the endometrium of patients with HPE can be regarded as one of the possible triggers for the development of hyperplastic processes.

KEY WORDS: hyperplastic processes of the endometrium; morphological changes of the endometrium; infectious agents

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INTRODUCTION

Endometrial hyperplasia (EH) is the most common pathology of the uterine body in women of childbearing age, manifested by uneven non-invasive proliferation of endometrial glands with a violation of the glandular-stromal ratio in comparison with the proliferative type endometrium, with its functional and morphological changes. According to the scientific literature, EH is 15-40% of all gynecological pathology [1–3].

Scientific publications on this issue allowed us to analyze some etiopathogenetic, ways in the formation of hyperplastic changes in endometrial tissue, in particular to assess the role of infectious factors as a provoking and etiological factor in the occurrence of HPE [4–9].

A number of authors consider HPE as a clinical and morphological syndrome caused by damage to the endometrium by pathogen infections, with numerous secondary morphological and functional changes that disrupt cyclic biotransformation and susceptibility of the mucous membrane of the uterus [10–14]. Other scientists also note the role of the infectious factor in the occurrence of EH despite the lack of growth of the microflora in crops from the uterine cavity [15–19].

A recent study showed that the herpes virus and HVI in endometrial biopsy persist in 40.7% of women with chronic endometritis, microbial viral associations – in 35% [20–23]. Some researchers have demonstrated the possible involvement of viral infection in the occurrence of atypia in HPE, in particular, reports of the presence of HPV in adenocarcinomas of endometrial tissue have been published [16, 24–27]. However, diseases caused by HVI in the female genitals are usually associated with specific areas: the vulva, vagina, cervix and uterine body, where there is a specific epithelium-stratified squamous cell, leading to epithelial cell proliferation and often to atypia. In contrast to the molecular diversity of microbes, morphological manifestations of tissue reactions to microorganisms, as well as morphogenetic mechanisms of these changes are often pathognomonic for a particular microorganism, which should certainly be considered in histological diagnosis. The question arises whether morphological changes can be detected in any tissues, indicating, for example, HPI [10, 25, 28].

The study of research results, in our opinion, indicates the feasibility of further study of the role of viral infection as an etiopathogenetic factor in the occurrence of HPE, as well as the study of the spectrum of viral infection and the role of individual viruses in the occurrence and recurrence of HPE. The study of these features will expand the possibilities of predicting the course and prospects of preventive treatment of relapses in HPE.

THE AIM

To conduct a morphological study of endometrial tissue to identify changes characteristic of viral lesions to develop improved antirelapse treatment of HPE in women of reproductive age.

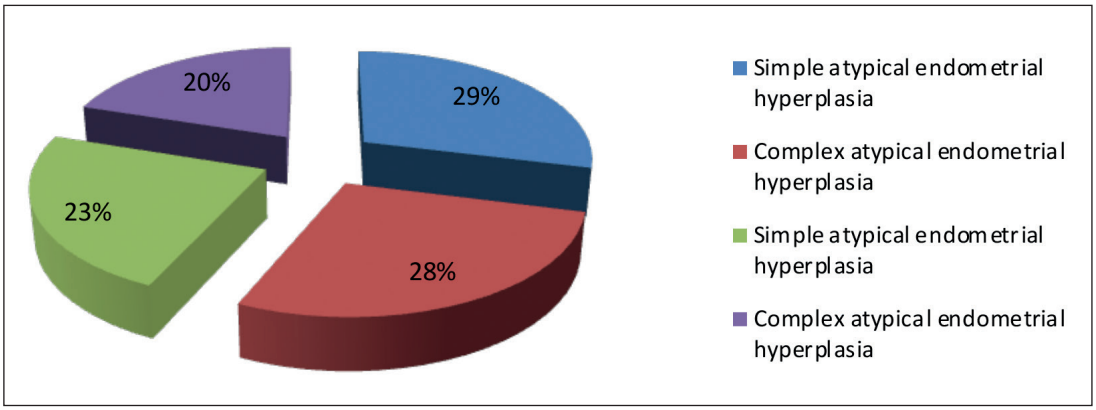

Fig. 1. Quantitative ratio of different types of hyperproliferative processes of the endometrium

MATERIALS AND METHODS

We examined 90 patients of the gynecological department of Ternopil Municipal City Hospital №2, who sought medical care during 2017-2019 for hyperplastic processes of the endometrium in reproductive age. The age of women ranged from 18 to 49 years (average 34.52 ± 2.51).

The criteria for inclusion of patients (group I) in the experiment was histologically confirmed the diagnosis of endometrial hyperplastic process (with simple and complex atypical and atypical endometrial hyperplasia according to the classification of WHO endometrial pathology (2014)) [29]. Control – II group consisted of 30 patients

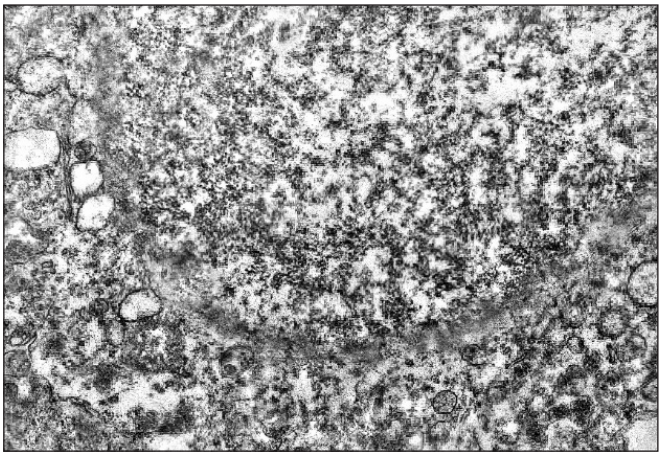

Fig. 2. The structure of the endometrium at the relative norm. Hematoxylin and eosin staining. x 200.

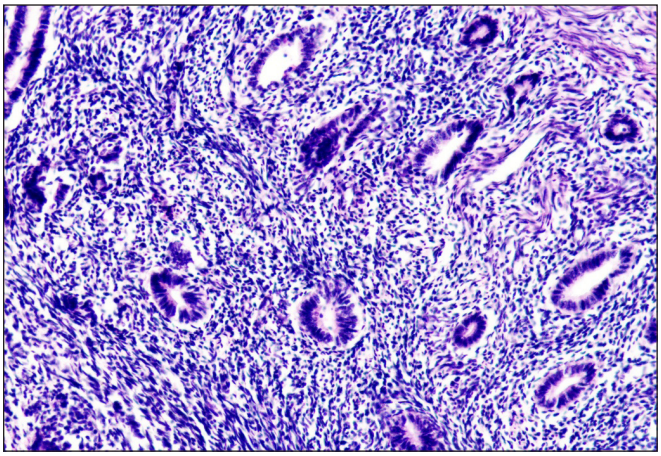

Fig. 3. Simple endometrial hyperplasia without atypia. Cytoplasmic vacuolation of glandular epithelium. Hematoxylin and eosin staining. x 400.

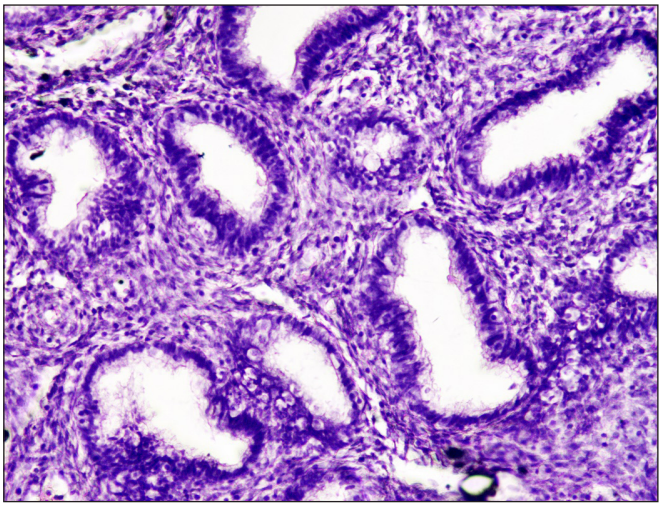

Fig. 4. Complex endometrial hyperplasia without atypia. Focal cytoplasmic vacuolation of endometrial cells. Hematoxylin and eosin staining. x 400.

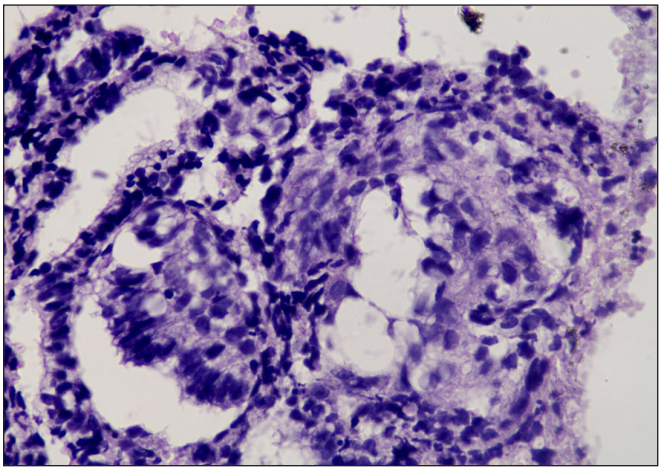

Fig. 5. Complex endometrial hyperplasia without epithelial atypia. Cytoplasmic vacuolation of endometrial cells. Hematoxylin and eosin staining. x 200

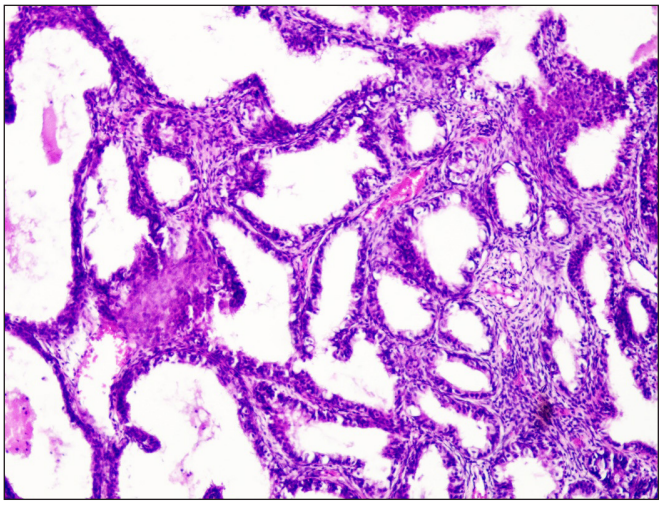

Fig. 6. Complex endometrial hyperplasia with cellular atypia. Cytoplasmic vacuolation of individual epithelial cells. Hematoxylin and eosin staining. x 400.

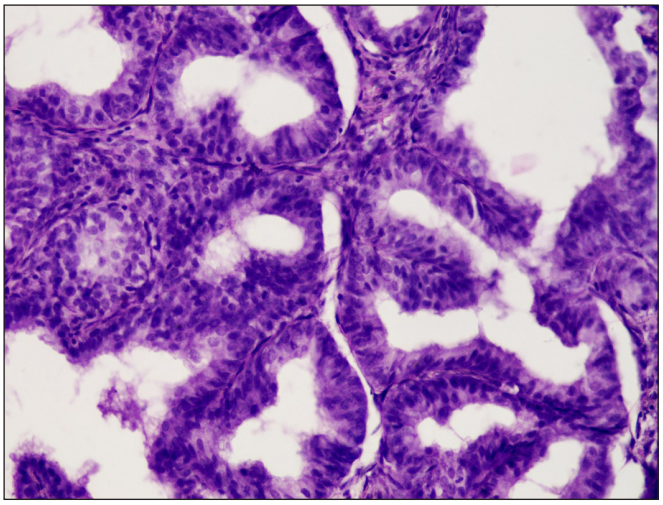

Fig. 7. Complex endometrial hyperplasia without cellular atypia. Cytoplasmic vacuolation of individual epithelial cells. Hematoxylin and eosin staining. x 400.

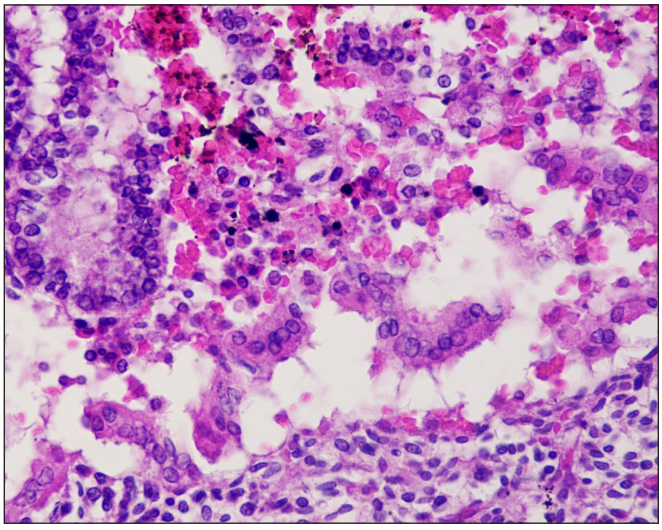

Fig. 8. Cytoplasmic vacuolation of endometrial cells. Focal squamous cell metaplasia is a large foam cell among the endometrial glands. Hematoxylin and eosin staining. x 400.

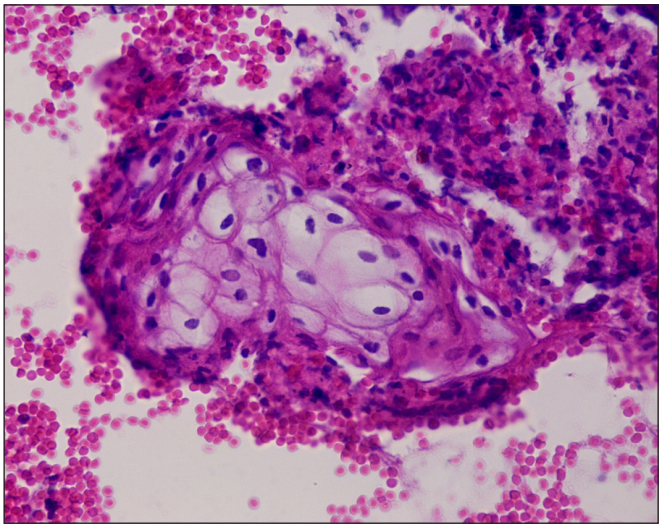

Fig. 9. Fibrillaryl granular nucleus with peripheral displacement of chromatin. Perinuclear nucleocapsids showing a palisading «rosary bead» formation.

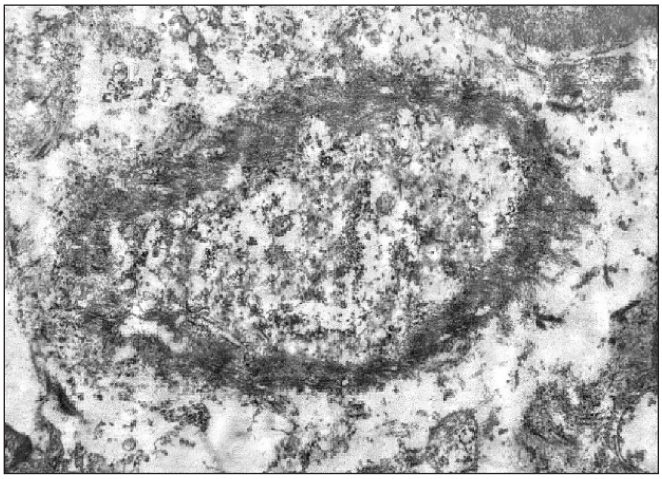

Fig. 10. Fibrillary nucleus (bottom left) and perinuclear cytoplasmic virions about 50 nm in diameter.

of the same age category, healthy patients – volunteers, confirmed by aspiration "Pipelle" (Pipelle de Cornier) endometrial biopsy, followed by histological examination of the evacuated material.

The exclusion criteria were medical records of women with alimentary-constitutional obesity (BMI>30.0), the presence of severe somatic or mental pathology in patients.

Hysteroscopy was performed in the operating room under intravenous anesthesia on hard fiber optics company "KARLSTORZ" from the 6th to the 11th day of the menstrual cycle (with a preserved menstrual cycle). After complete removal of pathologically altered endometrial tissue, its histological examination was performed. The material was fixed in a buffered 10% formalin solution.

Pipelle biopsy was performed on days 19-23 MC using an aspiration probe. The aspiration probe was inserted into the uterine cavity in an assembled form to the bottom area

and the contents were aspirated with a piston, resulting in the effect of "suction" to the walls of the uterine cavity and through the existing perforation material which enters the probe. After performing 2-3 aspirations, the conductor was removed and the probe was removed from the uterine cavity. The resulting material is placed in a vial with a 10% solution of neutral formalin.

Histological examination of endometrial tissue was performed according to the generally accepted method [30] on the basis of the Department of Morphology of the Interdepartmental Training and research laboratory of I. Ya. Horbachevsky Ternopil National Medical University.

The surgical material and endometrial tissue obtained by hysteroscopy and aspiration biopsy of the endometrium were subjected to morphological examination. Biopsy material was fixed in 10% neutral formalin. Further processing was performed according to the standard generally accepted unified method [30, 31]. Examination of histological specimens was performed using a Nikon Eclipse light microscope (Ci-E) using a digital video camera according to the software manufacturer's recommendations.

For electron microscopic examinations, pieces of endometrium were removed, fixed in 2.5% glutaraldehyde solution, and postfixed with 1% osmium tetraoxide solution on phosphate buffer. Further processing was performed according to the generally accepted method [30]. Ultrathin sections made on an ultramicrotome UMPT-7 were contrasted with uranyl acetate, lead citrate according to the Reynolds method and studied under an electron microscope PEM-125K.

For detailed analysis and photo documentation, the image from the microscope was transferred to a computer monitor using a VISION Color CCD Camera and InterVideoWinDUR.

All diagnostic and therapeutic manipulations were performed after the written consent of the patients for examination and treatment in accordance with the protocol approved by the Bioethics Committee (№53 / 03.06.2019). Consent to surgical treatment was obtained before treatment.

The obtained data were processed using standard statistical methods using a personal computer HP PREMIER EXPERIENCE with MicrosoftWord 2010, the graphics were built using Microsoft Excel. Statistical processing was performed by the program Statistics 10.

RESULTS

Hyperplastic processes of the endometrium (HPE) are manifestations of pathological proliferation of endometrial glands, which are manifested by a spectrum of atypical morphological, architectural and cytological disorders that lead to an increase in the glandular-stromal ratio in the endometrium.

By means of morphological research of an endometrium features of various types of HPE were revealed (Figure 1).

Group II patients were diagnosed with endometrial structure, which corresponded to the relative norm (Figure 2). No changes in the inherent hyperproliferative processes of the endometrium in the control group were detected.

At simple atypical HPE (Figure 3) the mucous membrane of a body of a uterus was characterized by numerous glands (increase in number) of various form and size which were unevenly distributed, including cystic-expanded, on separate sites with weakly expressed folds in the direction of a gleam of glands. The structure of the glandular epithelium differed little from the epithelium of the endometrial glands in the proliferation stage, but a significant amount of cytogenic stroma with liquid lymphocytic infiltration (a sign of inflammation of the uterine mucosa) was determined. Dilated capillaries and venules were observed in the superficial parts of individual areas of the endometrium. Simple endometrial hyperplasia without atypia, also known as cystic or mild dysplasia, contained glands of various sizes and shapes that were partially cystically enlarged. There was a moderate ratio of glands / stroma – slightly more than 1. The nature of epithelial growth and its cytological features are similar to those in the phase of endometrial proliferation, although the number of mitoses is minimal.

In simple atypical HE, the endometrium is similar to simple HE without atypia, but there are signs of atypia of the glandular epithelium in the form of loss of cellular polarity and the presence of vesicular nuclei with pronounced nucleoli. The glandular epithelium is rounded with lost normal perpendicular orientation to the basement membrane.

Structural and functional features of the epithelium in simple and complex atypical HPE (Figure 4,5,7,8) may be morphologically similar, but differs in glands, namely their configuration. Complex HE without atypia was characterized by an increase in the number and size of endometrial glands, their close location and branching. Histostructure – atypia in the form of a significant reduction of the stromal component and extremely complex, complex configuration of the glands. The glands increase in number, usually located "back to back", the minimum number of dividing stroma (in some cases, the stroma is reduced to thin strips of elongated cells), and the figures of mitosis are numerous. The glands have a bizarre, branched shape (complex architecture), but the invasion of the epithelium into the underlying stroma is absent, the glands are clearly separated from each other. The lumen of a number of glands is narrowed, irregularly shaped. In some cases, there are multinuclear papillary structures such as "gland in gland". However, the glands do not merge and the epithelial cells are cytologically normal.

In patients with atypical HPE (adenomatosis) (Figure 6), the endometrium has a more pronounced structural rearrangement of the glandular component compared to simple HPE: branched glands predominate with folds, which is directed into the lumen of the gland against the background of numerous compact glands. Multilayered glandular epithelium with different mitotic activity is

also fixed. The stroma contains a significant number of fibroblasts, moderate infiltration of lymphocytes, and in some areas there is swelling. In the superficial layers of the endometrium, the glands have dilated lumens with the effects of stasis, fibrin clots are visualized in the blood vessels.

At atypical HPE the endometrium is characterized by excessive growth of a glandular component and its dominance over a stroma, compact arrangement of glands, atypia of cells of glandular epithelium and change of their tectorial properties. In some glands there is a tendency to form "epithelial membranes". There is a hyperchromatosis of nuclei, the nuclear-cytoplasmic index increases. The glandular epithelium retains a multilayered structure with a basement membrane. The epithelial lining of the glands has an uneven inner contour, stratified with loss of polarity. Mitotic activity of cells is variable, pathological mitoses are observed. Blood vessels are mostly thin-walled, there are fibrin clots. Complex HE with atypia has great morphological similarity with highly differentiated endometria adenocarcinoma. Rounded vesicular nuclei with well-defined nucleoli are determined.

Simple atypical glandular HE differs from simple and complex atypical hyperplasia by the presence of atypia of gland cells, which is manifested by a loss of polarity of the location and an unusual configuration of the nuclei, which often acquire a rounded shape. The nuclei of cells in this type of hyperplasia are polymorphic, and large nucleoli are often isolated in them. Complex atypical HE is characterized by pronounced proliferation of the epithelial component, which is combined with tissue and cell atypia without invasion of the basement membrane of glandular structures. The glands usually lose their regularity for the normal endometrium, they are extremely diverse in shape and size. The epithelium lining the gland consists of large cells with polymorphic, rounded or elongated nuclei with broken polarity and a lot of order of their location.

Histological examination of various types of endometrial hyperplasia in 74.5% of group I ($p<0.05$) revealed a squamous cell component (signs of metaplasia) (Fig. 3, 6). Due to the fact that cytoplasmic vacuolation of the epithelium is often characteristic of koilocytes, which are formed under the influence of the virus in the ectocervical epithelium, we studied various manifestations of endometrial hyperplasia and tried to establish the presence of viral elements in patients with various types of hyperplasia.

Ultrastructural examination of epithelial koilocyte cells of the endometrial glands showed signs of viral infection. Nuclear inclusions consisted of fibrous and granular material, the thickness of the fibrils did not exceed 10 nm and there was a peripheral displacement of nuclear chromatin. Intranuclear viral particles were not identified, but many cells contained electron-dense perinuclear aggregates, not bound by a membrane, up to 55 nm in diameter (Figure 9,10). Of course, such structural manifestations are not sufficient for accurate verification of the process, but are very characteristic of virus-infected cells.

DISCUSSION

According to the scientific literature [2, 23, 26, 32] metaplastic processes are most often manifested in the altered endometrium, including hyperplasia, endometritis, atypical hyperplasia or combined pathology. Papillary syncytial metaplasia is an exophytic proliferation of eosinophilic cells that form small syncytia or micropapillary processes on the surface of the endometrium or inside the glands and is often associated with glandular and stromal disorders. Eosinophilic and ciliated cell metaplasias are characterized by epithelial cells with a saturated dense eosinophilic cytoplasm or numerous cilia on the surface. Mucinous metaplasia reflects the presence of a pale, basophilic cytoplasm that is either vacuolated or granular. Squamous cell metaplasia consists of a significant mass of polygonal cells with dense eosinophilic cytoplasm and signs of keratinization. Secretory metaplasia is characterized by the presence of cells that contain sub- or supranuclear vacuoles resembling the early secretory endometrium.

The source of squamous epithelium in endometrial hyperplasia is unclear, but cells are likely to arise as a result of metaplasia or through squamous differentiation of total stem cells or glandular cells and may be stimulated by estrogen [33, 34, 35, 36].

The defeat of epithelial cells by the virus in endometrial hyperplasia is possible in two ways – direct spread from the cervix, or infection through the blood.

Viruses are known to promote the development of tumors in both humans and animals, and HVI, for example, is known to frequently infect the ectocervical epithelium and is closely associated with the development of cervical cancer [23]. It became known that the virus is involved in the pathogenesis of endometrial hyperplasia [25]. It is likely that it exists in epitheliocytes not only as a "passenger", but also as an etiological factor. In our studies, we observed a predominance of squamous cell metaplasia of the glandular epithelium from 39 to 61% of the studied cases. It became known that it was in complex hyperplasia with atypia that the percentage reached the highest level, which is a precancerous condition (Fig. 2). In the control – II group there were no typical cells that could indicate a viral infection of the endometrium.

CONCLUSIONS

1. Typical morphological change of the endometrium – multinucleation, multinuclearity and koilocytotic atypia in women of childbearing age with HPE – was revealed.
2. The presence of infectious pathogens in the endometrium of patients with HPE can be regarded as one of the possible triggers for the development of hyperplastic processes.

Detection of morphological signs of viral lesions in hyperplasia of the endometrium indicates the need for additional research to identify ways of nuclear damage to the endometrium and justifies the search and study of other effects on the endometrium to improve comprehensive drug therapy in patients with HPE.

REFERENCES

1. Akrish S., Eskander-Hashoul L., Rachmiel A., Ben-Izhak O. Clinicopathologic analysis of verrucous hyperplasia, verrucous carcinoma and squamous cell carcinoma as part of the clinicopathologic spectrum of oral proliferative verrucous leukoplakia: A literature review and analysis. Pathol. Res. Pract. 2019;215(12):152670. https://doi.org/doi:10.1016/j.prp.2019.152670.
2. Farhane F.Z., Alami Z., Bouhafa T. et al. Primary squamous cell carcinoma of endometrium: case report and literature review. Pan Afr. Med. J., 2018;4 (30):8. https://doi.org/doi:10.11604/pamj.2018.30.8.8983.
3. Boichuk A.V., Shadrina V.S., Vereshchahina T.V. Hyperplasia of endometrium – a modern system-pathogenetic view on the problem (literature review): (literature review). Actual problems of pediatrics, obstetrics and gynecology, 2019;(1):67-72. https://doi.org/10.11603/24116-4944.2019.1.9906.
4. Upadhyaya J.D., Fitzpatrick S.G., Islam M.N., et al. A retrospective 20-year analysis of proliferative verrucous leukoplakia and its progression to malignancy and association with high-risk human papillomavirus. Head. Neck. Pathol., 2018;12(4):500-510. https://doi.org/doi:10.1007/s12105-018-0893-7
5. Li P., Ma J., Zhang X. Et al. Cervical small cell carcinoma frequently presented in multiple high risk HPV infection and often associated with other type of epithelial tumors. Diagn. Pathol., 2018;13(1):31. https://doi.org/doi:10.1186/s13000-018-0709-9.
6. Cicinelli E., Matteo M., Trojano G. et al. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. Reprod. Immunol., 2018;79(1):e12782. https://doi.org/10.1111/aji.12782.
7. Younes J.A., Lievens E., Hummelen R., et al. Women and their microbes: The unexpected friendship. Trends Microbiol., 2018,26(1)16-32. https://doi.org/10.1016/j.tim.2017.07.008.
8. Vovk I.B., Horban N.Y., Lysiana T.A. et al. The role of the inflammatory factor in the genesis of hyperplastic changes of endometrium in women of reproductive age. Actual Prob. Pediatr., Obstet. Gynecol., 2019;(1):73-80. https://doi.org/10.11603/24116-4944.2019.1.10180.
9. Gorban N.Y., Vovk I.B., Hoida N.H., Linchak O.V. Menstrual dysfunction: the situation in the world and in ukraine – in women of reproductive age and with atypical hyperproliferative pathology of the endometrium. Bull. Soc. Hygiene Health Protect. Organiz. Ukraine, 2019;(3): 77-83. https://doi.org/10.11603/1681-2786.2019.3.10596.
10. Zhao Y.H., Bai Y.P., Mao M.L. et al. Clinicopathological characteristics of HPV(+) oropharyngeal squamous cell carcinoma. Zhonghua bing li xue za zhi, 2019;48(2):127-131. https://doi.org/doi:10.3760/cma.j.issn.0529-5807.2019.02.010.
11. Du J., Liao X. Superficial spreading squamous cell carcinoma in situ of the cervix involving the endometrium: a rare case presentation and review of literature. Int. J. Clin. Exp. Pathol., 2019;12(11):4162-4166.
12. Dobrokhotova Yu.E., Yakubova K.K. Microbiota of the reproductive tract and hyperplastic processes of the endometrium (literature review). RMJ "Medical Review", Gynecology, 2018;10:14-16.
13. Gorban N.Y., Zadorozhna T.D., Vovk I.B., Zhulkevych I.V. Morphological features of uterine polyps in females of reproductive age. Bull. Sci. Res., 2019;(2):47-52. https://doi.org/10.11603/2415-8798.2019.2.10267
14. Chornenka G.M., Logach M.V. Morphological features of endometrium in women of reproductive age: analysis of morphometric indicators. Achievements of Clinical and Experimental Medicine, 2018;(3):146-150. https://doi.org/10.11603/1811-2471.2018.v0.i3.9275.
15. Adamane S.A., Mittal N., Teni T., et al. Human papillomavirus-related multiphenotypic sinonasal carcinoma with unique HPV type 52 association: A case report with review of literature. Head Neck Pathol., 2019;13(3):331-338. https://doi.org/doi:10.1007/s12105-018-0969-4.
16. Tingthanatikul Y., Lertvikool S., Rodratn N., et al. The effects of dienogest on macrophage and natural killer cells in adenomyosis: A randomized controlled study. Int. J. Fertil. Steril., 2018;11(4):279-286. https://doi.org/10.22074/ijfs.2018.5137.
17. Lacheta J. Uterine adenomyosis: Pathogenesis, diagnostics, symptomatology and treatment. Ceska gynekologie, 2019;84(3):240-246.
18. Gordts S., Grimbizis G.F., Campo R. Symptoms and classification of uterine adenomyosis, including the place of hysteroscopy in diagnosis. Fertil. Steril., 2018;109(3):380-388. https://doi.org/10.1016/j.fertnstert.2018.01.006
19. Choi E.J., Cho S.B., Lee S.R. et al. Chung, H. Comorbidity of gynecological and non-gynecological diseases with adenomyosis and endometriosis. Obstet. Gynecol. Sci., 2017;60(6):579-586. https://doi.org/10.5468/ogs.2017.60.6.579.
20. Yang F., Li H., Qi X., Bian C. Post-hysterectomy rare collision vulva tumor with long-term human papilloma virus infection composed of squamous cell carcinoma of the labia major and adenosquamous carcinoma of bartholin gland: A case report. Medicine (Baltimore), 2019;98(39):e17043. https://doi.org/doi:10.1097/MD.0000000000017043.
21. Thompson A.B., Flowers L.C. Human papillomavirus (HPV). In: Hussen S. (eds). Sexually transmitted infections in adolescence and young adulthood. Springer, Cham, 2020:279-297. https://doi.org/10.1007/978-3-030-20491-4_18.
22. Zhulkevych I.V., Kryvokulskyi B.D. Personalization in oncology: individual approach to the prevention of thromboembolic complications during hysterectomy. Bulletin of Social Hygiene and Health Protection Organization of Ukraine, 2018;4:11-18. https://doi.org/10.11603/1681-2786.2018.4.9875.
23. Wu Q., Chu Z., Han H. et al. Primary squamous cell carcinoma of the endometrium in a woman of reproductive age: a rare case report. J. Int. Med. Res., 2018;46(8):3417-3421. https://doi.org/doi:10.1177/0300060518776579.
24. Yuksel S., Bektas S., Salman S. Adenoid basal carcinoma combined with invasive squamous cell carcinoma of uterine cervix: A case report of a 37-year-old woman and literature review. Diagn. Cytopathol., 2019;47(10):1051-1054. https://doi.org/doi:10.1002/dc.24258.
25. Rintala M., Vahlberg T., Salo T. et al. Proliferative verrucous leukoplakia and its tumor markers: Systematic review and meta-analysis. Head Neck., 2019;41(5):1499-1507. https://doi.org/doi:10.1002/hed.25569.
26. Tetikkurt S., Çelik E., Taş H. et al. Coexistence of adenomyosis, adenocarcinoma, endometrial and myometrial lesions in resected uterine specimens. Mol. Clin. Oncol., 2018;9(2):231-237. https://doi.org/doi:10.3892/mco.2018.1660.
27. Vargas M.V., Huang K., Norwitz E. et al. Endometriosis and adenomyosis. Evidence-based Obstetrics and Gynecology. John Wiley & Sons, Ltd, 2019:8:75–87. https://doi.org/10.1002/9781119072980.ch8.
28. Baker J.M., Chase D.M., Herbst-Kralovetz M.M. Uterine Microbiota: Residents, Tourists, or Invaders? Front. Immunol., 2018;9:208. https://doi.org/doi:10.3389/fimmu.2018.00208.
29. Kurman R.J., Carcangiu M.L., Herrington C.S. World Health Organisation. Classification of tumours of the female reproductive organs. 4th Revised ed. International Agency for Research on Cancer, 2014.

30. Bagriy M.M., Dibrova V.A., Popadynets O.G.; Grishchuk, I.M.; Methods of morphological research: monograph. edited by M.M. Bagriya, V.A. Dibrovy Vinnytsia: New book, 2016:238 p.
31. Nikitina I. et al. Estimation to effigiency of the multifunction metod of endometriosis ovaries treatment. Wiad Lek. 2020;5:868-873.
32. Gorban N., Vovk I., Nikitina I. et al. Immunoglobulin indicators to viruses cytomegal and genital herpes in the blood serum of women with non-atiptcal endometrial hyperproliferative patology. Wiad Lek. 2020;8:1600-1605.
33. Kulavsky V.A., Pushkarev B.A., Kulavsky E.V. et al. Pathology of the endometrium: (diagnostic and therapeutic aspects). Ufa: Healthcare publishing house of Bashkortostan, 2018, p.328.
34. Moreno I., Cicinelli E., Garcia-Grau I., et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. Am. J. Obstet. Gynecol., 2018;218(6):602. https://doi.org/10.1016/j.ajog.2018.02.012.
35. Honcharenko G.Y. The role of steroid receptors in the pathogenesis of adenomyosis in the presence of concomitant endometrial pathology in postmenopause. Reports of morphology, 2019;1(25):45-54. https://doi.org/10.31393/morphology-journal-2019-25(1)-07
36. Horban N.Ye., Vovk I.B., Lysiana T.O. et al. Peculiarities of uterine cavity biocenosis in patients with different types of Endometrial Hyperproliferative Pathology. J. Med. Life, 2019;12(3):266-270. https://doi.org/doi:10.25122/jml-2019-0074.

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ORIGINAL ARTICLE

ECHOCARDIOGRAPHIC PREDICTORS OF THE EARLY POSTOPERATIVE PERIOD IN PATIENTS AFTER CORONARY ARTERY BYPASS GRAFT SURGERY

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ABSTRACT

The aim: The purpose of the present research was to study the results of coronary artery bypass graft (CABG) surgery depending on the degree of left ventricular ejection fraction (LVEF) reduction with the aim to identify additional echocardiographic predictors of the early postoperative period.

Materials and methods: Were fixed, the results of CABG in 97 patients operated on in the "Bikard" private clinic from March 2016 to December 2018 were the material of the research. All patients underwent CABG surgery under cardiopulmonary bypass and cardioplegia, and in the preoperative period underwent echocardiographic examination according to the standard technique on the Vivid 7 machine. Patients, in dependent of the LVEF, were divided into 3 groups: group 1 35 people (LVEF < 40%), group 2 32 people (40% < LVEF < 50%), group 3 30 people (LVEF > 50%).

Results: Our studies showed that the most important echocardiographic predictors of a complicated development of the disease in the early postoperative period, in addition to LVEF of the heart, can be the size of the left ventricle and left atrial, the presence and severity of mitral regurgitation and diastolic dysfunction of the left ventricle of the heart.

Conclusions: Comprehensive measurement of these echocardiographic parameters will allow more accurately predict the results of coronary artery bypass grafting in the early postoperative period.

KEY WORDS: echocardiography, cardiovascular disease, coronary artery bypass surgery, ventricular ejection fraction, mitral regurgitation

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INTRODUCTION

It is known that the results of coronary artery bypass graft (CABG) surgery depend on the state of cardiac contractile function, on the degree of coronary heart disease atherosclerosis, on the presence of concomitant diseases, and other factors. Echocardiography is currently the major method for the pre-operative heart condition assessment and its parameters can serve as prognostic predictors of the outcome of CABG surgery [1]. It is believed that the accuracy of the forecast increases if the complex of echocardiographic parameters and their dynamics in pharmacological tests are taken into account [2]. It is shown that patients with significant left ventricular systolic dysfunction (LVEF < 35%) have an increased risk of complications leading to death [3]. Additionally, evidence indicates that such patients after CABG surgery have satisfactory recovery in the early postoperative period [4].

THE AIM

In the present research, we have studied the results in the early postoperative period of surgical intervention in patients after underwent CABG depending on the degree of decrease in the contractile function of the left ventricle of the heart. The aim was to identify additional echocardiographic predictors of an unfavorable disease course. So, the ***purpose of the research*** is to study the results of CABG surgery depending on the degree of LVSD reduction with the aim to identify additional echocardiographic predictors of the early postoperative period.

MATERIALS AND METHODS

The material of the research was the results of CABG in 97 patients operated on in the "Bikard" private clinic from March 2016 to December 2018. All patients underwent CABG surgery under cardiopulmonary bypass and cardioplegia. All patients in the preoperative period underwent echocardiographic examination according to the standard technique on the Vivid 7 machine. Patients, depending on the left ventricular ejection fraction (LVEF), were divided into 3 groups. The first one consists of 35 people with LVEF < 40%, the second group 32 people with 40% < LVEF <

Table I. Biochemical parameters of patients depending on the reduction degree in heart's LVEF.

Parameter	Group 1	Group 2	Group 3	Differences in means		
				Group 1-Group 2	Group 1-Group 3	Group 2-Group 3
Creatinine, μmol/L	125,8±11,3	104,0±10,5	99,0±9,6	0,077	0,029	0,656
Urea, μmol/L	8,6±1,3	6,6±0,6	7,5±0,8	0,135	0,415	0,194
ALT, units/L	29,0±1,2	26,0±1,3	27,0±1,3	0,026	0,133	0,480
AST, units/L	27±1,3	29±1,2	26±1,3	0,156	0,480	0,026
Cholesterol, μmol/L	6,9±1,2	6,3±0,41	5,9±0,9	0,622	0,429	0,474
LDL, μmol/L	2,4±0,5	2,6±0,3	2,3±0,4	0,699	0,850	0,385
HDL, mmol/L	1,9±1,1	1,8±0,8	2,1±0,9	0,931	0,864	0,735
Triglycerides, μmol/L	2,2±1,0	1,7±0,5	1,9±0,7	0,490	0,774	0,793
Blood sugar, μmol/l	7,7±2,3	7,1±1,0	6,7±2,1	0,798	0,686	0,764

Table II. Echocardiographic parameters of the examined patients, depending on the reduction degree in heart's LVEF.

Parameter	Group 1 M±m	Group 2 M±m	Group 3 M±m	Differences in means		
				1st and 2nd group	1st and 3rd group	2nd and 3rd group
					ANOVA	
Age, units	63,6±1,6	63,3±1,3	61,7±1,5	0,983	0,665	0,778
Body mass index	31,2±1,2	30,2±3,0	32,3±2,1	0,566	0,461	0,949
EDD	57,3±0,8	56,0±0,7	50,4±0,7	0,518	0,000	0,000
ESD	43,1±0,9	40,8±1,2	34,3±0,7	0,245	0,000	0,000
Intraventricular septum	10,1±0,2	10,9±0,4	9,9±0,3	0,132	0,904	0,062
Posterior wall	10,0±0,2	10,8±0,3	9,4±1,8	0,057	0,995	0,056
Ejection fraction	35,7±0,7	45,1±0,3	54,9±0,8	0,000	0,000	0,000
Left atrium	43,0±1,1	39,5±0,8*	38,2±1,1	0,048	0,004	0,654
					z-criterion	
Diastolic function	0,571 (0,408-0,720)	0,656 (0,483-0,796)	0,800 (0,627-0,905)	0,476	0,049	0,205
Insufficiency (Regurgitation)	0,743 (0,579-0,869)	0,781 (0,612-0,890)	0,500 (0,331-0,668)	0,713	0,043	0,021

Note. M ± m - mean ± standard error

50%, and the third group 30 people with LVSD > 50%. Average, standard deviations and standard errors of the mean were calculated as descriptive statistics for quantitative variables. Differences in means were performed by one-way ANOVA test. The Scheffe test was used as a Post Hoc test. The critical level was taken equal to 0.05. The calculations were carried out in the SPSS 16 program environment.

The calculations for the qualitative variables were carried out on the website http://vassarstats.net/. Fractions were calculated; 95% confidence intervals for fractions and the proportions were compared using the Z-criterion according to the rules on the website.

RESULTS

The studied groups of patients did not significantly differ in age, body mass index (BMI), biochemical parameters (urea, aspattataminotranferase (AST), total cholesterol, low-density lipoproteins (LDL), high-density lipoprotein (HDL), triglyceride, blood sugar consists). A significant difference between the first and the third groups was in creatinine and between the first and second groups in alaninaminotranferase (ALT) (Table I).

According to echocardiographic parameters, a statistically significant difference between the groups was in LVEF, left ventricle end-diastolic dimension (LV EDD), left ventricle end-systolic dimension (LV ESD), left atrium (LA), mitral regurgitation, and left ventricular diastolic dysfunction (Table II).

After CABG surgery, three people died in the first group of patients with LVEF lower than 40%, (one person in the hospital, and two after being discharged from the hospital for a month), three patients felt worse after the surgery than before, and the rest of the patients showed improvement of general well-being. In the second and third groups, there were no fatal cases, and almost all of them

improved their functional condition in the postoperative period. Biochemical analysis data (increased creatinine and ALT) in patients with low LVEF (less of 40%) indicate that they have renal and hepatic dysfunction. The results of our echocardiographic researches showed that, along with a low LVEF, predictors of a complicated course of the disease in the early postoperative period can be LV dimensions (LV EDD and LV ESD), LA dimension, mitral regurgitation and left ventricular diastolic dysfunction. Thus, according to our data, the group of patients with low LVEF (< 40%), had statistically larger LV dimensions (LV EDD = 57.3 ± 0.8 mm and LV ESD = 43.1 ± 0.9 mm compared to the 3rd group: LV EDD = 50.4 ± 0.7 mm and LV ESD = 34.3 ± 0.7 mm, p <0.001), and LA (43.01 ± 1.1 mm compared to the 2nd 3rd groups, respectively, 39.5 ± 0.8 mm and 38.2 ± 1.1 mm; p <0.001). Mitral regurgitation and left ventricular diastolic dysfunction were recorded much more often in the 1st and 2nd groups of patients compared with patients with preserved left ventricular systolic function (p <0.05). It should be emphasized that the severity of mitral regurgitation and diastolic dysfunction was also higher in the group of patients with low LVEF.

DISCUSSION

It is known that patients with low LV contractility are at high risk and the mortality rate and complications after CABG surgery are higher than in patients with preserved LVEF [1-4]. Moreover, there is data about the family-depending of early heart disease development. There were registered more younger development of cardiovascular syndrome in case different heart diseases have been found in the family anamnesis [5]. Gratti G et al. [6] studied 4383 cases with multiple coronary heart disease who underwent CABG in the period from January 1999 to September 2014. 300 patients (mean age 66.1 ± 9.6 years) had LVSD ≤ 35%. Hospital mortality in the period of 6.2 ± 4 years was 5.3%. The most frequent postoperative complications were prolonged invasive ventilation (17.7%), acute renal failure (14.7%), and multiple hemotherapy (21.3%). Vickneson et al. [7] conducted a retrospective analysis of 346 patients with low EF (≤ 30%) in the period from 2019 to 2015. It was shown that patients with significant LV dysfunction who underwent CABG surgery had a full recovery within a month. The most significant predictors of mortality were hemodynamic instability and renal dysfunction.

According to our previous studies, the overall hospital mortality after CABG surgery was 3.4%, which is comparable to world standards, does not exceed 1-3% [6, 9]. At the same time, according to our materials, the mortality rate among patients with low LVEF (< 40%) reached 8.6% (3 persons out of 35 patients). The main cause of death in our patients after myocardial revascularization was heart failure caused by systolic or diastolic cardiac dysfunction. In recent years, the problem of diastolic dysfunction received greater attention. It was proved that in the postperfusion period, diastolic dysfunction can be caused by ischemic and reperfusion injury, hypothermia, metabolic disorders or myocardial edema and is an independent factor in the development of difficulties when disconnecting a patient from cardiopulmonary bypass machine, the use of high doses of inotropic support and an increased risk of complications [1]. It was shown that left ventricular diastolic dysfunction is a powerful predisposing factor for the development of postoperative atrium fibrillation. It was established that diastolic dysfunction in cardiac surgery patients increases postoperative mortality [8]. In more than 500 patients who underwent coronary artery bypass grafting surgery without the use of cardiopulmonary bypass machine, the E/e'> 15 according to tissue Doppler echocardiography and high diastolic pressure were associated with a high risk of postoperative complications [9]. According to a multivariate analysis of a number of researchers, left ventricular diastolic dysfunction is the best predictor of hemodynamics after cardiac surgery compared with systolic dysfunction [10]. The inclusion of measurement of indicators of diastolic function of the left ventricle in cardiac surgery patients can improve the prognosis after heart surgery and help prevent adverse events in the postoperative period [11, 12]. It was showed that low volume characteristics of the left ventricle against the background of restrictive diastolic myocardial dysfunction are the echocardiographic predictors of poor prognosis in patients with coronary heart disease in the near term after myocardial revascularization [1].

Patients with mitral regurgitation in combination with coronary heart disease have a higher mortality rate. Thus, more than 40% of patients with coronary heart disease with severe mitral regurgitation die during the first year after the mitral regurgitation was formed [13, 14]. In the case of a combination of coronary heart disease with moderate mitral regurgitation, the mortality rate is about 15-18%, mild mitral regurgitation is 8-11%, while the mortality rate in patients with coronary heart disease without mitral regurgitation is approximately 5% [15]. The results of several studies convincingly showed that moderate ischemic mitral regurgitation affects the increased risk of mortality from cardiovascular diseases, including in patients with heart failure [16]. Our data confirmed that the presence and severity of mitral regurgitation is one of the important prognostic predictors of complications in the early postoperative period after CABG surgery.

CONCLUSIONS

The results of our studies showed that the most important echocardiographic predictors of a complicated course of the disease in the postoperative period, in addition to LVEF of the heart, maybe the sizes of the LV and LA, the presence and severity of mitral regurgitation and diastolic dysfunction of the LV.

According to our data, LV EDD and LVEF between the 1st and 2nd groups of patients did not achieve a statistically significant difference. At the same time, the LA dimension in these groups of patients was significantly different. Based on these results, we can assume that LA size is a more sensitive prognostic criterion for the course of the disease in the early postoperative period than LV dimension.

Has to be noted, overall hospital mortality after CABG surgery in our study wasn't more than the all-world range and made up 3.4%, but the mortality rate among patients with low LVEF (< 40%) reached 8.6% (3 persons out of 35 patients).

So, left ventricular diastolic dysfunction is a powerful predisposing factor for the development of postoperative atrium fibrillation, and dysfunction in cardiac surgery patients increases postoperative mortality.

Comprehensive measurement of these echocardiographic parameters will allow more accurately predict the results of coronary artery bypass grafting in the early postoperative period.

REFERENCES

1. Bockeria L.A., Sokolskaya N.O., Kopylova N.S. Ekhokardiograficheskiye prediktory tyazhesti rannego posleoperatsionnogo perioda u bol'nykh posle khirurgicheskoy revaskulyarizatsii miokarda. [Echocardiographic predictors of the severity of the early postoperative period in patients after surgical myocardial revascularization]. AnesthesiologyReanimatology. 2015;5:8-11. (in Russian).
2. Bukhovets I.L., Vorozhtsova I.N., Lavrov A.G. et al. Ekhokardiograficheskiye aspekty remodelirovaniya levogo zheludochka u bol'nykh s ishemicheskoy bolezn'yu serdtsa do i posle korrigiruyushchikh operatsiy na serdtse. [Echocardiographic aspects of left ventricular remodeling in patients with ischemic heart disease before and after corrective heart surgery]. ComplexProblemsCardiovascularDiseases. 2013;4:109-116. (in Russian).
3. Bystrov D. Aortokoronarnoye shuntirovaniye na rabotayushchem serdtse bez iskusstvennogo krovoobrashcheniya u bol'nykh so snizheniyem fraktsii vybrosa levogo zheludochka. [Coronary artery bypass grafting off-pump without cardiopulmonary bypass in patients with reduced LVSD]. PhD dissertation. Moscow. 2014: 138 p.
4. Volkov A. Koronarnoye shuntirovaniye patsiyentov s vysokim riskom razvitiya oslozhneniy. [Coronary artery bypass grafting in patients with a high risk of complications]. Doctoral dissertation. St. Petersburg. 2014: 304 p.
5. Hrechanina O., Isayeva G., Kolesnikova O., Isakova Y. Relations between familial hypercholesterolemia and early ichemic heart disease: an analysis of medical documentation data. Serbian Journal Experimental Clinical Research. 2019 .doi: 10.2478/sjecr-2019-0056.
6. Gratti G, Maschietto L., Dell Angela L. et al. Predictors of immediate and long-term outcomes of coronary bypass surgery in patients with left ventricular dysfunction. Heart Vessels. 2016;31(7):1045-1055. https://doi.org/10.1007/s00380-015-0714-9.
7. Vickneson K., Chan S.P., Li Y. et al. Coronary artery bypass grafting in patients with low ejection fraction: what are the risk factors? J CardiovascSurg (Torino). 2019;60(3):396-405. DOI: 10.23736/s0021-9509.19.10670-2.
8. Rowlens M., Suri R.M., Seward J.B. et al. Patophysiological mechanism underlying initiation of new onset post-operative atrial fibrillation. J.AM. Coll Cardiol. 2011;58:9(23):953-961. doi: 10.1016/j.jacc.2011.05.021.
9. Jun N.H., Shim J.K., Kim J.C. Prognostic value of a tissue Doppler-derived index of left ventricular filling pressure on composite morbidity after off-pump coronary artery bypass surgery. BritishJournalAnaesthesia. 2011;107(4):1020-1029. https://doi.org/10.1093/bja/aer188.
10. Titova A.L., Saiganov S.A. Diastolicheskaya funktsiya levogo zheludochka u bol'nykh ishemicheskoy bolezn'yu serdtsa, podvergshikhsya operatsii aorto-koronarnogo shchuntirovaniya. [Diastolic function of the left ventricle in patients with ischemic heart disease who underwent coronary artery bypass grafting]. RussianFamilyDoctor. 2014;3:10-17. (in Russian).
11. Skubas N.J., Shernan S.K., Mahmood F. Prioperative assessment of diastolic dysfunction. A&A. 2011;11(3):449-472. doi: 10.1213/ANE.0b013e31822649ac.
12. Beran E., Kapitan M., Machler H. et al. Acurate preoperative echocardiography has more impact on prediction of long-term mortality than intra-operatively measured flow in coronary bypass grafts. Eur.Cardiothorac.Surg. 2011;40(1):245-248. doi: 10.1016/j.ejcts.2010.11.001.
13. Makhmudov Sh. Otsenka sostoyaniya mitral'nogo klapana v otdalennom periode posle korrektsii ishemicheskoy mitral'noy nedostatochnosti pri operatsiyakh AKSH u bol'nykh IBS. [Assessment of the state of the mitral valve in the long-term period after correction of ischemic mitral insufficiency during CABG surgery in patients with coronary heart disease]. PhD dissertation. Moscow. 2017: 104 p. (in Russian).
14. Isayeva G.S. The state of coronary arteries in perimenopausal women with chest pain. JClinMedRes. 2014;6(6):451–455. doi: 10.14740/jocmr1915w.
15. Sukhanov S.G., Timofeeva I.V. Dinamika umerennoy ishemicheskoy mitral'noy regurgitatsii v rannem i otdalennom periode posle koronarnogo shuntirovaniya. [Dynamics of moderate ischemic mitral regurgitation in the early and late period after coronary artery bypass grafting]. BulletinBakoulevCenterCardiovascularDiseases. 2007;8(1):47-51. (in Russian).
16. Khutsurauli E. Progressirovaniye nedostatochnosti mitral'nogo klapana u bol'nykh IBS posle operatsii AKSH i khirurgicheskoy rekonstruktsii levogo zheludochka: faktory riska, mekhanizmy razvitiya, strategiya profilaktiki. [Mitral valve insufficiency advance in patients with coronary artery disease after CABG and surgical reconstruction of the left ventricle: risk factors, mechanisms of development, prevention strategy]. Abstract of PhD Dissertation. Moscow. 2012: 23 p.

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ORIGINAL ARTICLE

KNOWLEDGE OF YOUNG WOMEN ABOUT NUTRIENTS INFLUENCING DEVELOPMENT OF FETAL NERVOUS SYSTEM

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ABSTRACT

The aim: To analyze the nutritional knowledge of women in the reproductive age about nutrients influencing the fetal nervous system's development.

Materials and methods: The study was conducted with a proprietary anonymous questionnaire using the CAWI (Computer Assisted Web Interviews) method among 263 women aged 18-51 years.

Results: In 35.4% of the surveyed women, the level of knowledge was insufficient, in 31.9% – sufficient, in 27% – good, and only in 5.7% – very good. The knowledge of the respondents was influenced by education ($p < 0.001$) and body mass index (BMI, $p < 0.001$). Women of reproductive age with higher education and/or lower BMI had a better understanding of nutrients influencing the fetal nervous system's development. Age, place of residence, family status, and professional status did not affect their knowledge.

Conclusions: The study showed that it is necessary to conduct nutritional education among women of reproductive age. Increasing the respondents' awareness of the importance of nutrients determining the fetus's proper development, both during the reproductive period and during pregnancy, is extremely important.

KEY WORDS: women of reproductive age, pregnancy, nervous system, fetus, nutrients

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INTRODUCTION

One of the most important factors influencing the proper development of the fetal nervous system is women's nutrition, both during pre-conception and during pregnancy. The diet should be based on the principles of rational nutrition in accordance with the latest recommendations developed by the Food and Nutrition Institute presented in the Healthy Nutrition Pyramid. When planning a diet, one should consider the energy demand and contents of carbohydrates, proteins, and fats [1].

Improper eating habits, as well as both too low and too high body weight, increase the risk of certain diseases, such as type 2 diabetes and arterial hypertension. Numerous studies were carried out to prove the importance of the phenomenon of nutritional programming. Developing proper eating habits in women during the procreation period determines, among other things, the correct course of pregnancy, reduces the risk of perinatal complications, and also conditions the development of the fetus and then the child in adulthood. The supply of vitamins and minerals in women of reproductive age, according to the current needs, is important in the development of the fetus's nervous system from conception. Nutrients that should be given special attention include vitamin D, iodine, iron, folic acid, and omega-3 polyunsaturated fatty acids. A proper, balanced diet in the pre-conception period enables the accumulation of essential nutrients and their effective use by the developing fetus. On the other hand, the inclusion of physical activity in accordance with the World Health Organization (WHO) recommendations allows one to maintain a healthy body weight. According to the latest recommendations, an adult should perform moderate physical activity for at least 150 minutes a week or intense exercise for a minimum of 75 minutes a week [2].

According to the latest recommendations of the Polish Gynecological Society of 2017, one should supplement folic acid for three months before a planned pregnancy and increase the consumption of food products that are its source. These include dry legume seeds, dark green leafy vegetables (Brussels sprouts, lettuce, spinach, broccoli), as well as liver. An increased risk of a fetus's neural tube defect and a cleft lip and palate in the newborn is one of the consequences of a folate deficiency in pregnancy. Several risk groups were taken into account, determining the recommended folic acid supplementation dose. The high-risk group includes women with certain gastrointestinal diseases, such as ulcerative colitis, celiac disease or Crohn's disease, carbohydrate metabolism disorders, including type 1 or 2 diabetes, insulin resistance, and diagnosed polycystic ovary syndrome. Some groups of drugs, such as antiepileptics or metformin, affect the metabolism of folic acid. Women with a history of alcoholism or smoking are at higher risk, which in turn is associated with the need to increase the dose of folic acid to 0.8 mg/day, taking into account active folates. In obese women, as well as after bariatric surgeries, attention should be paid to folate supplementation at a dose of 0.8

mg/day. Another risk factor predisposing to increasing the dose of folic acid to 0.8 mg/day is the MTHFR gene mutation. On the other hand, in women with diagnosed neural tube defects in their offspring, mother or father, the recommended dose of supplementation should be 5 mg/day, taking into account active folates, 12 weeks before the planned pregnancy, and also during the first and second trimesters [3-8].

Due to the increased risk of anemia in pregnancy, iron is the second essential nutrient. The demand for this micronutrient rises to 27 mg/day in pregnancy [9]. Even though the absorption of iron with food is only 10% – 15%, it is worth enriching the diet with products that are its source. These include dry seeds of legumes, red meat, and liver [9]. The consequence of the deficiency of this element in pregnancy is the occurrence of anemia. The effects of untreated anemia include increased risk of premature delivery, cesarean section, postpartum hemorrhage, or giving birth to a child with low body weight [10].

Another important micronutrient is iodine. It affects the thyroid gland's proper functioning and determines the production of the hormones: triiodothyronine and thyroxine. The demand in women of reproductive age is 150 μg/day, while during pregnancy, it increases to 250 μg/day [11]. Due to its widespread deficiency in society, it has been ordered to enrich table salt with iodine since 1997. Sea fish such as cod, salmon, and pollock, as well as rennet cheeses, are its main source in the diet. Currently, a frequently used practice in the food industry is the enrichment of mineral waters with iodine. When planning a menu during pregnancy, it is essential to remember about the existence of goitrogens, which reduce the absorption of iodine from food, while disturbing the production of thyroid hormones. These include cabbage, soy, turnips, broccoli, and cauliflower [12]. Adequate supply in line with the current needs reduces the risk of hypothyroidism in pregnancy, miscarriage, intrauterine fetal death, or premature birth [13].

Currently, due to the widespread deficiency of vitamin D in Polish society, the Polish Society of Gynecologists and Obstetricians recommends a dose of 2000 IU/day for women of reproductive age, as well as during pregnancy and breastfeeding. Consequences of insufficient supply include the development of pre-eclampsia, gestational diabetes, and an increased risk of premature delivery or having a low birthweight child [14, 15]. The effect of vitamin D deficiency for the fetus is the possibility of an enamel defect in childhood and skeletal mineralization deterioration [16].

Due to the fact that polyunsaturated fatty acids are a building component of the myelin sheaths of synaptic membranes, it is worth paying attention to their adequate supply. One of the most essential acids belonging to the group of omega-3 polyunsaturated fatty acids is docosahexaenoic acid (DHA). Its source is fatty sea fish and seafood, but when planning a pregnant woman's diet, it should be noted that some products that are the source of DHA may have potentially adverse health consequences. In 2010, guidelines were published not to recommend marine fish for pregnant women, such as smoked sprats, tuna, shark, and swordfish. A proper supply of omega-3 fatty acids reduces the risk of postpartum depression and determines the fetus's growth [17].

Pregnancy is a period of many changes taking place in a woman's body. Therefore, caring for its proper course, as well as the development of a child, one should take into account components that are of particular importance for the development of the fetal nervous system. When planning a diet, it is worth noting that the energy demand also increases in the second trimester by 360 kcal and by 475 kcal in the third trimester of pregnancy.

THE AIM

The study aimed nutritional knowledge of women of reproductive age about nutrients influencing the fetal nervous system's development.

MATERIALS AND METHODS

The survey was carried out using the CAWI method (Computer Assisted Web Interviews) in the period from March to May 2020. The questionnaire consisted of single-choice questions concerning knowledge about the nutrients required for the proper development of the fetal nervous system as well as concerning sociodemographic data, including questions about education, professional status, place of residence, age, height, and body weight.

Inclusion criteria for the study were: female gender, age 18-51, informed consent to participate. Exclusion criteria were as follows: male gender, pregnancy, lactation period, female age under 18 and over 51. Women's knowledge was assessed using the proprietary point scale. For each correct answer, the respondents obtained 1 point, and for incorrect or no answers, the respondents obtained 0 points. A maximum of 18 points could be obtained. The following grading scale was adopted: 16-18 points – very good; 13-15 points – good; 10-12 points – satisfactory grade; 0-9 points – insufficient grade.

The last question in the proprietary questionnaire concerned the subjective assessment of one's own knowledge about the nutrients for the fetal nervous system's proper development.

The obtained results were processed with the use of Microsoft Office Excel. Statistical analysis was performed using the Statistica 13 software. Measurable data were characterized using the mean (X) and standard deviation (SD), while nominal data were presented as percentages. The Kolmogorov-Smirnov test was used to check whether a given distribution meets the condition of a normal distribution. Non-parametric tests were applied when compared variables did not meet the requirement of normal distribution. The significance of differences in the results between the two groups was tested using the Mann-Whitney U test. The significance of differences in the results between more than two groups was checked using the Kruskal-Wallis test.

Table 1. The sociodemographic analysis of the surveyed women.

		Number	[%]
Place of residence	Rural area	56	21.4
	Town: < 50 thousand residents	43	16.4
	Town: 50-100 thousand residents	35	13.4
	City: > 100 thousand residents	128	48.9
Education	Primary	2	0.8
	Vocational	6	2.3
	Secondary	80	30.5
	Higher	174	66.4
Employment status	Pupil/student	50	19.0
	Student and professionally active	47	17.9
	Professionally active	149	56.7
	Unemployed	17	6.5
Social status	Single	84	31.9
	In a partnership/marriage	179	68.1

Further post hoc testing was checked by multiple pairwise comparisons, and significance levels were corrected by the Bonferroni method. Correlations between the variables were checked using the Spearman correlation coefficient. The level of significance was $p < 0.05$.

RESULTS

Two hundred sixty-three women aged 18-51 participated in the study. The mean age of the respondents was 28 years ± 6.62 years, mean body weight – 65.4 ± 13.2 kg, mean height – 167 ± 6.39 cm, while the mean BMI value was 23.4 ± 4.26 kg/m^2. The respondents with normal body weight constituted 64% of the respondents, followed by 7% of underweight women, 23% – overweight, and 6% – obese.

The sociodemographic analysis of the surveyed women is presented in Table 1.

Answers to questions about the knowledge of nutrients influencing the development of the fetus's nervous system are summarized in Table 2.

For each correct answer, the respondents obtained 1 point, and for incorrect or no answer – 0 points. The maximum number of points possible to earn was 18.

The examined women obtained an average of 10.6 ± 3.35 points. At least half of the respondents gave no more than 11 correct answers. Most often, the respondents were able to provide 13 answers correctly (33 people). Two respondents did not answer any of the questions correctly, and one person answered all the questions correctly.

Based on the obtained number of correct answers, the level of knowledge about nutrients influencing the development of the nervous system of the fetus was determined. 35.4% of women obtained an insufficient grade, 31.9% – satisfactory, 27% – good, and only 5.7% – very good.

There was no statistically significant relationship between the level of knowledge about nutrients influencing the development of the nervous system of the fetus and age ($p = 0.676$), place of residence ($p = 0.685$), employment status ($p = 0.093$), or family status (single or partnership) ($p = 0.057$).

However, a statistically significant relationship between BMI and the level of knowledge was demonstrated. The respondents who obtained high BMI values gave less correct answers ($p < 0.001$).

Moreover, a statistically significant relationship was found between the level of education and the level of knowledge. The respondents who had a higher education level provided more correct answers to the questions asked ($p < 0.001$).

The correlation made with the Spearman rank coefficient showed a statistically significant relationship between the self-assessment of knowledge and the level of knowledge about nutrients influencing the fetal nervous system's development. The respondents who stated that their self-esteem in the discussed topic was high, provided more correct answers to the questions asked ($r = 0.49$; $p < 0.001$).

DISCUSSION

Knowledge about preventive nutritional activities in women of reproductive age was the subject of research by many specialists. One of the most important factors in maintaining full health is using a properly balanced diet, in line with the current energy needs and the inclusion of physical activity. An adequate supply of nutrients not only prevents the consequences of their deficiency in reproductive age but also ensures the proper course of pregnancy. In order to provide the appropriate development of the fetus, with particular emphasis on the nervous system, one should ensure the correct supply of vitamin D, iodine, iron, polyunsaturated fatty acids, and folic acid.

Table 2. Answers to the survey questions concerning the nutritional knowledge of women of reproductive age in the field of nutrients influencing the fetal nervous system's development.

		Number of responses	[%]
Which of the listed minerals must be supplemented during pregnancy?	Calcium, magnesium, vitamin D	13	5.0
	Vitamin C, E, zinc	4	1.5
	Calcium, vitamin D	3	1.1
	Folic acid, vitamin D*	241	92.3
What time before a planned pregnancy should folic acid be supplemented according to the Polish Gynecological Society's latest recommendations?	4 weeks	33	12.7
	8 weeks	70	26.9
	12 weeks*	133	51.2
	16 weeks	24	9.2
What are the sources of folate in food?	Green leafy vegetables and whole grains*	230	88.1
	Milk and dairy products	2	0.8
	Meat and fish	17	6.5
	Fruit and vegetables rich in beta-carotene	12	4.6
What might be the effects of a folate deficiency in pregnancy?	Fetal vision and hearing defects	25	9.7
	Fetal neural tube defects*	166	64.1
	Deterioration of the psychomotor and cognitive development of the fetus	57	22.0
	Cretinism and hypothyroidism	11	4.2
Which of the listed ingredients increase folate absorption?	Iodine and vitamin D	77	30.1
	Iron, zinc, and vitamin C*	88	34.4
	Magnesium, zinc, and vitamin E	25	9.8
	Phosphorus, calcium, and vitamin D	66	25.8
How much vitamin D should be supplemented according to the latest recommendations?	400 IU/day	44	16.9
	800 IU/day	55	21.2
	2000 IU/day*	147	56.5
	4000 IU/day	14	5.4
Which of the following ingredients have the most significant impact on the development of the fetal nervous system?	Vitamin C, E, and magnesium	4	1.5
	Vitamin D, omega-3 fatty acids, iron, and folic acid*	167	64.2
	Folic acid, chlorine, phosphorus, and vitamin C	23	8.8
	Iron, calcium, omega-3 fatty acids and vitamin K	66	25.4
What are the sources of vitamin D in food?	Dark green leafy vegetables	27	10.4
	Fruits	9	3.5
	Oily sea fish*	182	70.3
	Legume seeds	41	15.8
What are the effects of iron deficiency in pregnancy on the fetus?	Cretinism and hypothyroidism	24	9.3
	Low birthweight*	122	47.5
	Development of metabolic disorders	86	33.5
	Increased risk of bone fractures	25	9.7
Which component might be vastly deficient in women who are menstruating profusely before pregnancy?	Calcium	9	3.5
	Magnesium	13	5.0
	Iron*	225	86.9
	Iodine	12	4.6
What are the effects of iodine deficiency on the woman and the fetus?	Hypothyroxinemia, the development of attention deficit hyperactivity (ADHD)*	49	19.1
	Megaloblastic anemia, lower intelligence quotient (IQ) in childhood	39	15.2
	Hypothyroxinemia, increased fracture frequency	41	16.0
	Cretinism accompanied by hypothyroidism, visual impairment	128	49.8
Which component deficiency can cause hypothyroxinemia?	iron	20	7.8
	zinc	37	14.5
	iodine*	180	70.3
	magnesium	19	7.4

What are the sources of iodine in the diet?	Dark green leafy vegetables	26	10.0
	Fish and seafood*	215	83.0
	Fruits	9	3.5
	Whole-grain cereal products	9	3.5
What is the sufficient sodium intake for women of reproductive age??	less than 0.5 g/day	67	26.2
	1.5 g/day*	141	55.1
	4 g/day	46	18.0
	more than 6 g/day	2	0.8
Which products inhibit iodine absorption due to the content of goitrogens?	Milk and dairy products	59	23.1
	Turnips, broccoli, Brussels sprouts, and cabbage*	98	38.4
	Pepper, tomato, parsley, and peach	24	9.4
	Orange, grapefruit, and lemon	74	29.0
The demand for which ingredient increases significantly during pregnancy?	Magnesium	58	22.7
	Zinc	14	5.5
	Iodine*	72	28.1
	Calcium	112	43.8
What are the sources of omega-3 fatty acids in the diet?	Milk and dairy products	4	1.6
	Whole-grain cereal products	10	3.9
	Rapeseed oil, olive oil, walnuts, and linseed*	204	79.4
	Sunflower oil, peanuts, evening primrose oil	39	15.2
Which medical conditions can cause a lower IQ and impaired speech development and cretinism?	Hypothyroidism*	124	48.4
	Iron deficiency anemia	45	17.6
	Vitamin B_{12} deficiency anemia	71	27.7
	Diabetes and insulin resistance	16	6.3
Self-assessment of knowledge about the nutrients influencing the development of the fetal nervous system	Insufficient	95	36.7
	Satisfactory	100	38.6
	Good	59	22.8
	Very good	5	1.9

*the correct answer

In 2017, Kamińska K, Zegan M, and Michota-Kotulska E researched women of reproductive age to test their knowledge about folic acid's role in pregnancy. Respondents knew the effects of folate deficiency, but they did not know what the recommended dose was in pregnancy and were not able to list the sources of folic acid in food products. Based on the above results, it was concluded that the respondents' knowledge was insufficient [18].

In 2015, Grzelak T. et al. conducted a study in which the respondents were divided into two groups. The first group consisted of 49 women planning pregnancy within the next year or pregnant women. The second group consisted of 205 adults. The mean age was 28.91 ± 5.05 years in the first group and 30.29 ± 11.81 years in the second group. The aim was to evaluate the supplements used among women of reproductive age planning to become pregnant or already pregnant women using an anonymous proprietary survey. 69.39% of pregnant women or women planning to become pregnant in the next 12 months declared regular or occasional intake of dietary supplements. In the second group, the same procedure was reported by 54.66% of the respondents. The use of supplementation was demonstrated in 56% of women of reproductive age planning pregnancy and as much as 89% of pregnant women [19]. Similar studies were conducted in 2008. The aim was to evaluate the consumption of vitamins and nutrients among 60 women from the Mazowieckie voivodeship in the reproductive age. The mean age of the respondents was 26.9 ± 4.2 years. As many as 75% of the respondents belonged to the age group of 21 – 30 years, 15% of women were over 30 years, and only 10% were under 20 years of age. The results were similar to the study conducted by Grzelak T et al. The use of supplements concerned 98.3% of pregnant women and 55% of women planning a pregnancy. There was a higher than the recommended intake of specific vitamins, such as vitamin D (157%), folic acid (128%), iodine and zinc (113%), and iron (120%.). Insufficient supply was found in the case of magnesium, calcium, and vitamin A. Most often, supplements were taken in the form of multi-component preparations. In the case of single-ingredient supplements, folic acid was most often used. In the conducted study, only every fifth woman showed a change in diet during pregnancy, of which 10% of the respondents introduced an easily digestible diet [20].

Pieszko M, Ciesielska-Piotrowicz J, Skotnicka M, and Małgorzewicz S conducted studies in which they examined the level of knowledge of women with higher and secondary education about proper eating habits influencing the development of the fetus. An anonymous questionnaire with 22 questions was used. The survey was divided into two parts. The first part consisted of a record, while the second part contained questions about the number

of meals, consumed food products, supplementation, feeling hungry, as well as the consumption of vegetables, fruit, sweets, and stimulants (coffee, alcohol, cigarettes). The authors assessed in the study the knowledge on the sources of folic acid and iron, as well as the recommended weight gain during pregnancy and physical activity. 58.1% of the surveyed women were in the 21 – 30 age group. In the studied group, the majority of the respondents were women who visited them for the first-time pregnancy, and constituted 86% of the respondents. 84% of respondents showed greater interest in proper eating habits during pregnancy. As many as 98% of women increased the consumption of vegetables and fruits, while 41.9% of the respondents increased the consumption of sweets during pregnancy. The most commonly used supplements were multi-component pharmaceutical preparations. 90.7% of women knew consequences of folic acid deficiency during pregnancy, while in our own work – only 64.1%. Most of the respondents in the study by Pieszo M et al. were women who were pregnant, while our own work did not take into account the respondents' experiences resulting from motherhood. 83.7% of the respondents knew the answer regarding the sources of folate in food. In our study, similar results were obtained – the correct answer was indicated by 88.1% of the respondents. One of the reasons for the knowledge of the effects of insufficient supply of folic acid is the introduction of the Primary Neural Tube Defects Prevention Program in 1997, which aimed to increase knowledge in the studied area [21].

Gieratka-Czernel M et al. researched 100 women between 5-38 weeks of pregnancy in Mazowieckie voivodship, of which 45% of the respondents were pregnant for the first time. Only every third respondent supplemented iodine, and 59% of women used food enriched with this element. In this study, only every 5th respondent knew the effects of iodine deficiency in pregnancy, which constituted 19.1% (n = 49). The results of the research by Gieratka-Czernel M et al. are consistent with our results [22].

In a study conducted by Pieczyńska J et al., nutrition was assessed using an anonymous proprietary questionnaire and a 24-hour interview from 7 consecutive days. The aim was to determine the relationship between pregnancy advancement and the intake of fatty acids with food and dietary supplements. The study involved 119 female residents of the Lower Silesia Province, who were divided into three groups according to the stage of pregnancy. The first group consisted of 79 women in the first trimester of pregnancy, the second group consisted of 54 women and the third group of 44 respondents. The study showed insufficient fat consumption in the various trimesters of pregnancy, while the average consumption was at the level of 29%, which was in line with the norms. Overly high consumption of saturated fatty acids was also shown.

In the presented conclusions, it was stated that the supply of particular groups of fatty acids is insufficient, both with food and in the form of dietary supplements. Simultaneously, it was shown that the consumption of fish increases and fried food consumption decreases in the next trimesters of pregnancy, which was a positive phenomenon. Women showed a willingness to change their eating habits during pregnancy, but no significant differences in the supply of particular groups of fatty acids were noted. One of the proposed solutions was the introduction of nutritional education among women of reproductive age in order to increase knowledge and awareness of proper eating habits [23].

CONCLUSIONS

1. In more than one-third of the surveyed women of reproductive age, the level of knowledge in the field of nutrients influencing the development of the nervous system in the fetus was insufficient, while only in slightly more than half – sufficient or good.
2. Body mass index and education were the factors influencing the nutritional knowledge of the surveyed women. Women of reproductive age with higher education and/or lower BMI had a better understanding of nutrients influencing the fetal nervous system's development.
3. Age, place of residence, family status, and professional status did not significantly impact the nutritional knowledge of women of reproductive age.
4. The conducted research indicates the need for nutritional education, led by specialists among women of reproductive age, on nutrients influencing the development of the nervous system in the fetus.

REFERENCES

1. Moszak M. Żywienie kobiet ciężarnych i karmiących. [W:] Grzymisławski M. Dietetyka kliniczna. Warszawa: Wydawnictwo Lekarskie PZWL; 2019.
2. Pudło H, Respondek M. Programowanie żywieniowe – wpływ odżywiania kobiet w ciąży na zdrowie dziecka. J Edu Health Sport 2016;6(7):589-600.
3. Cieślik E, Kościej A. Kwas foliowy-występowanie i znaczenie. Probl Hig Epidemiol 2012;93(1): 1-7.
4. Bomba-Opoń D, Hirnle L, Kalinka J, Seremak-Mrozikiewicz A. Suplementacja folianów w okresie przedkoncepcyjnym, w ciąży i połogu. Rekomendacje Polskiego Towarzystwa Ginekologów i Położników. Ginekol Perinatol Prakt 2017, 2(5): 210-214.
5. Karolewicz-Bilińska A, Nowak-Makwitz E, Opala T et al. Rekomendacje Polskiego Towarzystwa Ginekologicznego w zakresie stosowania witamin i mikroelementów kobiet planujących ciążę. Ginekol Pol 2014, 85(5) ; 395-399.
6. Wolski H, Kocięcka M, Mrozikiewicz A, Barlik M, Kurzawińska G. Coexistence of the 677-C>T and 1298 A>C MTHFR polymorphizm and its significance in the population of Polish women. Ginekol Pol 2015;86(10):742-747.
7. Lorenc A, Kurzawińska G, Kraśnik W, Wolski H, Seremak-Mrozinkiewicz A. Znaczenie współwystępowania polimorfizmów genów MTHFR, MTR, MTRR, TCII w hipotrofii płodu. Perinatol Neonatol Ginekol 2014;7(2):72-80.
8. Wronka L, Małachowska A, Grochowska A. Suplementacja w ciąży według rekomendacji Polskiego Towarzystwa Ginekologów i Położników. Lek Pol 2019;29(6):5-8, 10-15.
9. Paurenpaa P, Heliovaara-Peippo S, Frasu I, Paavonen J, Hurskainen R. Effects of anemia and iron deficiency on quality of life in women with heavy menstrual bleeding. Acta Obstet Gynecol Scand. 2014;93(7):654-660.

10. Pietrzak B, Seremak-Mrozinkiewicz A, Marciniak B. Niedokrwistość z niedoboru żelaza w ciąży w położnictwie i ginekologii. Ginekol Perinatol Prakt 2016;1(3):115-121.
11. Pyka B, Zieleń-Zynek I, Kowalska J, et al. Zalecenia dietetyczne dotyczące spożywania jodu-w poszukiwaniu konsensusu między kardiologami a endokrynologami. Folia Cardiol 2019;14(2):156-160.
12. Czerwińska E. Ocena spożycia jodu i sodu przez kobiety ciężarne. Ginekol Perinatol Prakt 2018;3(1):10-15.
13. Kowalska A. Niedoczynność tarczycy podczas ciąży. Med Dypl. 2014;26(4):78-83.
14. Bomba-Opoń D, Karowicz-Bilińska A, Laudański P, Spaczyński RZ. Suplementacja witaminy D w położnictwie i ginekologii. Ginekol Perinatol Prakt 2016;1(1):20-29.
15. Paszkowski T. Rola witaminy D w ciąży – przegląd najnowszych doniesień. Forum Poloz Ginekol. 2017;32:8-11.
16. Misiorowska J, Misiorowski W. Rola witaminy D w ciąży. Post Nauk Med 2014;267(12):865-871.
17. Dębski R, Karowicz-Bilińska A, Oszukowski P, Paszkowski T, Spaczyński M. Rekomendacje Polskiego Towarzystwa Ginekologicznego dotyczące stosowania suplementacji kwasem dokozaheksaenowym w profilaktyce porodu przedwczesnego. Ginekol Pol. 2014;85:318-320.
18. Kamińska K, Zegan M, Michota-Kotulska E. Wiedza wybranych grup kobiet w wieku prokreacyjnym na temat znaczenia kwasu foliowego. Wiedza Zywien Metabol 2017;44.
19. Grzelak T, Suliga K, Sperling M, Pelczyńska M, Czyżewska K. Ocena stosowania suplementów diety wśród kobiet ciężarnych lub planujących ciążę. Forum Zab Metabol 2016;7(1):8-15.
20. Hamułka J, Wawrzyniak A, Pawłowska R. Ocena spożycia witamin i składników mineralnych z suplementami diety przez kobiety w ciąży. Roczn PZH. 2010;61(3):269-275.
21. Pieszko M, Ciesielska-Piotrowicz J, Skotnicka M, Małgorzewicz S. Zachowania zdrowotne kobiet ciężarnych z wyższym i średnim wykształceniem-badania wstępne. Pediatr Med Rodz, 2018;13(1):94-102.
22. Gietka-Czernel M, Dębska M, Kretowicz P, et al. Iodine status of pregnant women from central Poland ten years after introduction of iodine prophylaxis programme. Endokrynol Pol. 2010;61(6):646-651.
23. Pieczyńska J, Sozański R, Kłósek A, Grajeta H. Wpływ zaawansowania ciąży na strukturę spożycia kwasów tłuszczowych z dietą przez kobiety ciężarne. Probl Hig Epidemiol 2017;98(1):73-80.

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Conflict of interest

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

HEALTHCARE ASSOCIATED DEEP PELVIC TISSUE INFECTION AND OTHER INFECTIONS OF THE FEMALE REPRODUCTIVE TRACT IN UKRAINE

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ABSTRACT

The aim: To obtain the first estimates of the current prevalence of healthcare-associated deep pelvic tissue infection and other infections of the female reproductive tract (vagina, ovaries, uterus) including chorioamnionitis, and antimicrobial resistance of causing pathogens in Ukraine.

Materials and methods: We performed a retrospective multicenter cohort study was based on surveillance data. The study population included 3,053 women's who underwent gynecological surgery or other procedures from 2017 to 2019 in 7 women hospitals in Ukraine. Definitions of healthcare-associated reproductive tract infection were used from the CDC/ NHSN.

Results: The prevalence of healthcare-associated deep pelvic tissue infection and other infections of the female reproductive tract was 26.3%. Incidence of infection was: 13.3% Pelvic abscess or cellulitis, 14.6% Adnexa utery, 9.5% Salpingitis, 7.1% Oophoritis, 12.2% Parametritis, 4.6% Chorioamnionitis, and 38.8% Bacterial Vaginitis. The predominant pathogens were: *Escherichia coli* (25.6%), *Enterobacter* spp. (11.4%), *Klebsiella pneumoniae* (10.6%), *Staphylococcus aureus* (8.5%), *Enterococcus faecalis* (7.2%) and *Pseudomonas aeruginosa* (7.1%). Methicillin-resistance was observed in 14.3% of S. *aureus* (MRSA). The overall proportion of extended spectrum beta-lactamases (ESBL) production among *Enterobacteriaceae* was 20.8%. The prevalence of ESBL production among *E. coli* isolates was 24.7% and among *K. pneumoniae* 11.9%. Resistance to third-generation cephalosporins was observed in 12.8% *E.coli* and 9.2% *K. pneumoniae* isolates.

Carbapenem resistance was identified in 8.5% of *P.aeruginosa* isolates.

Conclusions: Healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine is a common occurrence and many cases are caused by pathogens that are resistant to antibiotics.

KEY WORDS: Healthcare-associated infection, deep pelvic tissue infection, female, vagina, ovaries, uterus, chorioamnionitis, pathogens, antimicrobial resistance

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INTRODUCTION

Healthcare-associated reproductive tract infection (HARTI) in female is a major problem for public health care in worldwide, causing increased morbidity and mortality in women. These infections may result in infertility, ectopic pregnancy and chronic pelvic pain and other disease [1-4]. The prevalence of RTIs in women varies from country to country and ranges from 1.8% [5] to 48% [6,7]. In the United States, 1 million women are diagnosed with RTI each year and the cost of their treatment is estimated at $ 4.2 billion [8]. Many studies show that despite the introduction into medical practice of new diagnostic technologies for diagnosis and treatment, as well as broad-spectrum antibiotics, the number of healthcare-associated infections is not decreasing.

HARTIs in female include Endometritis, Episiotomy, Vaginal cuff infections and other infections of the female reproductive tract [9]. Other infection of the female reproductive tract involves the Deep pelvic tissue infection or other infection of the female reproductive tract (for example, vagina, ovaries, uterus) including chorioamnionitis, but excluding Endometritis or Vaginal cuff infections [9]. In the available literature, there are many studies devoted to Endometritis or Vaginal cuff infections. However, there are a limited number of studies on other reproductive tract infection in female.

One of the reasons for healthcare-associated infections is the wide spread of conditionally pathogenic microorganisms in Ukraine that are resistant to antibiotics [10,11]. The emergence and spread of antimicrobial resistance has become a major public health threat in Ukraine, and the number of infections caused by resistant pathogens causing of HARTIs continues to increase [12,13].

Antimicrobial resistance creates obstacles for effective prevention and treatment of patients with infections. Every year resistant healthcare-associated infection is becoming more and more pressing for medical specialists in Ukraine. Microbiological monitoring of the prevalence of etiologic agents of HARTIs and antimicrobial resistance is necessary to enhance our knowledge of its epidemiology. To our knowledge, the prevalence of and causative agents of most HARTIs (besides Endometritis and Episiotomy infections)

among female in-patients and out patients have not been studies in Ukraine. This was the basis for our study.

THE AIM

The aim of this study was to obtain the first estimates of the current prevalence of infection of the female reproductive tract involves the Deep pelvic tissue infection or other infections of the female reproductive tract including Chorioamnionitis, but excluding Endometritis or Vaginal cuff infections and antimicrobial resistance of responsible pathogens in Ukraine.

MATERIALS AND METHODS

SETTING

We performed a retrospective multicenter cohort study was based on surveillance data of Reproductive Tract Infections in women's from January 1st, 2017 to December 31st, 2019 in 7 regional (tertiary) women hospitals of Ukraine. The hospitals had 1250 beds. They are similar in terms of medical equipment, staff and number of beds. All participating hospitals were required to have at least one full-time infection-control professional, a clinical microbiology laboratory with the capacity to process cultures.

PARTICIPANTS

Inclusion criteria: This study included 3,053 women's aged 16-55 years admitted to hospitals with the diagnosis of acute pelvic inflammatory disease (PID). All women were local residents. Diagnosis of acute PID was based on criteria from the Centers for Disease Control and Prevention (CDC) [4].

Exclusion criteria: endometritis or vaginal cuff infections; current pregnancy; positive serological test for syphilis or other sexually transmitted infections; inflammatory bowel disease or significant renal or hepatic disease; had a history of colitis; neoplasms or haematological malignancy.

DEFINITIONS OF HARTI

Diagnosis of Deep pelvic tissue infection or other infection of the female reproductive tract (vagina, ovaries, uterus) including chorioamnionitis was based on criteria from the CDC/NHSN Surveillance Definitions for Specific Types of Infections [9]. Women had uterine curettage, or delivery, abortion, instrumentation of the upper genital tract, pelvic or abdominal surgery within the last month (30 days) prior to admission.

DATA COLLECTION

In this study, we analyzed the inpatient data and ambulatory medical records to identify deep pelvic tissue infection or other infections of the female reproductive tract including chorioamnionitis. We collected the data using structured NHSN Reproductive Tract Infection (REPR) Checklist. Full-text ambulatory medical records and relevant hospital records were reviewed for the all women's. Additional data form was created to extract demographic and clinical data, microbiology (isolated pathogens and antibiograms) and outcome information from inpatient data and ambulatory medical records.

MICROBIOLOGICAL METHODS

All samples were obtained from women with clinical symptoms of PID. Microbial isolates were identified using standard microbiological techniques, including automated microbiology testing (Vitek-2; bioMe´rieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was performed by using the disk diffusion method (Kirby – Bauer antibiotic testing) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Strains in the intermediate range were classified as resistant for data analysis.

ETHICS

The Shupyk National Medical Academy of Postgraduate Education of Ukraine ethics committee approved the waiver of informed consent to participate in this study due to its retrospective design. All pregnant women's data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

Prevalence of healthcare-associated deep pelvic tissue infection or other infections of the female reproductive tract was reported as the percentage of the total number of women who had been submitted to PID cases. We analyzed samples from women's in the context of a study about microbiology of HARTI and antimicrobial resistance of responsible pathogens. The analysis of statistical data was performed using Excel. Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. Statistical significance was defined as $P<0.05$.

RESULTS

PREVALENCE AND TYPE OF INFECTION

During the study period (from 2017 to 2019), 804 of 3,053 patients were found to have healthcare-associated infections (HAI). The prevalence of healthcare-associated deep pelvic tissue infection and other infections (n=804) of the female reproductive tract in women hospitals in Ukraine was 26.3% [95% confidence interval (CI) 25.5-27.1]. Of these cases, 13.3% Pelvic abscess or cellulitis, 14.6% Adnexa utery, 9.5% Salpingitis, 7.1% Oophoritis, 12.2% Parametritis, 4.6% Chorioamnionitis, and 38.8% Bacterial Vaginitis

were identified (Table I). Of 804 cases healthcare-associated deep pelvic tissue infection and other infections of the female reproductive tract, 78.6% were detected after hospital discharge. None of the patients died as a result of these infections. The prevalence of healthcare-associated deep pelvic tissue infection and other infections of the female reproductive tract in women differed according to the procedure types. Gynecologic patients had a higher incidence of healthcare-associated deep pelvic tissue infection and other infections of the female reproductive tract compared with pregnant women. The largest number of cases were identified after Abortion (35.8%, 95% CI 34,1 – 37.5), Instrumentation of the upper genital tract (20.1%, 95% CI 18,7 – 21.5) and vaginal surgery (19.4%, 95% CI 18,0 – 20.8). The incidence of HAI was 10.9% after abdominal surgery, 8.3% after laparoscopic surgery, and 5.3% after delivery (Table II). Vaginal delivery represented a low risk of HAIs compared cesarean delivery.

A total of 3053 women in reproductive age (16 – 55 years) participated in the study. The median age of the women was 28 years. The proportion of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract was higher in divorced (37.9 %) than marred women (14.4 %). Demographic and Social characteristics of patients with healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine are presented in Table III.

ANTIBIOTIC PROPHYLAXIS

For Ukraine antibiotic prophylaxis at surgery and gynecological procedure is standard practice. In this study of 3,053 patients with PID who underwent chart review, 3,012 (98.7%) were prescribed Ceftriaxone (75%) or Cefotaxime (25%). However, 804 (26.3%) of 3,053 patients developed infections of the reproductive tract.

RESPONSIBLE PATHOGENS

In this study, a total of 1857 pathogens isolated from 804 women with healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine were analyzed using culture based methods. Of these pathogens, gram-negative bacilli make up 73.6% (1367/1857) and 26.4% (490/1857) gram-positive cocci from of all isolates. The predominant pathogens of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract were: *Escherichia coli* (25.6%), *Enterobacter* spp. (11.4%), *Klebsiella pneumoniae* (10.6%), *Staphylococcus aureus* (8.5%), *Enterococcus faecalis*

Table I. Types of healthcare-associated deep pelvic tissue infection and other infections (n=804) of the female reproductive tract in Ukraine ($P < 0.05$)

HAI[a] type	Cases of HAI[a]		95% CI[b]
	n	%	
A pelvic abscess or cellulitis	107	13.3	12.1 – 14.5
Infection of the uterine appendages (Adnexa utery)	117	14.6	13.4 – 15.8
Infection of the Fallopian tubes (Salpingitis)	76	9.5	8.5 – 10.5
Infection of the ovaries (Oophoritis)	57	7.1	6.2 – 8.0
Infection of the supporting ligaments (Parametritis)	98	12.2	11.1 – 13.3
Chorioamnionitis	37	4.6	3.9 – 5.3
Infection of the Vagina (Bacterial Vaginitis)	312	38.8	37.1 – 40.5

Note
[a]HAI, healthcare-associated infection
[b]CI, confidence interval.

Table II. Distribution of type procedure associated deep pelvic tissue infection and other infections (n=804) of the female reproductive tract in Ukraine

Type of procedure	Cases of HAI[a]		95% CI[b]
	n	%	
Surgery			
Abdominal	88	10,9	9,8 – 12.0
Vaginal	156	19,4	18,0 – 20.8
Laparoscopic	67	8,3	7,3 – 9.3
Delivery (vaginal and cesarean delivery)	43	5,3	4,5 – 6.1
Abortion	288	35,8	34,1 – 37.5
Instrumentation of the upper genital tract	162	20,1	18,7 – 21.5

Note
[a]HAI, healthcare-associated infection
[b]CI, confidence interval.

Table III. Demographic and Social characteristics of patients with healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine (P < 0.05)

Variables	Number of participants *n*	HAI[a]		95% CI[b]
		n	%	
Age of participants				
16–20	117	16	13,7	13,1 – 14.3
21–25	338	71	21,0	20,3 – 21.7
26–30	417	162	38,8	37,9 – 39.7
31–35	374	137	36,6	35,7 – 37.5
36–40	596	144	24,2	23,4 – 25.0
41–45	488	87	17,8	17,1 – 18.5
46–50	402	76	18,9	18,2 – 19.6
51-55	321	111	34.6	33,7 – 35.5
Marital status				
Single	1578	461	29,2	28,4 – 30.0
Married	918	132	14,4	13,8 – 15.0
Divorced	557	211	37,9	37,0 – 38.8
Residence				
Rural	857	112	13,1	12,5 – 13.7
Urban	2196	692	31,5	30,7 – 32.3
Educational status				
College and above	618	137	22,2	21,5 – 22.9
Highs school	2435	667	27,4	25,6 – 28.2
Occupational status				
Employed	633	151	23,9	23,1 – 24.7
Merchant	675	163	24,1	23,3 – 24.9
Students	421	112	26,6	25,8 – 27.4
Unemployed	1324	378	28,5	27,7 – 29.3
Total	3053	804	26,3	25,5 – 27.1

Note
[a]HAI, healthcare-associated infection
[b]CI, confidence interval.

(7.2%), *Pseudomonas aeruginosa* (7.1%). The distribution of microorganisms causing deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine is shown in Table IV.

ANTIMICROBIAL RESISTANCE

Staphylococcal strains displayed a most resistance to penicillin (78.1%) and erythromycin (58.7%). In this study methicillin-resistance was observed in 12.9% of *S. aureus* (MRSA). However, Staphylococcal isolates showed susceptibility to most of the other antimicrobials tested. No strains resistant to linezolid, teicoplanin, vancomycin, tigecycline, and fusidic acid were found.

Streptococcal isolates demonstrated a noteworthy resistance against erythromycin (54.7%) and benzylpenicillin (42.3%), followed by ampicillin (29.8%). Most of the Streptococcal strains were sensitive to clindamycin (89.1%), gentamycin (93.2%), cefuroxime (95.1%), and linezolid (100%).

E. faecalis isolates were not sensitive to cefuroxime, clindamycin, and trimethoprim-sulfamethoxazole. Approximately, 20% of the E. faecalis isolates displayed resistance to aminoglycosides and 11.4% was resistant to quinolones.

The overall proportion of extended spectrum betalactamases (ESBL) production among Enterobacteriaceae was 20.8%. The prevalence of ESBL production among *E. coli* isolates was 24.7%. *E. coli* was most sensitive (>90%) to ertapenem, cefotaxime, ceftazidime, imipenem, piperacillin/tazobactam and high (>60%) resistance to cefuroxime, amoxicillin, and levofloxacin. Antimicrobial resistance to third-generation cephalosporins was observed in 12.8% *E.coli* isolates.

Isolates of *Enterobacter* spp. was most sensitive (>90%) to ciprofloxacin, cefotaxime, piperacillin/tazobactam,

Table IV. Types of microorganisms (n=1857) isolated from 804 patients with healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine

Microrganism[a]	All isolates	
	n	%
Gram-positive cocci	490	26.4
Staphylococcus aureus	157	8.5
Enterococcus faecalis	134	7.2
Streptococcus spp.	112	6.0
Staphylococcus epidermidis	73	3.9
Enterococcus faecium	14	0.8
Gram-negative bacilli	1367	73.6
Escherichia coli	476	25.6
Enterobacter spp.	211	11.4
Klebsiella pneumoniae	197	10.6
Pseudomonas aeruginosa	132	7.1
Proteus mirabilis	112	6.0
Acinetibacter spp.	103	5.5
Serratia spp.	77	4.1
Citrobacter spp.	43	2.3
Klebsiella oxytoka	16	0.9

Note:
[a]Used "The Bergey's Manual of Determinative Bacteriology" 9th Edition

ceftazidime and ticarcillin. No strains of *Enterobacter* spp. resistant to cefepime, meropenem, imipenem, and ertapenem were found. *Enterobacter* spp. strains were high (>50%) resistance to ampicillin/sulbactam, ampicillin, amoxicillin/clavulanic acid, clindamycin, ciprofloxacin, and high percentage resistance to gentamycin (41.2%), cefaperazon (38.5%) and ceftriaxon (29.2%).

K. pneumoniae isolates showed high susceptibility to piperacillin/tazobactam, ertapenem, meropenem, imipenem, levofloxacin, and gentamycin. However, these stranis while ones exhibited a noticeable percentage (>30%) of resistance to ampicillin, amoxicillin/clavulanic acid, ofloxacin, and ciprofloxacin. The prevalence of ESBL production among *K. pneumoniae* isolates was 11.9%. Resistance of *K. pneumoniae* strains to third generation cephalosporins was observed in 9.2%.

P. aeruginosa isolates demonstrated remarkable resistance (>35%) to cefepime, gentamycin, and cefoperazone. This isolates was most sensitive (>90%) to meropenem, imipenem, piperacillin/tazobactam, and ceftazidime. Carbapenem resistance was identified in 8.5% of *P.aeruginosa* isolates.

DISCUSSION

This study presents the first estimates of the current prevalence of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract and antimicrobial resistance of responsible pathogens in Ukrainian women hospitals. The prevalence of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract was 26.3%. The incidence of infection was: 13.3% Pelvic abscess or cellulitis, 14.6% Adnexa utery, 9.5% Salpingitis, 7.1% Oophoritis, 12.2% Parametritis, 4.6% Chorioamnionitis, and 38.8% Bacterial Vaginitis. Gynecologic patients had a higher incidence of HAIs compared with pregnant women. Of these cases, 78.6% were detected after hospital discharge. The prevalence of HAIs in women differed according to the procedure types. In our study gynecologic patients had a higher incidence of HAIs compared with pregnant women. Vaginal delivery represented a low risk of the female reproductive tract infection compared cesarean delivery. Available literatures there are no epidemiologic studies (prevalence or incidence) of different types of healthcare-associated deep pelvic tissue infection and other infections of the female reproductive tract. Therefore, we were unable to compare our results with other studies in other countries.

Microbiological monitoring of the prevalence of etiologic agents of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract and antimicrobial resistance is necessary to enhance our knowledge of its epidemiology. Therefore, this was the basis for this study. In present study, the predominant pathogens of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract were *E.coli*, *Enterobacter* spp., *K. pneumoniae*, *S.aureus*, *E.faecalis*, *P.aeruginosa*. Our results was coherent with reports other studies [7, 14, 15].

Current international guidelines for the management of pelvic inflammatory disease recommend the prescription of antibiotics for prophylactic and treatment [16-18]. According to the literature, the appointment of an inadequate starting therapy decreases the effectiveness of treatment [19, 20]. In our study, 26.3% of patients who were prescribed antibiotics developed reproductive tract infections. Possibly, this is due to the high levels of antimicrobial resistance of causing pathogens of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine. In present study, overall proportion of extended spectrum betalactamases (ESBL) production among *Enterobacteriaceae* was 20.8%. The prevalence of ESBL production among *E. coli* isolates was 24.7% and among *K. pneumoniae* isolates was 12.8%. Antimicrobial resistance to third-generation cephalosporins was observed in 12.8% *E.coli* isolates and in 9.2% *K. pneumoniae* isolates. Carbapenem resistance was identified in 8.5% of *P.aeruginosa* isolates. Methicillin-resistance was observed in 12.9% of *S. aureus* (MRSA). There is therefore need the treatment and prevention of pelvic infection and other infections of the female reproductive tract with Gram negative bacteria needs to be changed in Ukraine. However, the data are limited and more studies are needed to guide a change in first-line antibiotic treatment or prophylactics.

STUDY LIMITATIONS

The absence of national surveillance data in Ukraine compelled us to rely entirely on data from the only study prevalence of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract. The strengths of the study lie in the application of CDC/NHSN methodology. Indicators of HAIs provided by surveillance activities require comparison with adequate reference data to stimulate further infection control actions and to enhance quality of health care. The limitations of this study include in conduct at a in seven (29.2%) regional women hospitals in Ukraine. Therefore, the results present study not is representative of other hospitals of Ukraine.

CONCLUSIONS

Healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract in Ukraine is a common occurrence and many cases are caused by pathogens that are resistant to antibiotics. Results this study suggest that the prevention and treatment of pelvic infection and other infections of the female reproductive tract in Ukraine needs to be changed. However, the data are limited and more studies are needed. The present study provides valuable data as a first study for national surveillance of healthcare associated deep pelvic tissue infection and other infections of the female reproductive tract and potential for comparison with data from other countries.

REFERENCES

1. Ruggeri M., Cannas S., Cubeddu M. et al. Bacterial agents as a cause of infertility in humans. New Microbiol. 2016;39(3):206-209.
2. Mascarenhas M.N., Flaxman S.R., Boerma T. et al. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012;9(12):e1001356. doi: 10.1371/journal.pmed.1001356.;
3. Chayachinda C., Rekhawasin T. Reproductive outcomes of patients being hospitalised with pelvic inflammatory disease. J Obstet Gynaecol. 2017 Feb;37(2):228-232. doi: 10.1080/01443615.2016.1234439.
4. Workowski K.A., Bolan G.A. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recommendations and Reports 2015;64(RR-03):1-137.
5. Salmanov A., Vozianov S., Kryzhevsky V. et al. Prevalence of healthcare-associated infections and antimicrobial resistance in acute care hospitals in Kyiv, Ukraine. J Hosp Infect. 2019;102(4):431-437. doi: 10.1016/j.jhin.2019.03.008.
6. Ross J. Antibiotic therapy for pelvic inflammatory disease. Cochrane Database Syst Rev. 2017 Apr 24;4(4):CD010285. doi: 10.1002/14651858.CD010285.pub2.
7. Omoregie R., Egbe C.A., Igbarumah I.O. et al. Prevalence and etiologic agents of female reproductive tract infection among in-patients and out-patients of a tertiary hospital in Benin city, Nigeria. N Am J Med Sci. 2010;2(10):473-477. doi:10.4297/najms.2010.2473
8. Quan M. Pelvic inflammatory disease: diagnosis and management. J Am Board Fam Pract. 1994;7(2):110-23
9. Horan T.C., Andrus M., Dudeck M.A. CDC/NHSN surveillance definition of health care-associate9 infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-332. doi:10.1016/j.ajic.2008.03.002.
10. Salmanov A.G., Vdovychenko S.Y., Litus O.I. et al. Prevalence of healthcare-associated infections and antimicrobial resistance of the responsible pathogens in Ukraine: Results of a multicenter study (2014-2016). Am J Infect Control. 2019;47(6):e15-e20. doi: 10.1016/j.ajic.2019.03.007.
11. Salmanov A., Vozianov S., Kryzhevsky V. et al. Prevalence of healthcare-associated infections and antimicrobial resistance in acute care hospitals in Kyiv, Ukraine. J Hosp Infect. 2019;102(4):431-437. doi: 10.1016/j.jhin.2019.03.008.
12. Salmanov A.G., Voitok T.G., Maidannyk I.V. et al. Episiotomy infections in the puerperium and antimicrobial resistance of responsible pathogens in Ukraine. Wiad Lek. 2020;73(11):2325-2331. doi: 10.36740/WLek202011101.
13. Salmanov A.G., Vitiuk A.D., Zhelezov D. et al. Prevalence of postpartum endometritis and antimicrobial resistance of responsible pathogens in Ukraine: results a multicenter study (2015-2017). Wiad Lek. 2020;73(6):1177-1183. doi: 10.36740/WLek202006119.
14. Wójkowska-Mach J., Pomorska-Wesołowska M., Romanik M. et al. Prevalence and Antimicrobial Susceptibility Profiles of Microorganisms Associated with Lower Reproductive Tract Infections in Women from Southern Poland-Retrospective Laboratory-Based Study. Int J Environ Res Public Health. 2021;18(1):335. doi: 10.3390/ijerph18010335.
15. Gregor M., Paterová P., Buchta V. et al. Healthcare-associated infections in gynecology and obstetrics at a university hospital in the Czech Republic. Int J Gynaecol Obstet. 2014;126(3):240-3. doi: 10.1016/j.ijgo.2014.04.001.
16. Ross J., Judlin P., Nilas L. European guideline for the management of pelvic inflammatory disease. Int J STD AIDS. 2007;18(10):662-6. doi: 10.1258/095646207782193911.

17. Sweet R.L. Treatment of acute pelvic inflammatory disease. Infect Dis Obstet Gynecol. 2011;2011:561909. doi: 10.1155/2011/561909.
18. Savaris R.F., Fuhrich D.G., Maissiat ., et al. Antibiotic therapy for pelvic inflammatory disease. Cochrane Database Syst Rev. 2020;8:CD010285. doi: 10.1002/14651858.CD010285.pub3.
19. Laterre P.F., Levy H., Clermont G. et al. Hospital mortality and resource use in subgroups of the Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial. Crit Care Med. 2004;32(11):2207-18. doi: 10.1097/01.ccm.0000145231.71605.d8.
20. Tumbarello M., Sanguinetti M., Montuori E. et al. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. Antimicrob Agents Chemother. 2007;51(6):1987-94. doi: 10.1128/AAC.01509-06.

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ORIGINAL ARTICLE

REPARATIVE REGENERATION AT THE END OF BONE FILING AFTER OSTOPLASTIC AMPUTATION

DOI: 10.36740/WLek202103106

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ABSTRACT

The aim: To study the role and place of bone grafting in the formation of bone stump after amputation.

Materials and methods: 3 series of experiments were carried out on 44 rabbits with amputation of the thigh in the middle third and stump grafting using osteoplastic hermetic closure of the canal with a thin cortical plate (series I), closure of the canal with a spongy bone (series II), and loose closure of the canal with a cortical graft located at the entrance to the canal at an angle of 30° (III series). Observation period: 1, 3, 6 months. Histological examination method with vascular filling with 10% mascara-gelatin mixture.

Results: In series I, in the majority of observations, a stump of a cylindrical shape with a bone locking plate of an osteon-beam structure and normalization of intraosseous microcirculation was formed. A slight displacement of the graft caused a violation of microcirculation. In series II, organotypic stumps were formed in all observations. In series III, incomplete closure of the bone marrow cavity led to sharp microcirculatory disorders and the course of the reparative process with pathological bone remodeling.

Conclusions: The parameters of the favorable course of the reparative process and the formation of the organotypic bone stump are the safety of its cylindrical shape, the presence of a compact bone structure, normalization of intraosseous microcirculation.

KEY WORDS: amputation, bone grafting, reparative regeneration, intraosseous microcirculation

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INTRODUCTION

Amputation of a limb with the intersection of soft tissues, blood vessels, nerves, skin significantly violates the static-dynamic stereotype, which contains a potential threat of various complications. In addition, such an intersection substantially complicates the formation of a rational relationship between these anatomical formations in the future organ of support and movement. In recent years, researchers have focused on such particular issues of amputations as predicting wound healing [1], treatment of pain [2-8], and various modifications of known amputation methods. Unfortunately, such fundamental questions as stump healing, features of reparative processes, and factors influencing their course were not reflected in these works. According to the data of [9], unsatisfactory outcomes of bone stump healing were observed in 97.1% of the examined, and the formation of a functional bone stump within 1-1.5 months after amputation was noted in only 10% of cases [10]. Such disappointing results of operations prompted us to experimentally study the features of reparative regeneration at the end of bone filing. Amputation leads to a sharp change in the blood supply to the bone. The system of periosteal vessels is damaged, which penetrate from the periosteum into the compact substance of the bone through the Folkman and Haversian canals and feed the outer third of the cortical layer. The intersection of the periosteum and bone is accompanied by damage to the feeding artery. In the middle of the diaphysis, it, being one of the muscle branches, penetrates the bone and is divided into proximal and distal branches, which branch into many small vessels, in the medullary canal. The latter in the form of precapillaries and postcapillaries penetrate into the inner layers of the cortical plate through the system of Folkman channels, and along the bones of the Haversian channels are distributed along the bone and feed the inner two-thirds of the thickness of the cortical layer of the diaphysis.

In addition, amputation due to a breach of hermeticity in the medullary canal, it causes a drop in pressure necessary for pushing blood through the vessels of the narrow vascular channels of the cortical plate. In the formation of the future stump, this pressure should be higher than the level of interstitial pressure, which will ensure the pushing of blood through narrow intraosseous vessels. Considering that the tubular bone is normally closed, when the stump is formed after amputation, a closure bone plate must be formed, which ensures hermetic bone marrow cavity. Initially, the filing of the bones were covered with a fascia flap – the fascioplastic method. Subsequently, and until now, in amputation surgery, the myoplastic method is used – stapling of antagonist muscles under the filing of the bone. It should be noted that neither the first nor the second methods involve closing the bone marrow cavity. Their use is achieved only shelter of the truncated bone and the subsequent expectation of wound healing. The

influence of these methods of plastics on reparative processes in the bone stump is very insignificant. Considering this circumstance, osteoplasty of the end of bone filing in various versions was applied. We expected that this technique should help improve blood circulation and improve reparative regeneration at the end of filing.

THE AIM

The aim was to to study the role and place of bone grafting in the formation of bone stump after amputation.

MATERIALS AND METHODS

3 series of experiments on 44 rabbits were conducted. Amputation of the right hind limb in the middle third was performed under intravenous thiopental anesthesia. A 1% solution of novocaine was perineurally injected into the nerve trunks and crossed highly with an acute razor. The vessels were ligated with catgut. The bone was sawn aperiostally with a hand saw. In series I, during stump amputation, stump plastic surgery was performed using a bone-plastic hermetic closure of the canal with a thin cortical plate. In the second series, the canal was closed with a spongy bone; in the third series, the canal was closed loosely with a cortical graft located at the channel entrance at an angle of 30°.

Observation period was 1, 3, 6 months. Histological examination method with vascular filling with 10% mascara-gelatin mixture. Before removing from the experiment, the animal was intra-arterially injected with 5 thousand units of heparin in saline, after 15 minutes a lethal dose of hexenal was rapidly administered intravenously and the abdominal aorta was ligated. Below the ligature, a cannula was introduced from the system for intra-arterial injection, fixing it in the lumen of the vessels, and a 10% mascara-gelatin mixture was filled. After a day, the femur in the hip joint was isolated, a visual assessment of the relationship of the soft tissues with the bone was given, after which the thigh stump was freed from the soft tissues, leaving them only along the end surface. The drug was fixed in a 12% formalin solution and decalcified with a 5% nitric acid solution. From the obtained preparation of the whole bone stump of the femur, the articular end was cut off. The remaining plot was taken for research. A sagittal section was made through the middle of the bone, which was poured into a block of integoidin. Sections 15-30 mkm thick were stained with hematoxylin and eosin according to Van Gieson. The obtained histotopograms were studied using light microscopy.

The experiments were carried out in accordance with the principles of humane treatment of animals, set out in European Community directives (86 (609) EEC) and the Helsinki Declaration on the Humane Treatment of Animals.

RESULTS

First episode. The experimental group made a tight closure of the filing with a thin cortical plate.

The duration of 1 month, 7 observations. The shape of the end of the stump in five observations remained cylindrical. The thickness of the cortical diaphyseal layer in the distal and proximal parts is the same. At the end of the filing revealed sparse network of endosteal bone beams, along the lower edge of which is a locking plate. It consisted of mature and not quite mature bone tissue. In the inter-beam spaces of the end of the stump and in the proximal sections of the stump, the microcirculatory network corresponded to that of the diaphysis normally.

In two observations, a slight resorption of the cortical diaphyseal plate in the filing area was noted, and its thickness even far from the end was uneven. The end of the stump is beveled. The bone locking plate consisted of not quite mature bone tissue. In the medullary canal near the filing, single sinusoids were detected. In the proximal section, adipose bone marrow with a characteristic microcirculatory network for the diaphysis. It should be noted that in these two observations in the postoperative period there was a displacement of the bone graft.

The duration of 3 months, 6 observations. The stump shape retained the shape of the diaphysis in all observations. The thickness of the cortical diaphyseal layer saved all over. Bone cortical locking plate consisted of mature bone tissue. The mascara-filled vessels of the microcirculatory network corresponded to the norm. The reparative process is complete.

The duration of 6 months, 6 observations. In 5 observations, the stump end shape is cylindrical. The bone pinch plate consisted of mature bone tissue. The cortical diaphyseal plate is basically uniform in thickness. It has the characteristic structure of a compact bone with a longitudinal arrangement of vascular channels. In the proximal and distal sections, the adipose bone marrow with a microcirculatory network corresponding to the bone is normal. In one observation, the shape of the end of the stump has slight sloping due to the resorption of the cortical diaphyseal plate at the end. The bone tissue of the bone pinch plate is not quite mature. In the proximal bone marrow cavity, adipose bone marrow. The microcirculatory network here corresponds to normal bone microcirculation. In the distal vessels of the sinusoidal type, sinusoids. The reparative process in this observation is not completed.

Series II – closure of the medullary canal with a trabecular bone, 15 observations.

The duration of 1 month, 5 observations. The bone stump shape in all preparations is cylindrical (Fig. 1, 2, 3) with a locking bone plate of the osteon-beam structure (Fig. 4). Cortical diaphyseal plates are well contoured all over. In the inter-beam spaces, the adipose bone marrow with mascara-filled microvessels close to the vessels of normal bone. In the proximal part of the medullary canal, adipose bone marrow with a characteristic microvascular network.

The duration of 3 months, 5 observations. The shape of the bone stump is cylindrical. The base of the stump is flat. In all cases, the cortical diaphyseal plate maintains a normal structure. The bone locking plate of the osteon-beam structure is represented by mature bone tissue. The condition of

Fig. 1. Histotopogram of a cylindrical stump. Hematoxylin and eosin staining. X6.

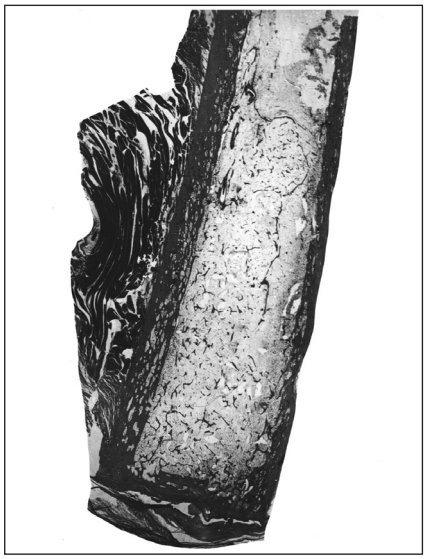

Fig. 2. Histotopogram of a cylindrical stump with sponged cortical diaphyseal plate. Hematoxylin and eosin staining. X6.

Fig. 3. Histotopogram of a cylindrical stump. Hematoxylin and eosin staining. X6.

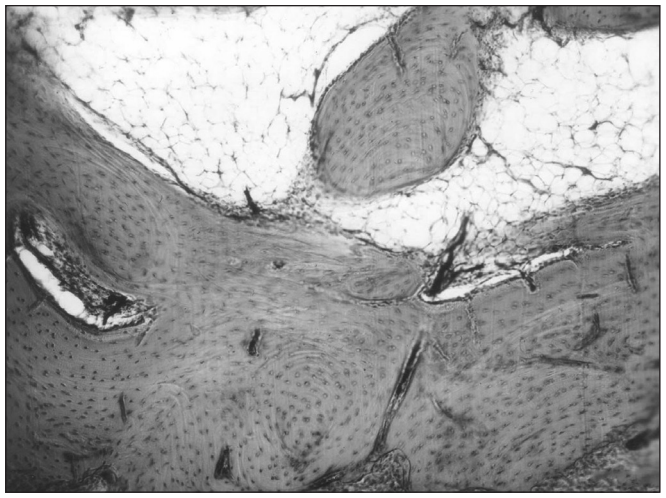

Fig. 4. Microphotograph. Bone locking plate of osteon-beam structure. Hematoxylin and eosin staining. X90.

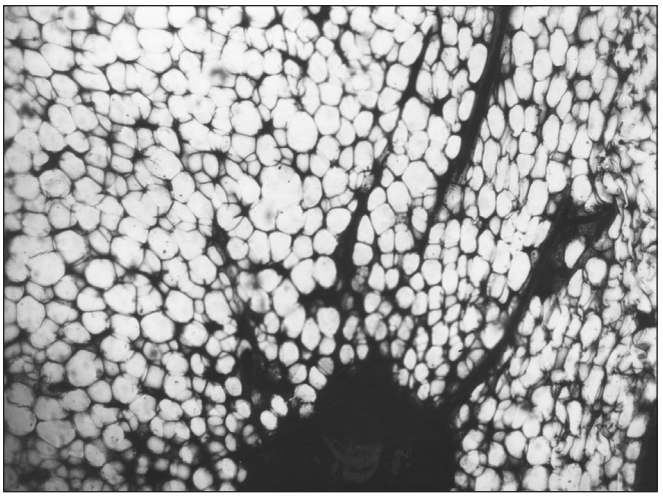

Fig. 5. Microphotograph. Bone marrow with mascara-filled microvessels in the proximal bone marrow canal. A large branch with mascara-filled venous sinus. Hematoxylin and eosin staining. X90.

the bone marrow in the terminal and proximal regions is normalized (Fig. 5, 6). The reparative process is complete.

The duration of 6 months, 5 observations. In all observations, the morphological picture was identical with the previous period.

Series III – bone grafting with a thin graft implanted in the medullary canal to a depth of 0.3 cm, 10 observations.

The duration of 3 months, 5 observations. The stump shape is sharply deformed. In four preparations, the stump form is conical. The cortical diaphyseal plate in these observations is unevenly thinned, there are its breaks and focal rarefication. In the conical part, the elongated bone beams forming it are revealed. There are no bone beams near the apex of the cone. There is dense and loose fibrous tissue with the inclusion of primitive rare bone beams and a diffuse arrangement of lymphoid-plasma cells. In one observation, a narrowing of the diameter of the stump in the lower part and closure of the medullary canal with a violation of the structure of the diaphysis are determined. In the preserved part of the medullary canal, the bone marrow is replaced by a swollen, loose fibrous tissue with a large number of sinusoidal vessels filled with mascara and tissue cysts. The gaps of the branches of the feeding artery filled with mascara are also revealed. Among the structures of endosteal bone formation, foci of immature bone tissue are revealed.

The duration of 6 months, 5 observations. In all observations, the stump was characterized as sharply deformed, conical. Three of them showed fractures of the cortical diaphyseal plate (Fig. 7, 8). At the end of the stump, the end sections of the cortical diaphyseal plate below and slightly above the level of the breaks underwent partial resorption

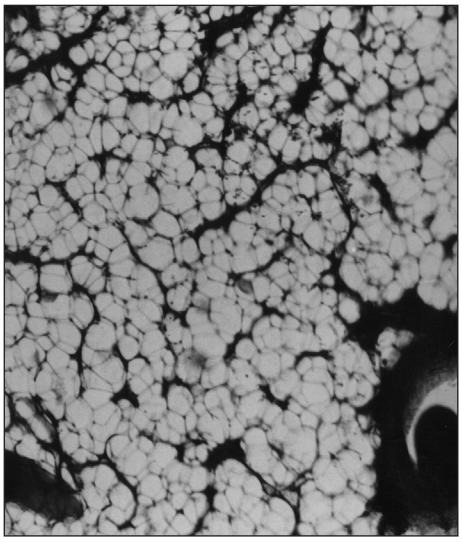

Fig. 6. Microphotograph. Adipose bone marrow of the end of the stump with mascara-filled vessels of the microcirculatory channel. The state of intraosseous microcirculation is close to normal. Hematoxylin and eosin staining. X90.

Fig. 7. Histotopogram of a cylindrical stump with a cone-shape end and a break in the cortical diaphysial plate. Hematoxylin and eosin staining. X6.

Fig. 8. Histotopogram of cone-shape stump. Hematoxylin and eosin staining. X6.

starting from the periosteal surface and replaced by newly formed immature bone tissue along the endosteal surface. Large contours were determined by its contours. In the medullary canal from its end and proximal over a large extent, loose edematous fibrous tissue with many large tissue cysts and thin-walled vessels of large diameter filled with mascara, as well as branches of the feeding artery, is revealed. In the medullary canal in the area of resorption of the diaphyseal plate in the edematous loose fibrous tissue, diffusely located macrophages, lymphoid and plasma cells are visible. A large number of ink-filled sinusoids is detected. The connective bone plate consists of immature bone tissue, not always expressed. Thus, even in the long term, in the reparative process at the end of the stump of the bone against the background of disturbed intraosseous microcirculation, pathological rearrangement of bone tissue took place.

The completeness of the reparative process was not noted in any observation of this series.

DISCUSSION

The formation of a bone stump after amputation involves the creation of an organotypic organ, which in its physical and physiological parameters should approach the bone normally. It is permissible to assume that in this organ the form that is provided by the cortical diaphyseal layer, its normal structure and adequate intraosseous circulation must be preserved. The latter largely depends on the level of intraosseous pressure, which should be sufficient to push blood through the vascular tubules of the cortical diaphyseal plate. Since depressurization of the bone marrow cavity occurs during amputation, the need to close it becomes obvious. Until recently, this was done by blocking the canal with fascia or muscle. However, in fact, it was the overlap of the bone filing, and not the gaping bone marrow cavity, in which it was necessary to restore the disturbed hermeticism. Studies have confirmed this assumption. In experiments on the use of various options for osteoplastic closure of the medullary canal with additional fixation of muscles by the end of the filing, various healing results were revealed. At the same time, we were convinced that the main constants of the created organ were: the preservation of the normal form and structure of the stump of the bone, the presence at the end of the bone cortical closure plate, the normalization of intraosseous microcirculation, and the completeness of the reparative process. In the first series of experiments, for a month and beyond, while maintaining the cylindrical shape of the stump at its end, in most cases, a bone locking plate was formed. Within a month, it was not quite mature, and subsequently – mostly mature, osteon-beam structure. The cortical diaphyseal plate changed slightly. Intraosseous microcirculation returned to normal. The slight displacement of the grafts that took place at the stage of development of the technique in several cases violated the nature of the reparative process. Immediately there was a slight resorption of the edge of the cortical diaphyseal plate, and not quite mature bone beams were revealed in the bone marrow canal along the endostal surface. In its distal section, single tissue cysts were detected. The base of the stump became beveled. The bone locking plate consisted of not quite mature bone tissue. It can be concluded that any, even a minor violation of the technique of performing amputation leads to significant changes in the reparative process.

The good stump formation results obtained in the second series of experiments are explained by the tight closure of the canal with a spongy graft, which is deprived of

the possibility of displacement. In all the observations of this series, cylindrical stumps were obtained with a bone locking plate made of compact bone tissue at the end of the filing, normalization of intraosseous microcirculation and complete completion of the reparative process. Bone grafts in tissue regenerates were not detected in any case.

Lack of density of closure of the canal and even slight destruction of the bone marrow by a cortical graft placed in the plane of the filing at an angle 30°, it does not ensure the integrity of the bone marrow cavity. In the formation of the stump revealed large deviations from the above morphological standards of previous experiments. In all the observations of this series, the studied stump was characterized as sharply deformed. Serious violations of intraosseous microcirculation were revealed. Significant resorption of the cortical diaphyseal plate with its fractures led to changes in the shape of the stump. Bone closure plate is not formed in any case. The completion of the reparative process did not occur. This condition was regarded as a pathological rearrangement of bone tissue.

CONCLUSIONS

1. The parameters of the favorable course of the reparative process and the formation of the organotypic bone stump are the safety of its cylindrical shape, the presence of a compact bone structure, normalization of intraosseous microcirculation, and the completeness of the reparative process already by 1 month.
2. The most important factor in the optimal course of the reparative process at the end of the stump of the bone after amputation at the level of the diaphysis is the tight closure of the opened bone marrow cavity with a thin cortical or spongy graft.
3. Bone grafting with a cortical and spongy graft, provided that the bone marrow canal is tightly closed, contributes to the rapid restoration of intraosseous circulation and the formation of an organotypic stump.
4. The lack of hermetic closure of the bone marrow cavity leads to significant violations of the intraosseous circulation and reparative process.

REFERENCES

1. Bosse M.J., Morshed S., Reider L. et al. METRC. Transtibial Amputation Outcomes Study (TAOS): Comparing Transtibial Amputation With and Without a Tibiofibular Synostosis (Ertl) Procedure. J Orthop Trauma. 2017;31(l):S63-S69.
2. Kahle J.T., Highsmith M.J., Kenney J. et al. The effectiveness of the bone bridge transtibial amputation technique: A systematic review of high-quality evidence. Prosthet Orthot Int. 2017; 41(3):219-226.
3. Nijmeijer R., Voesten H.G.J.M., Geertzen J.H.B. et al. Disarticulation of the knee: Analysis of an extended database on survival, wound healing, and ambulation. J Vasc Surg. 2017;66(3):866-874.
4. Preißler S., Htielemann D., Dietrich C. et al. Preliminary Evidence for Training-Induced Changes of Morphology and Phantom Limb Pain. Front Hum Neurosci. 2017; 11:319.
5. Tosun B., Selek O., Gok U. et al. Medial gastrocnemius muscle flap for the reconstruction of unhealed amputation stumps. J Wound Care. 2017;26(8):504-507.
6. Bezsmertnyi Y.O., Shevchuk V.I., Grushko O.V. et al. Information model for the evaluation of the efficiency of osteoplasty performing in case of amputations on below knee. Proc. SPIE 10808, Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments. 2018: 108083H; doi. 10.1117/12.2501558.
7. Vasil'ev A.Ju., Egorova E.A., Smyslenova M.V. Luchevaja diagnostika izmenenij kul'tej nizhnih konechnostej pri protezirovanii [Radiation diagnosis of changes in the stump of the lower extremities during prosthetics]. Klinicheskaja medicina. 2013;5(1):51-7. (in Russian).
8. Vojnovskij E.A., Pil'nikov S.A., Kovaljov A.S. et al. Rezul'taty amputacij nizhnih konechnostej v sovremennyh vooruzhennyh konfliktah. Bolezni i poroki kul'tej [The results of amputation of the lower extremities in modern armed conflicts. Diseases and vices of stumps]. Medicinskij vestnik MVD. 2015; 78(5):10-14. (in Russian).
9. Weidong W., Bin Zh., Dingshen L. et al. Significance of alpha smooth muscle actin expression in traumatic painful neuromas: a pilot study in rats. Sci Rep. 2016; 6: 23828.
10. Xin Zh., Yongming Xu., Jin Zh. et al. Ultrasound-guided alcohol neurolysis and radiofrequency ablation of painful stump neuroma: effective treatments for post-amputation pain. J Pain Res. 2017;10:295-302.

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ORIGINAL ARTICLE

MORPHOLOGICAL CHARACTERISTICS OF ACELLULAR DERMAL MATRIX MANUFACTURING

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ABSTRACT

The aim is to develop a method of the acellular dermal matrix manufacturing from pig's skin dermis while preserving the native structure.

Materials and methods: Combination of physical and chemical effects on the dermis underlies in the process of an acellular dermal matrix manufacturing. Dermal collection of 1.0-1.3 mm thickness in pigs under 1 year of age from the back and partially from the lateral parts of the body was carried out. The 0.3-0.4 mm thickness layer of skin was previously removed from the relevant areas with help of a dermatome, which was physically and chemically treated. The maximum acellularization of the dermal matrix was achieved step-by-step and included four stages of skin processing: 1 – freeze-thaw process; 2 – glycerin dehydration; 3 – osmotic stress; 4 – cell residue removal by detergent.

Results: Histological analysis of the of the pig's skin dermis revealed that after freeze-thaw cycles the collagen scaffold of the dermal matrix maintains its structural organization that was obtained as a result of the first stage of decellularization. On the second stage of decullalarization, the decreased number of fibroblastic cells was indicated. By means of this, the connective tissue elements that are represented by collagen fibers' multidirectional bundles retained their structural organization.

Fibroblasts lysis as basophilic stained elements was revealed in small amount of dermis on the third stage of the decellularization. Washing of lyophilized skin with nonionic detergent sodium dodecyl sulfate the complete absence of fibroblasts, epidermocytes in the hair follicles, endothelial cells in the wall of blood vessels was detected indicating the effectiveness of this reagent in removing residual products.

Conclusions: Suggested protocol for decullalarization of the pig's skin dermis is effective in removing nuclear and cellular structures from dermis. Particular protocols can be modified by increasing the temperature difference or changing the number of freeze-thaw cycles.

KEY WORDS: pig's skin, derma, acellular dermal matrix, morphology

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INTRODUCTION

The influence of chemical compounds (about 5000), plants, physical factors, infectious agents, ectoparasites' bites and other insects, skin invasion by helminthes and other agents can lead to skin lesions [1, 2]. Disease manifestations mostly depend on the irritant type, intensity and duration of its impact, repeated contacts, localization of the pathological process, physiological condition of tissues, general condition of the body and its protective and adaptive capabilities, whereas the curing depends on the depth and location of the lesion [3, 4, 5].

Nowadays, more than 700 artificial wound dressings that are recommended for treatment of skin lesions are known, while the intensity of the new wound dressings development is not reduced. Wound dressings as well as drugs used in traditional treatment must be adapted to the stage of the wound process are currently available for clinicians [6].

Due to special mechanisms of influence, modern wound dressings actively affect the healing process. They are able to regulate the amount of exudate due to sorption, remove its excess by keeping the wound surface moist, provide gas exchange, maintain a certain temperature, change the pH of the burned surface, and prevent mechanical tissue injury [7].

Perspective way of various etiologies skin injuries repairment is the usage of decellularized (acellular) pig's skin. The main advantages of this biological material are the composition and structure of the pig' dermis that are as much as the patient's dermis [8]. It has been presented in various researchers that skin substitutes improve the quality of wound healing and functional outcomes. They are a useful tool in plastic and reconstructive surgery [9-13].

Currently, physical and chemical methods of decellularization are proposed that will allow the preserving of the structure and properties of xenografts [14, 15].

Acellular dermal matrix supports fibroblasts' infiltration, neovascularization, and epithelialization in the absence of a recipient immune response has been shown in conducted investigations [16].

It is also shown that cosmetic and functional results after usage of cutaneous xenografts for the treatment of skin defects significantly exceed the results after skin grafting with a perforated flap. Moreover, it promotes faster healing

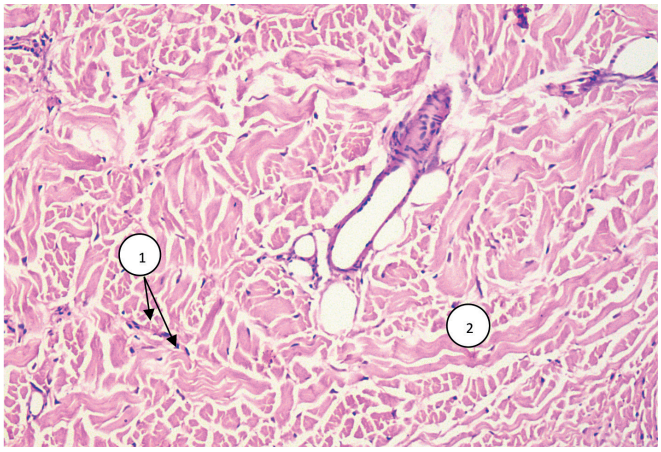

Fig. 1. Microscopic condition of lyophilized derma after freeze-thaw cycles. 1 – fibroblasts, 2 – collagen fibers. Hematoxylin and eosin. x200

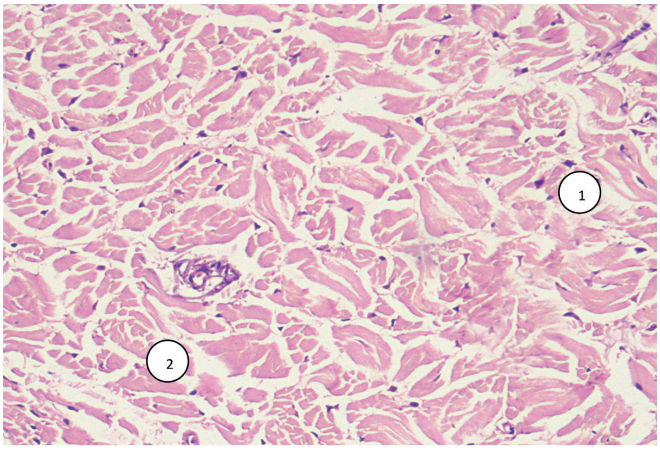

Fig. 2. Histological picture of the lyophilized derma after glycerol dehydration. 1 – damaged fibroblasts, 2 – collagen fibers. Hematoxylin and eosin. x200

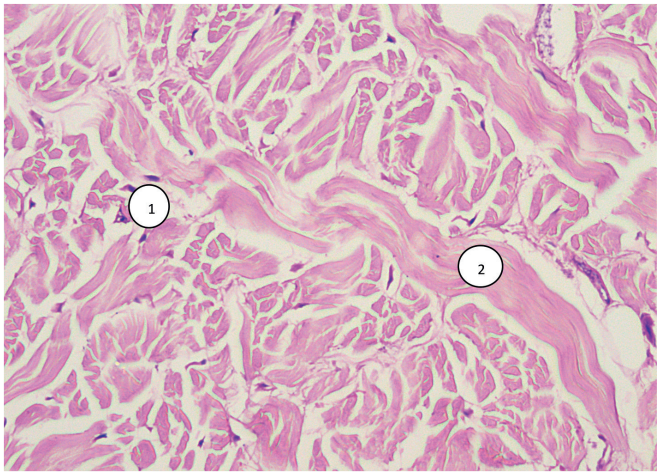

Fig. 3. Microscopic picture of the lyophilized derma after osmotic stress. 1 – residues of cellular material, 2 – collagen fibers. Hematoxylin and eosin. X100

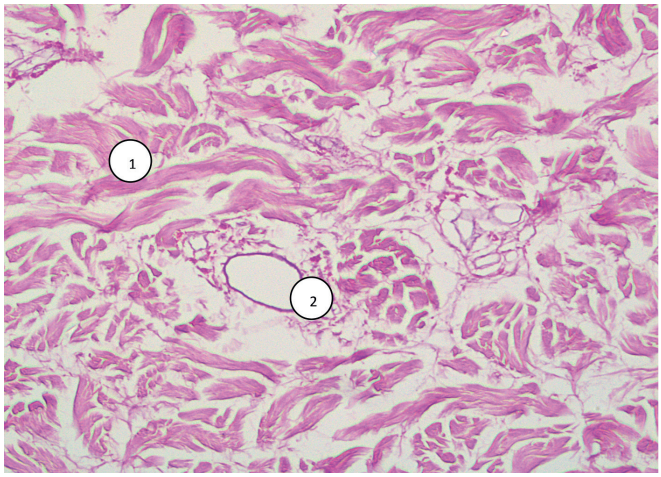

Fig. 4. Histological condition of reticular layer of lyophilized derma after rinsing with sodium dodecyl sulfate (SDS). 1 – collagen scaffold, 2 – vessels' wall. Hematoxylin and eosin. X100

of donor wounds, as it requires thinner autodermal grafts [17, 18]. However, decellularization methods usually have opposite effects: extremely aggressive removal of immunogenic components can destroy the structure and composition of the tissue, while more gentle techniques can preserve the immunogenicity of the tissue.

Particularly, methods of obtaining skin substitutes do not have a common carrier of cellular structures, which would possess: biocompatibility, would create an optimal microenvironment for wound regeneration, would have the absorption capacity for wound exudate, would prevent entering and developing of microorganisms, would be permeable to water vapor and air, wouldn't dry the bottom of the wound, would be elastic, would simulate a surface with a complex texture.

THE AIM

To create a method of acellular dermal matrix (ADM) manufacturing from dermis of pig skin with preservation of native structure.

MATERIALS AND METHODS

The process of ADM manufacturing has been a combination of physical and chemical effects on the dermis. In pigs under 1-year of age 1.0-1.3 mm thickness derma was collected from the back and partially from the lateral parts of the body**.** Previously with help of dermatome the particular 0,3-0,4 mm thickness skin layer was removed and was proceeded by physical and chemical influence. Step by step the achieving the maximum acellularity of the dermal matrix was carried out and included 4 stages of skin treatment: 1 – the process of freezing-thawing; 2- dehydration with glycerin; 3 – osmotic stress; 4 – washing with detergent of the remaining cells. Freezing was conducted in liquid nitrogen (-196 ° C), thawing in 37°C water. This cycle was repeated three times. Subsequently, three times, the skin was covered with a solution of glycerin, osmotic stress was performed by alternating immersion of the dermis in distilled water and hypertonic solution. The skin was then treated with 1M NaOH solution.

On the final stage, residues of cell breakdown products, proteins and other components of the extracellular matrix were removed by 1% sodium dodecyl sulfate (SDS).

Afterwards, the skin underwent freeze-drying.

To evaluate the effectiveness of the decellularization protocol, morphological analysis of the dermis was performed using light microscopy. For this purpose, samples of native (n = 6) and decellularized (n = 6) dermis were fixed in 10% neutral formalin, filled with paraffin. The obtained 5 μm samples were dewaxed and stained with hematoxylin and eosin (H&E; Sigma-Aldrich, Inc., St Louis, MO, USA) for detection of nuclear, cellular and extracellular materials.

RESULTS

According to the results of light optical microscopy in the lyophilized pigs' dermis before the beginning of decellularization, any signs of autolytic and necrobiotic changes, as well as morphological disorders of its structural organization were observed.

It was revealed under microscopic examination of lyophilized skin that the dermis and hypodermis are located under the multilayered squamous corneal epithelium (epidermis) lying on the basement membrane. The papillary and the reticular layers of the dermis are represented by connective tissue. In the bulk of the cells that form these layers, well-defined nuclei and cytoplasm of fibroblasts, as well as clearly visualized nuclei of epidermocytes in the hair follicle.

In overwhelmingly of histological specimens, the collagen fibers of the dermis are contoured, form a network and are differently localized in the papillary layer of the dermis.

The deeper dermis parts (reticular layer) represented with an insignificant swelling and homogenization of collagen fibers' particular sections, and in some cases – their fragmentation. The connective tissue framework is also depicted by clearly contoured thin elastic fibers.

The reticular and papillary layers of the dermis are formed by fibrous connective tissue, where collagen fibers are in dominance was revealed in the dermis of pig skin after freeze-thaw cycles. Thick bundles that are located in different directions in the intercellular matrix and have an oxyphilic color represent the fibrous component of the reticular layer (Fig. 1).

The cellular composition is illustrated mainly by fibroblasts, the nuclei of which have a basophilic color after hematoxylin and eosin staining.

It is shown that the collagen scaffold of the dermal matrix obtained as a result of the first stage of decellularization retains its structural organization.

A decreased number of fibroblastic cells was revealed on the second stage of decellularization – dehydration with polyhydric alcohol glycerol (Fig. 2). In this case, the connective tissue elements of the dermis, which are represented by multidirectional bundles of collagen fibers in the papillary and reticular layers, retain their structural organization, which is confirmed by light microscopy.

The second stage of decellularization – dehydration with polyhydric alcohol glycerol – revealed decreased number of fibroblastic cells.

The second stage of decellularization – dehydration with polyhydric alcohol glycerol – found a decrease in the number of fibroblastic cells (Fig. 2).

In this case, the connective tissue elements of the dermis, which are represented by multidirectional bundles of collagen fibers in the papillary and reticular layers, retain their structural organization, which is confirmed by light microscopy.

Meanwhile, light microscopy confirmed that the connective tissue elements of the dermis represented by multidirectional bundles of collagen fibers in the papillary and reticular layers, maintain their structural organization.

As a result of the third stage of decellularization (osmotic stress) lysis of fibroblastic cells under microscopic examination of histological specimens was revealed. This can be explained by the fact that hypotonic solutions easily cause the dissolution of cellular material due to simple osmotic effects with minimal changes in the molecules of the matrix and its architecture [19], hypertonic saline dissociates DNA from proteins [20]. The data obtained by light microscopy revealed a small number of basophilically stained elements in the dermis, which can be regarded as residues of structural components of the cells (Fig. 3).

The final stage of the study involved rinsing the lyophilized skin with a nonionic detergent sodium dodecyl sulfate (SDS). This contributed to the removal of residues of cell decay products, which indicates the effectiveness of this reagent.

Light microscopy data show the complete absence of fibroblastic cells, epidermocytes in the hair follicles, endothelial cells and smooth myocytes in the wall of blood vessels (Fig. 4).

DISCUSSION

It is known that freeze-thaw cycles do not affect the content of collagen and glucosaminoglycans (GAG), as well as the mechanical strength of biological material [21]. However, 88% of DNA in cells remains unchanged after such physical exposure. Thus, this physical effect on lyophilized skin can be considered as a trigger for damaging effects on the cellular structures of the dermis**.**

At the stage of decellularization of the dermis, alcohols, in particular glycerin, contribute to the structural reorganization of cells by dehydration and lysis [22, 23]. Alcohols will also dissolve phospholipids, which are a part of cell membranes. However, attention should be paid to the negative effects of alcohols on tissues due to their ability to precipitate proteins, as well as ultrastructural extracellular matrix damage they can cause [24, 25, 26].

To obtain the maximum osmotic effect (third stage of decellularization), the tissues should alternatively be immersed in hyper- and hypotonic solutions for several cycles, which helps to leach the cells' residues from the tissue after lysis.

The step of the lyophilized skin washing with a nonionic sodium dodecyl sulfate (SDS) detergent proved to be effective for the complete removal of cellular material from the dermis, as this led to the solubilization of cell membranes and their complete leaching from the tissues. Increasing the exposure and concentration in the decellularization pro-

tocol by SDS also leads to complete removal of cell nuclei [27]. Removing of extracellular matrix proteins and DNA by usage of detergents increases with exposure time and varies depending on the organ subunit, tissue type and donor age [28]. SDS is generally more effective for elimination of cell residues from tissue than other detergents, although is also more destructive for the extracellular matrix [29, 30]. Therefore, the SDS concentration and exposure for each tissue should be carefully and accurately fitted.

Removal of cellular material from the dermis of pig skin as a potential coating for the wound surface significantly reduces the antigenic properties of the material, ensuring its constant engraftment to the wound area with vascular germination and gradual filling of the recipient's own cells. Preservation of the structure of the collagen matrix promotes angiogenesis and cell migration [31, 32]. In the future, decellularized dermal matrices are considered as one of the main scaffold options for creating a bioengineered autologous skin equivalent.

CONCLUSIONS

Proposed protocol of pig' skin derma decellularization is effective for nuclear and cellular particles elimination from the cell, as it has been concluded in this research. Particular protocols could be changed by increasing the temperature differences or changing the number of freeze-thaw cycles.

REFERENCES

1. Bojchuk T.M., Yermolenko S.B., Fedonyuk L.Ya., et al. The magnitude of linear dichroism of biological tissues as a result of cancer changes. Proc. SPIE. Materials of the 10th International Conference Correlation Optics. 2011;8338,ID8338-54:83381 K1-K7.
2. Vaĭnshteĭn S.G., Masik A.M., Zhulkevich I.V. Food fiber-research results and outlook. Vopr Pitan. 1988;6:8-12.
3. Xu S.J. et al. Differences of wound contraction and apoptosis in full-thickness burn wounds repaired with different artificial dermal stent in pigs. Chinese journal of reparative and reconstructive surgery. 2010;48(11):856-860.
4. Bosco F. et al. The use of banked skin in the Burns Centre of Verona. Blood Transfus. 2011;9(2):156-161. DOI: 10.2450/2011.0107-09.
5. Vaĭnshteĭn S.G., Zhulkevich I.V., Petropavlovskiĭ G.A., Kotelnikova N.E. Protective properties of microcrystalline cellulose in experimental diabetes mellitus in rats. Biull Eksp Biol Med. 1987;103;2:167-168.
6. Fedonyuk L.Ya., Oleshchuk A.M., Sas L.M., et al. Polypragmasy: from paediatrics to geriatrics. Clinical Practice in Pediatrics. 2018;1(13):77-82.
7. Percival S.L. et al. The effects of pH on wound healing, biofilms, and antimicrobial efficacy. Wound Repair and Regeneration. 2014;22(2):174-186.
8. Debeer S., Le Luduec J.B., Kaiserlian D. et al. Comparative histology and immunohistochemistry of porcine versus human skin. Eur. J Dermatol. 2013;23(4):456–466.
9. Chua A.W., Khoo Y.C., Tan B.K. et al. Skin tissue engineering advances in severe burns: review and therapeutic applications. Burns Trauma. 2016;4:3.
10. Tracy L.E., Minasian R.A., Caterson E.J. Extracellular matrix and dermal fibroblast function in the healing wound. Adv. Wound Care (New Rochelle). 2016;5:119–136.
11. Chaudhari A.A., Vig K., Baganizi D.R. et al. Future prospects for scaffolding methods and biomaterials in skin tissue engineering: a review. Int J Mol Sci. 2016;17:1974.
12. Sheikholeslam M., Wright M.E.E., Jeschke M.G. et al. Biomaterials for skin substitutes Adv. Healthc Mater. 2018;7:1700897.
13. Cui H., Chai Y., Yu Y. Progress in developing decellularized bioscaffolds for enhancing skin construction. J Biomed Mater Res A. 2019;107(8):1849-1859.
14. Hrebikova H., Diaz D., Mokry J. Chemical decellularization: a promising approach for preparation of extracellular matrix. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015;159(1):12-7.
15. Crapo P.M., Gilbert T.W., Badylak S.F. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011;32 (12):3233–3243.
16. Riau A.K., Beuerman R.W, Lim L.S. et al. Preservation, sterilization and de-epithelialization of human amniotic membrane for use in ocular surface reconstruction. Biomaterials. 2010;31(2):216–225.
17. Olczyk P., Mencner L., Komosinska-Vassev K. The role of the extracellular matrix components in cutaneous wound healing. BioMed Res Int. 2014; 2014:747584.
18. Volk S.W., Iqbal S.A., Bayat A. Interactions of the extracellular matrix and progenitor cells in cutaneous wound healing. Adv Would Care. 2013;2(6):261–272.
19. Xu C.C., Chan R.W., Tirunagari N.A. Biodegradable, acellular xenogeneic scaffold for regeneration of the vocal fold lamina propria. Tissue Eng. 2007;13(3):551–566.
20. Cox B., Emili A. Tissue subcellular fractionation and protein extraction for use in mass-spectrometry-based proteomics. Nat Protoc. 2006;1(4):1872–1878.
21. Xing Q., Yates K., Tahtinen M. et al. Decellularization of fibroblast cell sheets for natural extracellular matrix scaffold preparation. Tissue Engineering–Part C: Methods. 2015;21(1):77–87.
22. Prasertsung I., Kanokpanont S., Bunaprasert T. et al. Development of acellular dermis from porcine skin using periodic pressurized technique. J Biomed Mater Res B Appl Biomater. 2008;85(1):210–219.
23. Alekseyenko N. S. , Andriychuk V. M., Fedoniuk L. Ya., et al. Peculiarities of trunk skin and fat flexures changes of rural and city youth in the conditions of the educational process. Wiad. Lek. 2020;9(73):2017-2020.
24. Levy R.J., Vyavahare N., Ogle M. et al. Inhibition of cusp and aortic wall calcification in ethanol- and aluminum-treated bioprosthetic heart valves in sheep: background, mechanisms, and synergism. J Heart Valve Dis. 2003;12(2):209–216.
25. Gorschewsky O., Klakow A., Riechert K. et al. Clinical comparison of the Tutoplast allograft and autologous patellar tendon (bone-patellar tendon-bone) for the reconstruction of the anterior cruciate ligament: 2- and 6-year results. Am J Sports Med. 2005;33(8):1202–1209.
26. Gorschewsky O., Puetz A., Riechert K. et al. Quantitative analysis of biochemical characteristics of bone-patellar tendon-bone allografts. Biomed Mater Eng. 2005;15(6):403–411.
27. Alekseyenko N.S., Andriychuk V.M., Radoha R.V., et al. Comparative characteristics of the parameters' changes of skin and fat flexures thickness of extremeties at youth under the condition of higher education. Wiad. Lek. 2020;10(73):2214-2218.
28. Nakayama K.H., Batchelder C.A., Lee C.I., Tarantal A.F. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. Tissue Eng Part A. 2010;16(7):2207–2216.
29. Yang B., Zhang Y., Zhou L. et al. Development of a porcine bladder acellular matrix with well-preserved extracellular bioactive factors for tissue engineering. Tissue Eng Part C Methods. 2010;16(5):1201–1211.

30. Du L., Wu X., Pang K., Yang Y. Histological evaluation and biomechanical characterisation of an acellular porcine cornea scaffold. Br J Ophthalmol. 2011;95(3):410-4.
31. Funamoto S., Nam K., Kimura T. et al. The use of high-hydrostatic pressure treatment to decellularize blood vessels. Biomaterials. 2010;31(13):3590–3595.
32. Riau A.K., Beuerman R.W., Lim L.S. et al. Preservation, sterilization and de-epithelialization of human amniotic membrane for use in ocular surface reconstruction. Biomaterials. 2010;31(2):216–225.

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

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ORIGINAL ARTICLE

CLINICAL AND CYTOLOGICAL CHARACTERISTICS OF THE GUMS IN CHILDREN OF PRIMARY SCHOOL AGE WITH NORMAL BODY WEIGHT AND OVERWEIGHT

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ABSTRACT

The aim of the research was to study the cellular composition of the gums in children of primary school age with normal body weight and overweight for further use of this data in the early diagnostics of periodontal diseases.

Materials and methods: We examined 81 children aged from 6 to 12 years. Cytological examination of gingival cytograms was performed in all examined children.

Results: Based on the analysis of the quantitative content of epithelial cells in children with normal body weight, their ratio was established, which is determined by the percentage of 0: 6: 94 (parabasal, intermediate, superficial). The obtained data completely coincide with the percentage of the differentiated ratio of epitheliocytes of multilayered squamous epithelium in children with normal body weight with inflammation and without it in the periodontal tissues. Our cytological examinations of gingival scrape smears in overweight children in contrast to the results of the study of epithelial scrape smears in children with normal body weight have some differences. Thus, in the process of calculation, the degree of differentiation of various epitheliocytes determines their percentage as follows – 3: 7: 90 (parabasal, intermediate, superficial) for children with gingivitis, and 2: 5: 93 (parabasal, intermediate, superficial) for children without inflammation in the periodontal tissues.

Conclusions: The obtained results allowed us to conclude that in overweight children, in contrast to children with normal body weight, the number of parabasal cells decreases, and the number of superficial and intermediate cells increases.

KEY WORDS: cytogram, children of primary school age, overweight

Wiad Lek. 2021;74(3 p.I):423-428

INTRODUCTION

Inflammatory and dystrophic disorders of the periodontal tissues are so far predominant in the overall structure of pathological processes of the oral cavity in children [1]. It is known that the foci of chronic periodontal infection not only cause contamination of the tissues of the maxillofacial area, but also initiate the emergence of pathological processes in other organs and systems [2]. The results of numerous clinical and epidemiological studies indicate a tendency for an increase in the prevalence of overweight among children and adolescents due to the presence of concomitant somatic diseases [3,4,5]. An important role in the development of inflammatory changes in the periodontal tissues in overweight children belongs to a decrease in the overall reactivity of the body [6]. However, the analysis of literature showed the ambiguity of studies on the specific mechanisms of periodontal tissue damage in overweight children and adolescents, especially in the early stages of gingivitis, which is important for both its prevention and treatment. The morphological examination is one of the precise methods to detect pathology in the early stages of development. This method is widely used in the diagnosis of diseases of the oral mucosa and periodontal tissues. Due to its minimally invasive nature and highly informative value, the cytological method has a great advantage in the use of pediatric dentistry [7]. At present, the issues of determining the peculiarities of the cellular composition of the gingival mucosa by the cytological method in primary school children are insufficiently covered, and the comparative analysis of cellular composition in primary school children with normal body weight and overweight has not been performed at all.

THE AIM

The aim of the research was to study the cellular composition of the gums in children of primary school age with normal body weight and overweight for further use of this data in the early diagnostics of periodontal diseases.

MATERIALS AND METHODS

We examined 81 children aged from 6 to 12 years, who according to outpatient records did not have a concomitant pathology and underwent a preliminary examination by a pediatrician. The study of anthropometric data and assessment of physical development was conducted in ac-

Table I. Anthropometric data in the examined children with overweight and normal body weight

Indicators	Groups			
	1a	1b	2a	2b
The number of schoolchildren	17	8	40	16
Age	8.75±0.29	8.44±0.72	8.38±0.17	8.86±0.30
Height (m)	1.35±0.02	1.38±0.04	1.38±0.01	1.38±0.03
Weight (kg)	30.76±1.54	32.96±2.54	38.90±1.18***	40.41±2.38****
BMI (kg/m^2)	16.61±0.35	17.05±0.53	20.37±0.39***	20.77±0.61****
Waist circumference (m)	0.54±0.01	0.54±0.01	0.61±0.01***	0.63±0.02****
Thigh circumference (m)	0.36±0.01	0.36±0.01	0.41±0.01***	0.41±0.02
Neck circumference (m)	0.25±0.01	0.25±0.01	0.27±0.003	0.26±0.01
Fat fold (mm)	10.35±0.69	11.75±2.81	19.35±1.17***	24.44±2.49**, ****

Note: * the probable difference between groups 1a-1b, ** – between groups 2a-2b, *** – between groups 1a-2a, **** – between groups 1b-2b, p <0.05

Table II. The intensity of caries, the hygienic status of the oral cavity and periodontium in children with normal body weight and overweight

Indicators	Groups			
	1a	1b	2a	2b
Dysgnathia	0.71±0.12	0.88±0.13	0.48±0.78	0.43±0.13****
cf	1.18±0.43	0.5±0.5	1.85±0.35	1.13±0.52
CFR	0.29±0.14	0.5±0.33	0.18±0.09	0.25±0.25
cf + CFR	1.47±0.44	1±0.53	2.03±0.36	1.25±0.54
OHI, point	1.64±0.08	1.75±0.06	1.53±0.06	1.58±0.08****
PMA, %	0	25±0.03	0	30± 0.03 ****

Note: * the probable difference between groups 1a-1b, ** – between groups 2a-2b, *** – between groups 1a-2a, **** – between groups 1b-2b, p <0.05

Table III. Cytological characteristics of scrape smears in children aged 6-12 years (in the field of view)

Indicators	Groups							
	1a		1b		2a		2b	
	Cells in the field of view	%	Cells in the field of view	%	Cells in the field of view	%	Cells in the field of view	%
Basal	0		0		0		0	
Parabasal	0.13±0.09	0.13%	0.38±0.15*	0.37%	2.25±0.49***	2.21%	3±0.01**, ****	2.93%
Intermediate	6.31±0.32	6.31%	6.58±0.38	6.58%	5±0.53***	4.92%	7.41±0.51**, ****	7.24%
Superficial	93.56±0.79	93.56%	93.05±0.52	93.05%	94.38±1.25	91.88%	91.94±0.42**, ****	89.83%

Note: * the probable difference between groups 1a-1b, ** – between groups 2a-2b, *** – between groups 1a-2a, **** – between groups 1b-2b, p <0.05.

cordance with the Order of the Ministry of Public Health of Ukraine as of 13.09. 2013 No. 802 "Criteria for assessing the physical development of school-age children" [8] for further formation of research groups.

Clinical dental examination was performed according to the WHO method, 2013 [9]. The hygienic condition of the oral cavity was assessed using the simplified Green-Vermillion hygienic index [10]. To detect the inflammatory process and assess its intensity in the periodontal tissues, the PMA index was determined. [11].

Cytological study of gingival cytograms was performed in all examined children. Smears were obtained by scraping the mucous membrane of the gums, rotating the working part of the spatula, the resulting material was applied to a degreased glass slide. Drying of the smears was performed by the dry fixation method at room temperature under conditions of open air access. Smears were placed in a container for glasses and lowered into a special reservoir with May-Grünwald fixative stain [12]. The smears were fixed for 5 minutes, washed with water and laid out in a special tripod for drying. Cytogram analysis was performed using a Biorex–3 BM–500T microscope with a DCM–900 digital photomultiplier with programs adapted for the study data, using a magnification of 1000. Quantitative parameters were determined by counting cellular elements in 5 fields of view, recording the amount in absolute numbers, and determining averages using Excel software [13].

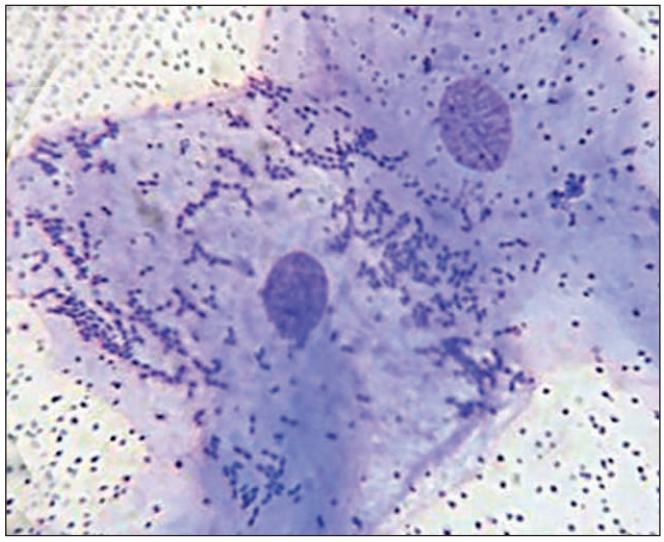

Fig. 1a. Intermediate cells in the cytogram of the gums in a child with normal body weight, without inflammation and with a subcompensated form of caries. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

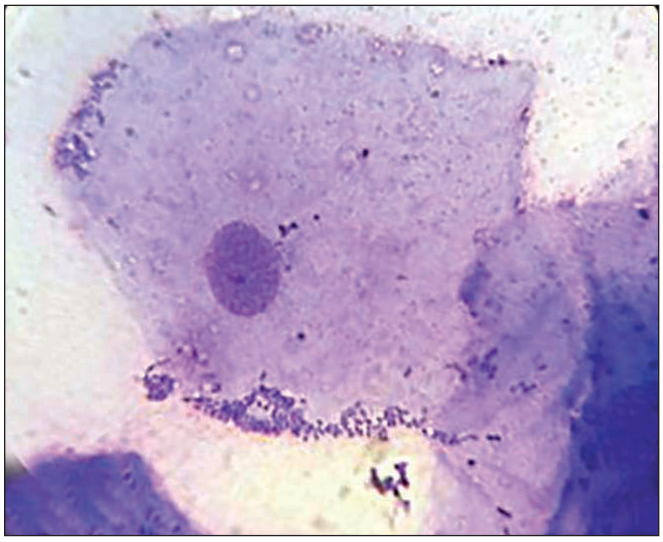

Fig. 1b. A superficial maturing cell in the cytogram of the gums in a child with normal body weight, without inflammation. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

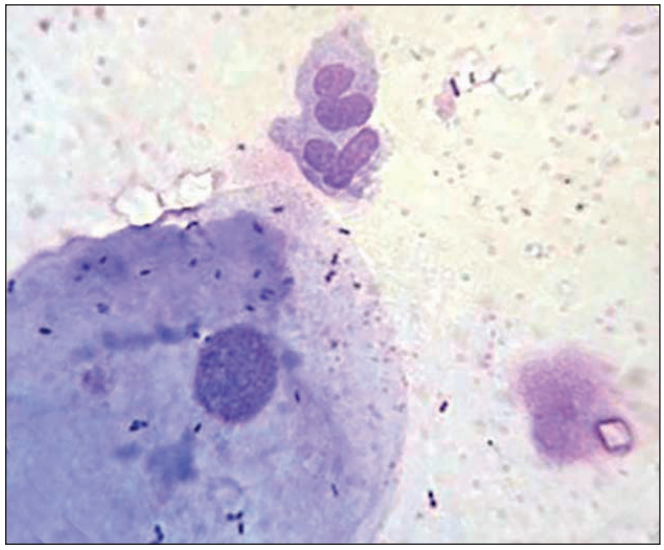

Fig. 2a. Superficial cells and segmental leukocytes in the cytogram of the gums in a child with normal body weight, with inflammation in the periodontal tissues. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

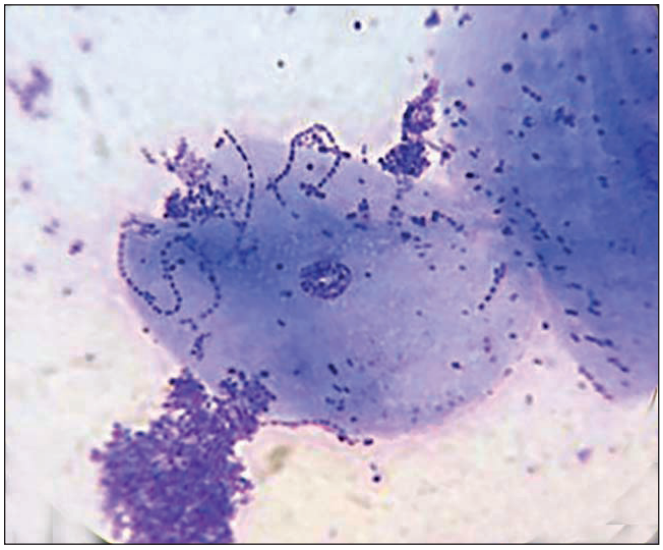

Fig. 2b. Contamination of microorganisms in the cytogram of the gums in a child with normal body weight, with inflammation in the periodontal tissues and a compensated form of caries. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

RESULTS AND DISCUSSION

The conducted examination of children with overweight and normal body weight allowed us to divide the two study groups into subgroups (1a, 1b and 2a, 2b). The first group consisted of 25 subjects whose general development and weight corresponded to the age norm, 8 of them had the phenomena of catarrhal gingivitis. 56 children were overweight, 16 of them showed signs of periodontal inflammation, and 40 children had clinically healthy gums.

After analyzing the main indicators of anthropometric studies, we concluded that the height in all studied groups corresponds to the age norm and ranges from 1.33 kg / m^2 to 1.42 kg / m^2. BMI (kg / m^2) differs significantly due to body weight indicated, which is interpreted according to the WHO centile tables.

The ratio of waist circumference (m) to thigh circumference (m) and thickness of the fat fold are reliable for diagnosis. That is, in the future one can use the WC / TC ratio and the quantitative value of the fat fold for the diagnosis of obesity in children of primary school age (Table I).

The study of caries intensity showed that in the examined children with normal body weight the index of cf + CFR is 1.47 ± 0.44 teeth for children without gingivitis and 1 ± 0.53 teeth for children with gingivitis. In overweight children, this figure was higher (2.03 ± 0.36 and 1.25 ± 0.54, respectively, $p<0.05$). The prevalence of caries in children

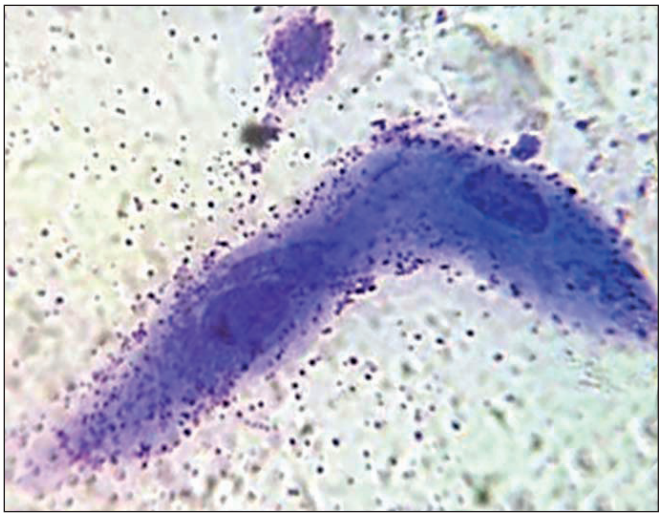

Fig. 3a. Parabasal cells in the cytogram of the gums of an overweight child without inflammation with a compensated form of caries. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

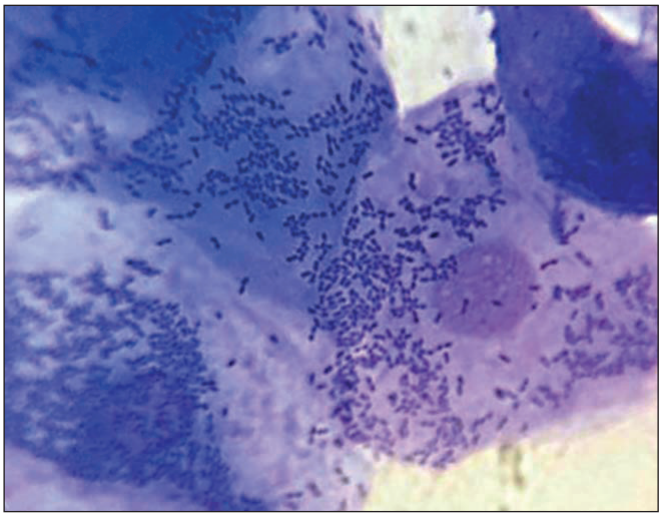

Fig. 3b. Contamination of microorganisms, karyopyknosis in superficial cells in the cytogram of the gums of an overweight child with inflammation in the periodontal tissues and a compensated form of caries. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

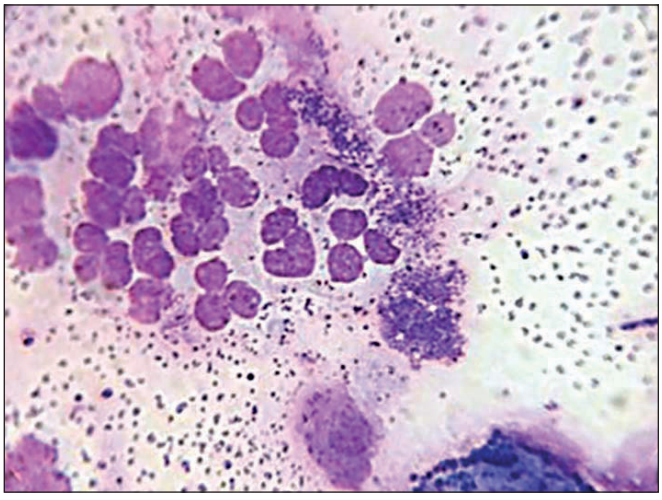

Fig. 4a. Leukocyte, lymphocyte, mycelium of fungi in the cytogram of the mucous membrane of the gums in an overweight child with inflammation in the periodontal tissues. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

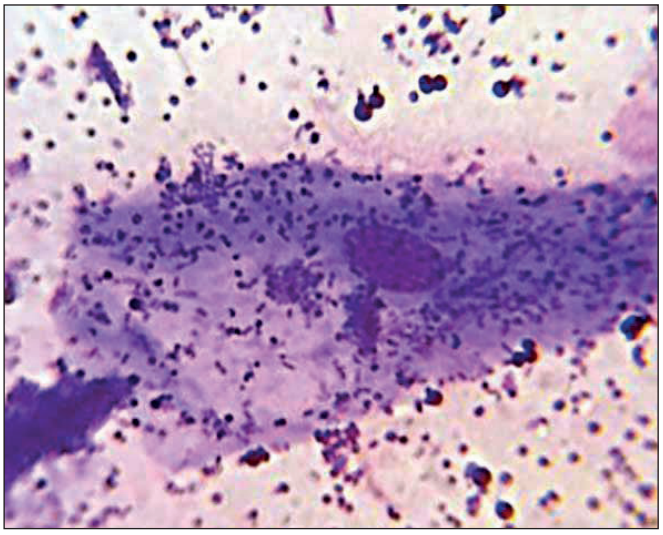

Fig. 4b. The navicular cell in the cytogram of the gums of an overweight child with inflammation in the periodontal tissues. May-Grünwald's stain. Magn.: obj. x 100, eyepiece x 10.

with normal body weight was 53% and 38%, respectively, and in the group of overweight children, it was slightly higher – about 60% and 44%, respectively (Table II).

The oral hygiene index in children with normal body weight and overweight corresponded to satisfactory hygiene regardless of the degree of inflammation in the periodontal tissues. Children with normal body weight, diagnosed with gingivitis, had worse hygiene, which was on the verge of satisfactory and poor hygiene as compared to the group of overweight children with gingivitis.

To assess the intensity of the inflammatory process in the periodontal tissues of children in the study groups, the data of the PMA index were analyzed. It was found that in children with normal body weight, the PMA index was 25 ± 0.5%, while in overweight children the index data were higher and amounted to 30 ± 0.03%, which was also characterized as moderate gingivitis.

In the epithelium of the mucous membrane of the human gums, there are defined 4 classes of cells – basal, parabasal, intermediate and superficial. In cytograms from the gingival mucosa of children, parabasal epitheliocytes are almost absent, which is due to their functional features [14]. According to the results of cytological studies of the cellular composition of the cytograms in the gums of school-age children, we found that they were dominated by superficial cells (Table III).

In children of group 1b, the number of parabasal cells was slightly greater than in 1a, though very small. We did not find any other quantitative differences in the cytograms of children with normal body weight.

The average number of intermediate cells in groups 1a and 1b had no significant difference. In children with normal body weight, the intermediate cells in the cytograms had a polygonal shape, a centrally located oval nucleus with structured chromatin. There was weak contamination of microorganisms on the cell surface (Fig. 1a). Superficial epitheliocytes in the cytograms of the gums in children of group 1a were smaller in size than intermediate cells, characterized by a rounded shape and an uneven contour of the plasmolemma. The nuclei of superficial epitheliocytes were located eccentrically in the cytoplasm and were hyperchromic (Fig. 2b).

For children with normal body weight and inflammation in the periodontal tissues, the cytograms were characterized by the presence of epitheliocytes, mainly of the superficial layer, in the scrape smears from the gingival surface. Their cytoplasm was weakly basophilic, lumps of keratohyalin were observed. Round nuclei were found at different stages of karyopyknosis. This group of children was characterized by the presence of segmental leukocytes and single lymphocytes in the cytograms (Fig. 2a).

The greater intensity of contamination of microorganisms on the surface of superficial epitheliocytes attracted our attention (Fig. 2b). This cytological presentation is observed in chronic gingivitis and in exacerbation of chronic gingivitis of lesser intensity.

Cytomorphological studies of scrape smears from the gingival mucosa revealed that in the group of overweight children without inflammation of the periodontal tissues, the number of parabasal was significantly greater than in groups 1a and 1b, whereas the intermediate ones were significantly fewer ($p<0.05$). The number of superficial cells was relatively larger, but no statistical difference was found (see Table 3).

In the group of overweight children with inflammation of the periodontal tissues, the average number in the field of view of parabasal cells (Fig. 3a) was significantly higher by 33%, and the number of the intermediate ones – by 48.2% than in the group without inflammation. The average number of superficial cells in group 2b was significantly lower by 2.59% (see Table 3).

We detected epitheliocytes with the signs of karyolysis, epithelial cells with destroyed nuclei, and epitheliocytes containing nuclei with unevenly distributed fine-grained chromatin. The greatest degree of manifestation of contamination of microbial flora attracted our attention, sometimes microorganisms covered the entire surface of cells (Fig. 3b).

In addition to epithelial cells, lymphocytes and neutrophilic granulocytes were visualized in the cytograms of overweight children, and the number in the field of view was higher in children with inflammation of the periodontal tissues (Fig. 4a).

It was found that in the group of overweight children with inflammation in the periodontal tissues, there was a significantly higher number of epitheliocytes with degenerative changes (Fig. 4b) as compared to the number of such cells in the group of children without inflammation of the periodontal tissues. It should be noted that degenerative changes in the squamous epithelial cells in inflammatory diseases can lead to cell death.

Cytological diagnosis allowed us to evaluate the obtained cytological presentation of scrapes from the oral mucosa, represented by multilayered squamous epithelium without keratinization, in the intact periodontium and in the occurrence and development of inflammatory changes therein.

The study of cells of the multilayered squamous epithelium of the oral mucosa in overweight children proved the presence of parabasal, intermediate and superficial cells, but with a predominance of intermediate and superficial type of cells.

In the group of children with inflammation in the epithelial layer, neutrophilic leukocytes and small lymphocytes are found. Single lymphocytes have no significant diagnostic value, but an increase in their number in cytological specimens over 5% indicates the involvement of the body's immune system and the transition from acute to chronic inflammatory reaction [6]. Monocytes in smears are very rare.

Most often in the smears of children with normal body weight, there were individual segmental neutrophilic granulocytes (normal, sometimes degeneratively altered – with a hypersegmented nucleus, without intersections between the segments that have lost specific granularity). In children with inflammation in the periodontal tissues, the number of leukocytes in the cytograms was higher. Thus, we can assume that the increased content of neutrophils in the epithelium and on its surface is an additional protective antimicrobial mechanism in areas of the mucous membrane that are not protected by the stratum corneum.

The literature data demonstrate that the appearance of neutrophilic granulocytes in cytological specimens in the amount equal to or exceeding 10% of the total number of cells (especially morphologically unaltered) indicates an acute inflammatory process in the oral cavity [13].

Based on the analysis of the cellular composition of cytograms in children with normal body weight without inflammation and with inflammation of the periodontal tissues, their ratio was 0: 6: 94 (parabasal, intermediate, superficial).

Our cytological examinations of gingival scrape smears in overweight children have some differences as compared to the results of the study of epithelial scrape smears in children with normal body weight. Hence, in the process of calculation, the degree of differentiation of various epitheliocytes determines their percentage as follows: 2: 5: 93 in children without inflammation of periodontal tissues and 3: 7: 90 in children with inflammation.

In overweight children, in contrast to children with normal body weight, the number of parabasal cells increases, the number of superficial cells decreases and the number of intermediate cells increases.

Obviously, such changes in cytological components are related to the characteristics and condition of the entire organism. This is why parabasal cells appear, which are smaller than intermediate cells. In parabasal cells, the nucleus is not clearly contoured, surrounded by tonofibrillar structures.

CONCLUSIONS

The cytological presentation is an important indicator and the assessment of cellular composition allows us to obtain information that enables determining the presence of preconditions for inflammation at the pre-nosological level for the prevention and prophylaxis of periodontal disease in primary school children.

REFERENCES

1. Sanz M., Herrera D., Kebschull M., et al; EFP Workshop participants and methodological consultants. Treatment of periodontitis – The EFP S3-level clinical practice guideline. J Clin Periodontol. 2020;47(S22):4-60. https://doi.org/10.1111/jcpe.13290
2. Peres K.G, ThomsonW.M., Chaffee B.W., et al. Rugg-Gunn Oral Health Birth Cohort Studies: Achievements, Challenges, and Potential Journal of Dental Research, 17 Jul 2020. https://doi.org/10.1177/0022034520942208.
3. Bezwushko Ye.V., Zhuhina L.F., Narykova A.A. et al. Porivnialna ocinka stomatologicznogo zdorovia ditwj shkilnogo viku za Yevropejskymy indykatoramy zdorovia porozhnyny rota. Nowyny stomatologii. 2013;3:76-80.
4. Ogijenko V.P. Statystyczni dani po poshyrenniu ozhyrinnia v Ukraini i sviti. [Elektronnyi resurs] : http://medstat.gov.ua/ukr
5. Sheshukova O.V., Onyshchenko A.V. vmist interleikinu -10 u rotovii ridyni ditej molodshogo shkilnogo viku z normalnoju ta nadmirnoiu masoiu tila. Visnyk problem Biologii i medycyny. 2020; 157 (3): 374- 377.
6. Zahajnova N.N. Koreliacyonnaia swiaz mezhdu pokazateliami intensivnosti karijesa zubov, sostojanijem tkaniej paradonta i gigijeny polosti rta u detej. Visnyk stomatologii. 2017;2:51-52.
7. Sheshukova O.V., Yeroshenko G.A., Trufanova V.P. et al. Method of preclinical determination of probability of inflammation of periodontal tissues in children. Ukraine patent 130471. 2018 June 4.
8. Pro zatverdzhennya Kryteriyiv otsinky fizychnoho rozvytku ditey shkil'noho viku [On approval of Criteria for assessment of physical development of school children]. 2013. Available from:http://zakon.rada.gov.ua/laws/show/z1694-13.
9. World Health Organization. Oral Health Surveys Basic Methods, 5th Ed. – Geneva : WHO, 2013, p.125
10. Homenko L.O. et al. Terapevtyczna stomatologiia dytiachogo viku. Karijes zubiv ta jogo uskladnennia. Kyiv. Knyga Plus, 2014, p.132.
11. Kostura V.L. Indeksna ocinka stanu tkanyn paradonta u ditej z nadmirnoiu masoiu tila. Visnyk problem biologii ta medycyny. 2018; 1 (142):360-363.
12. Gasyuk N.V., Yeroshenko G.A. Osobennosti kletochnogo sostava desen pri generalizovannom parodontite. [Feature of cellular composition of the gums in generalized periodontitis.] Svit meditsini I blologyi. 2015; 1(48): 17–2.
13. Lapach S.N., Chubenko A.V., Babich P.N. Statisticheskie metodyi v mediko-biologicheskih issledovaniyah s ispolzovaniem Exel. [Statistical data in biomedical research using Exel.] Kiev; 2000. 320 p.
14. Sheshukova O.V., et al. Cytologichna kharakterystyka klitynnogo skladu slyzovoi obolonky jasen u ditej shkilnogo viku. Aktualni problemy suchasnoi medycyny: Visnyk Ukrainskoi medycznoi stomatologichnoi akademii. 2019;2 (66):146–150.

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ORIGINAL ARTICLE

QUALITY OF LIFE IN WOMEN AFTER MASTECTOMY. CLINICAL AND SOCIAL STUDY

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ABSTRACT

The aim: To assess the quality of life in women after mastectomy.

Materials and methods: The study included 25 women after mastectomy. The patients were aged 31 to over 50 years and were members of a breast cancer support group at the Holy Cross Cancer Centre in Kielce. During group meetings, the patients underwent rehabilitation and worked with psychologists and social workers.

Results: Most women after mastectomy who underwent breast reconstruction or wore breast prostheses reported a better quality of life. Mastectomy affects ipsilateral upper limb function and causes difficulty with activities of daily living, such as cleaning, cooking, brushing hair, bathing, and dressing.

Conclusions: 1. All women who rated their health as poor experienced such negative emotions as sadness, low mood, dejection. 2. Mastectomy affects ipsilateral upper limb function. 3. Breast reconstruction after mastectomy improves patient-rated quality of life. 4. Pain in the ipsilateral upper limb is considerably more common in women with a limited range of motion. 5. All women who participated in breast cancer support group meetings found support there and the time they spent together resulted in an improved quality of life.

KEY WORDS: mastectomy, clinical problems, quality of life

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INTRODUCTION

Breast cancer is one of the most important epidemiological problems and the second most common malignancy in women worldwide. In Poland, the risk of developing a malignant breast tumour is high, with 18,000 to 19,000 new cases and 6,000 deaths per year. The incidence rate has been steadily increasing and the mortality rate rose by 7.2% in 2019. Breast cancer usually affects women over the age of 50 years, but 33% of cases are reported in patients aged 30 to 49 years. In the recent years, the incidence of breast cancer in young women has increased two-fold [1-6].

RISK FACTORS

The most important risk factors for breast cancer include age, gender, a family history of breast cancer, diagnosed intraductal hyperplasia, genetic factors, hormonal factors, ionising radiation, and long-term use of hormone replacement therapy and hormonal contraception [4-6].

The recent years have seen dynamic developments in the treatment of breast cancer. Currently, patients are managed with combination therapy that includes surgery, radiotherapy, and systemic methods, i.e. chemotherapy and hormone therapy. Treatment can be divided into less invasive surgical procedures (breast-conserving surgery), removal of the whole breast (mastectomy), and adjuvant treatment (radiotherapy, chemotherapy, hormone therapy) [1-3].

Mastectomy is associated with negative consequences that considerably limit ipsilateral upper limb function and cause postural changes and lymphoedema. Lymphoedema develops in the upper limb due to impaired lymph drainage after axillary lymph node removal. Axillary web syndrome, or cording, manifests as pain in the axillary fossa and results from the disruption of superficial veins and lymphatic vessels in this region [6-8].

Treatment of patients after radical breast cancer surgery should always include comprehensive in-hospital and out-patient rehabilitation; patients will also benefit from participating in breast cancer support group meetings and activities.

QUALITY OF LIFE IN WOMEN AFTER MASTECTOMY

SOCIAL, PSYCHOLOGICAL, AND EMOTIONAL CONSEQUENCES OF MASTECTOMY

Consequences of breast cancer include not only functional, physical impairment, but also psychological and social issues. Cancer can suddenly change one's entire life. In most cases, a breast cancer diagnosis is associated with negative emotions, for example anxiety, fear, the feeling of being in danger, irritability, hypersensitivity, anger, and sometimes depression. This has a significant effect on patients' ability

Table 1. Age of study patients

	N	[%]
31-40 years	2	8.0
41-50 years	8	32.0
Over 50 years	15	60.0
Total	25	100.0

Table 2. Patient-rated health

	N	[%]
Very good	3	12.0
Good	13	52.0
Fair	8	32.0
Poor	1	4.0
Total	25	100.0

Table 3. Reaction of study patients to cancer diagnosis.

	N	[%]
Shock, it is difficult to believe	12	48.0
I want to start treatment as soon as possible	13	52.0
Total	25	100.0

Table 4. Time from surgery.

	N	[%]
Less than a year	3	12.0
1-5 years	9	36.0
6-10 years	8	32.0
More than 10 years	5	20.0
Total	25	100.0

Table 5. Difficulty dressing.

	N	[%]
No difficulty	6	24.0
Slight difficulty	4	16.0
Moderate difficulty	10	40.0
Considerable difficulty	5	20.0
Total	25	100.0

Table 6. Ipsilateral limb pain.

	N	[%]
Yes, I experience pain	8	32.0
I do not experience pain	12	48.0
I have occasional pain	5	20.0
Total	25	100.0

Table 7. Limited range of motion after surgery.

	N	[%]
Yes, I have a limited range of motion	7	28.0
I do not have a limited range of motion	16	64.0
I have a slightly limited range of motion	2	8.0
Total	25	100.0

Table 8. Change in the way patients cared about their appearance as a result of mastectomy.

	N	[%]
Yes	11	44.0
No	14	56.0
Total	25	100.0

Table 9. Breast reconstruction after mastectomy.

	N	[%]
Yes	6	24.0
No	19	76.0
Total	25	100.0

Table 10. Patient-rated influence of breast reconstruction on everyday life.

	N	[%]
Significant influence	4	66.7
Moderate influence	2	33.3
Total	6	100.0

Table 11. Frequency of rehabilitation.

	N	[%]
Once a week	4	16.0
2-3 times a week	12	48.0
4 or more	9	36.0
Total	25	100.0

to accept the diagnosis and adjust to the new, changed life situation; in addition, it may affect the process of physical and mental recovery. Consequently, acceptance and self-assessment are very important in cancer treatment, which is usually complex and relatively long-term. Breast cancer patients worry about how they will respond to treatment and are afraid of recurrence or death. As a result, they may feel helpless, sad, and anxious about their changing life situation. It is important to assess whether they will be able to cope with the limitations associated with their disease and remain independent in their everyday life [7-16].

Moreover, breast cancer patients have to not only accept themselves in a new situation and come to terms with their disease, but also find their place among family members and friends. Mastectomy leaves both physical and mental scars and may result in such problems as the so-called half-woman complex, where women feel 'defective', unable to accept their body, anxious about no longer being sexually attractive, and worried their partner might leave them. Social interactions and support from family and friends influence recovery and prolong patients' lives [17-20].

Table 12. Effects of rehabilitation on everyday functioning.

	N	[%]
Yes	23	92.0
No	2	8.0
Total	25	100.0

Table 13. Wearing a breast prosthesis.

	N	[%]
Yes	16	64.0
No	9	36.0
Total	25	100.0

Table 14. Difficulty carrying groceries

	N	[%]
Slight	10	40.0
Fairly considerable	4	16.0
Considerable	11	44.0
Total	25	100.0

Table 15. Difficulty cleaning the house

	N	[%]
Slight	11	44.0
Fairly considerable	7	28.0
Considerable	7	28.0
Total	25	100.0

Table 16. Meetings with a psychologist.

	N	[%]
Yes	18	72.0
No	7	28.0
Total	25	100.0

Table 17. Negative emotions.

	N	[%]
Often	5	20.0
Rarely	8	32.0
Very rarely	12	48.0
Total	25	100.0

Table 18. Acceptance of physical appearance.

	N	[%]
I do not accept it	1	4.0
I somewhat accept it; I am no longer attractive	2	8.0
I moderately accept it; I got used to it	4	16.0
I mostly accept it	9	36.0
I fully accept it	9	36.0
Total	25	100.0

Table 19. Participation in breast cancer support group meetings and improvements in the quality of life

	N	[%]
Yes, it improved my quality of life	25	100.0

THE AIM

The aim of the present study was to assess the quality of life in women after mastectomy.

MATERIALS AND METHODS

PROBLEMS AND HYPOTHESES

The main research problem addressed in the study can be expressed as follows: does rehabilitation have a positive effect on the quality of life in women after mastectomy?

The following hypotheses were formulated and then verified:

1. Limb pain occurred more often in women who had a limited range of motion after surgery and were aged over 50 years.
2. Women after mastectomy rarely experience such emotions as sadness, low mood, anxiety, and dejection, which is associated with their relationship with family members.
3. Most women found support and understanding in a breast cancer support group and believe that their participation in group meetings improved their quality of life.

RESEARCH METHODS

The data collected from study patients were systematically entered into an Excel database created for the study. All calculations were performed with the SPSS Statistics 21.0. software. Participation in the study was voluntary. The study data were used to perform descriptive, graphical, and statistical analyses. The statistical relationship between the characteristics analysed in the study was tested with a chi-squared test.

The study included 25 women who were members of a breast cancer support group (Amazon Club) at the Holy Cross Cancer Centre in Kielce. The follow-up period was 5 months. The women participated in rehabilitation and met with psychologists and social workers.

Most study patients were aged either over 50 years (60%) or between 41 and 50 years (32%). Only two women underwent mastectomy before the age of 40 years (Table1).

Most study patients lived in a city with over 100,000 inhabitants (52%); others lived in rural areas (28%), a town with 10,000 to 100,000 inhabitants (12%) or a town with up to 10,000 inhabitants (8%).

Most study patients were married.

48% of study patients had higher education, 40% had secondary education, and 12% had vocational secondary education.

Most study patients rated their current health as good (52%) or fair (32%) (Table 2).

Usually, patients wanted to start cancer treatment as soon as possible (52%); others (48%) were deeply shocked and could not believe that they were affected by breast cancer (Table 3).

Table 20. Fisher's test for correlation between pain and surgery.

			Do you have a limited range of motion in your arm on the operated side after surgery?			Test result
			yes, I have a limited range of motion	I do not have a limited range of motion	I have a slightly limited range of motion	
Do you experience pain in your arm on the operated side?	yes, I experience pain	N	6	2	0	$p = 0.001$
		[%]	85.7	12.5	0.0	
	I do not experience pain	N	0	11	1	
		[%]	0.0	68.8	50.0	
	I have occasional pain	N	1	3	1	
		[%]	14.3	18.8	50.0	
Total		N	7	16	2	
		[%]	100.0	100.0	100.0	

p – significance; χ 2 –test statistic; df – degrees of freedom

Table 21. Acceptance of physical appearance.

Do you accept your physical appearance?		
	N	[%]
I do not accept it	1	4.0
I somewhat accept it; I am no longer attractive	2	8.0
I moderately accept it; I got used to it	4	16.0
I mostly accept it	9	36.0
I fully accept it	9	36.0
Total	25	100.0

Most study patients underwent breast cancer surgery either between 1 and 5 years before the study (36%) or between 6 and 10 years before the study (32%); in five women (20%), the surgical procedure had been performed more than 10 years before they entered the study (Table 4). Most study patients (60%) had no difficulty eating meals. Four women (16%) had slight difficulty with this activity, three patients (12%) had moderate difficulty, and another three (12%) had considerable difficulty eating meals.

A large group of study patients admitted they had moderate difficulty bathing (40%). One patient (4%) had very considerable difficulty; four patients (16%) complained of considerable difficulty and three women (12%) had slight difficulty with this task.

Most study patients had moderate difficulty (40%) dressing and five women (20%) had considerable difficulty with this task (Table 5).

Most study patients either did not have any difficulty walking up the stairs (44%) or experienced slight difficulty with this activity (36%). Two women complained of considerable difficulty walking up the stairs.

Most study patients had difficulty carrying groceries.

The majority of study patients either did not have difficulty walking longer distances (44%) or had slight difficulty with this activity (36%).

The majority of study patients responded that they experienced frequent (32%) or occasional (20%) pain in their ipsilateral upper limb (Table 6).

Seven women (28%) declared they had a limited range of motion. Two study patients (8%) described their range of motion as slightly limited (Table 7).

The majority of study patients worried about possible health problems after mastectomy (40%) and about decreased physical fitness (36%). Some patients were also anxious that their partners might not accept them (24%).

Only one study patient (4%) underwent bilateral mastectomy.

A vast majority of study patients declared that the way they cared about their physical appearance did not change after mastectomy (56%) (Table 8).

Most study patients did not undergo breast reconstruction after mastectomy (76%). The majority of patients who did receive breast reconstruction underwent this procedure a year after mastectomy (83.3%) (Table 9)

The majority of study patients (66.7%) after breast reconstruction believed that the procedure had a significant influence on their everyday life (Table 10)

The majority of study patients underwent physical therapy either 2 to 3 times a week (48%) or 4 or more times a week (36%) (Table 11). A vast majority of patients declared that they performed self-massage of the ipsilateral upper limb every day.

The majority of the women assessed in the study believed that rehabilitation had a positive effect on their everyday functioning (92%) (Table 12)

Most study patients declared that they had and used a breast prosthesis (Table 13)

A large group of study patients (48%) accepted their breast prosthesis and felt good wearing it. Four women (16%) were unable to accept their breast prosthesis.

The majority of study patients believed that their breast prosthesis had an effect on their quality of life and visibly improved it (52%).

Most patients had slight difficulty brushing their hair (52%). However, many patients (44%) had fairly considerable difficulty with this activity and one patient (4%) had very considerable difficulty.

One patient had considerable difficulty dressing; the other patients had either slight difficulty (48%) or fairly considerable difficulty (48%) with this task.

Table 22. Fisher's test for assessment of health vs. negative emotions.

			How would you rate your current health?				Test result
			very good	good	fair	poor	
How often do you experience such emotions as sadness, low mood, dejection?	Often	N	0	1	3	1	$p = 0.002$
		[%]	0.0	7.7	37.5	100.0	
	Rarely	N	0	3	5	0	
		[%]	0.0	23.1	62.5	0.0	
	Very rarely	N	3	9	0	0	
		[%]	100.0	69.2	0.0	0.0	
Total		N	3	13	8	1	
		[%]	100.0	100.0	100.0	100.0	

p – significance; χ 2 –test statistic; df – degrees of freedom

Table 23. Support from breast cancer support group

	N	[%]
Yes, I received support	25	100.0

Table 24. Participation in breast cancer support group meetings and improvements in the quality of life.

	N	[%]
Yes, it improved my quality of life	25	100.0

Many study patients had considerable difficulty carrying groceries (44%); 40% of the women assessed in the study had slight difficulty with this task (Table 14)

Most study patients admitted that they had slight difficulty during sleep (64%); eight patients (32%) had fairly considerable difficulty.

The majority of study patients admitted that they had slight difficulty with personal hygiene activities (64%); nine women had fairly considerable difficulty with this task.

Most study patients had considerable (52%) or fairly considerable (28%) difficulty doing sports.

Most study patients had either fairly considerable (28%) or considerable (28%) difficulty cleaning the house (Table 15)

The majority of study patients participated in meetings with a psychologist (72%) (Table 16).

Most patients believed that the psychologist helped them accept their new life situation (56%). Unfortunately, four women (16%) were unable to accept their changed life situation.

20% of study patients experienced sadness, grief, and low mood (Table 17)

Most study patients either fully accepted (36%) or partly accepted (36%) their physical appearance. Four study patients (16%) said they had managed to get used to it (Table 18).

All study patients confirmed that during treatment, they had the full support of their loved ones and that their relationship with family members did not deteriorate during treatment.

Most study patients found out they could join a breast cancer support group on the Internet (40%) or from their acquaintances (28%) or healthcare professionals (24%).

All study patients found support in the breast cancer support group.

All study patients responded that taking part in meetings of the breast cancer support group improved their quality of life (Table 19).

RESULTS

Hypothesis: Upper limb pain occurred more often in women who had a limited range of motion after surgery.

An analysis was performed to check for the presence of a statistically significant correlation between two variables (Do you experience pain in your arm on the operated side? and Do you have a limited range of motion in your arm on the operated side after surgery?). Fisher's test for R x C tables was used. There is a correlation between the variables (Table 20).

Pain was definitely most common in patients with a limited range of motion; as many as 85.7% of these patients experienced pain. In the other patients, the incidence of pain was up to 12.5%.

Study patients usually accepted their physical appearance (36%) (Table 21)

The p value was lower than 0.05, which means that there was a relationship between the variables. All women who rated their current health as poor often experienced negative emotions (Table 22).

Hypothesis: Most women found support and understanding in a breast cancer support group and believe that their participation in group meetings improved their quality of life.

All study patients found support in the breast cancer support group and believed that the group improved their quality of life (Table 23-24).

DISCUSSION

The aim of the present study was to assess the quality of life in women after mastectomy and to determine the influence of rehabilitation on their quality of life.

The study included a total of 25 women who were members of the breast cancer support group (Amazon Club) at the Holy Cross Cancer Centre in Kielce. Most study patients described their health as good (52%). Nevertheless, when asked about their quality of life, many patients

admitted they had difficulty bathing, dressing, carrying heavy objects, shopping, cleaning, brushing their hair, and cooking; eating meals was the least difficult activity.

Breast reconstruction after its removal is one of the methods of improving the quality of life in women after mastectomy. The majority of study patients who underwent breast reconstruction believed that the procedure had a significant positive influence on their everyday life.

Breast prostheses are used as an alternative to breast reconstruction and serve a similar purpose. Only half of the study group (48%) felt good wearing a breast prosthesis and used and accepted it. Four study patients (16%) wore a breast prosthesis out of necessity, but were unable to accept it. The other patients did not use breast prostheses. The majority of women who wore and accepted a breast prosthesis believed that the prosthesis improved their quality of life.

Rehabilitation is as important as breast reconstruction and prostheses. The majority of the women assessed in the study (96%) underwent rehabilitation with a physiotherapist after mastectomy and most patients described their therapy as either very good (56%) or good (28%). In addition, study patients believed that rehabilitation had a positive influence on their quality of life and everyday functioning (92%).

Physical activity is very important in the process of rehabilitation. More than half of the study group admitted that they had considerable (52%) or fairly considerable (28%) difficulty doing sports and engaging in physical activity. Consequently, it would be beneficial to create conditions that would allow women after mastectomy to take up physical activity and promote the positive influence of physical activity on the motor function in the upper limb on the side of surgery.

Psychologists play a very important role in breast cancer treatment as they help patients accept and understand their disease and find motivation to continue fighting it. The majority of study patients met with a psychologist (72%) and believed that the psychologist helped them accept their new life situation.

The study showed that all women who rated their health as poor often experienced negative emotions.

The study formulated a hypothesis that most women found support and understanding in their breast cancer support group and believed that their participation in group meetings improved their quality of life. All study patients found support in the breast cancer support group and believed that the group improved their quality of life.

A study conducted by Jankau et al. [14] showed that mastectomy has a negative effect on the general condition and quality of life in women. The quality of life is the best when it comes to the physical aspects and the worst when it comes to mental wellbeing. Age and physical activity also have an influence on the quality of life. Breast reconstruction surgery and breast prostheses improve the quality of life in women after mastectomy.

According to a study by Szczepańska-Gieracha et al. [19], breast cancer has a negative influence on patients' emotional state, causes an increase in anxiety levels, and contributes to negative emotions.

A study by Zegarski W et al. [20] revealed that the quality of life in breast cancer patients deteriorates during chemotherapy and patients experience sadness, anxiety, fear, and physical symptoms such as nausea, vomiting, and pain. Nevertheless, support from loved ones helps most patients cope with their cancer. Women receive significant help from breast cancer support groups.

CONCLUSIONS

1. Mastectomy affects ipsilateral upper limb function and causes difficulty with activities of daily living, such as cleaning, cooking, brushing hair, bathing, and dressing.
2. All women who rated their health as poor experienced such negative emotions as sadness, low mood, dejection.
3. Breast reconstruction after mastectomy improved patient-rated quality of life.
4. All women who participated in breast cancer support group meetings found support there and the time they spent together resulted in an improved quality of life.

REFERENCES

1. Manghan KL, Lutterbie MA, Ham PS. Treatment of breast cancer. Am Fam Physician. 2010;81(11):1339-46.
2. Merino Bonilla JA, Torres Tabanera M, Ros Mendoza LH. Brest cancer in the 21st century: from early detection to new therapies. Radiologia 2017;59(5):368-379.
3. Anastasiadi Z, Lianos GD, Ignatiadou E et al. Brest cancer in young woman: an overview updates surg. 2017;69(3):313-317.
4. Fahad Ullah M. Brest cancer: Perspectives on the disease status. Adv Exp Med Biol. 2019;1152:51-64.
5. Desantis C, Siegel R, Bandi P, Jemal A. Brest cancer statistic, 2011 CA. Cancer J Clin. 2011;61(6):409-18.
6. Matsen CB, Neumayer LA. Brest cancer: a review for the general surgeon. JAMA Surg. 2013;148(10):971-9.
7. Dauplat J, Kwiatkowski F, Rouanet P et al. Quality of life after mastectomy with or without immediate breast reconstruction. Br J Surg. 2017;1049(9):1197-1206.
8. Browne JP, Jeevan R, Gulliver-Clarke C et al. The association between complications and quality of life after mastectomy and breast reconstruction for breast cancer. Cancer. 2017;123(18):3460-3467.
9. Lederc AF, Foidart-Dessalle M, Tomasella M et al. Multidisciplinary rehabilitation program after breast cancer: benefits on physical function, antropometry and quality of life. Eur J Phys Rehab Med. 2017;53(5):633-642.
10. Aygin D, Cengiz H. Life quality of patients who underwent breast reconstruction after prophylactic mastectomy: systematic review. Brest Cancer. 2018;25(5);497-505.
11. Velikova G, Williams LJ, Willis S et al. Quality of life after postmastectomy radiotherapy in patients with intermedicate-risk breast cancer (SUPREMO); 2-year follow-up results of a randomized controlled trial. Lancet Oncol. 2018;19(11):1516-1529.
12. Salibasic M, Delibegovic S. The quality of life and degree of depression of patients suffering from breast cancer. Med Arch. 2018;72(3):202-205.
13. Hansdorfer-Korzon R, Burakowska A. Zmiany w obrębie skóry u pacjentek po mastektomii z powodu raka sutka. Forum Med Rodz. 2010;4(3):174-178.
14. Jankau J, Trus-Urbańska M, Renkielska A. Zmiana jakości życia po zabiegu rekonstrukcji piersi. Forum Med Rodz. 20115(5):414-419.

15. Kalinowski P, Krawulska A. Rola fizjoterapii po mastektomii w opinii pacjentek. Med Ogol Nauk Zdr. 2012;18(4):291-292
16. Kulpa M, Owczarek K, Stypuła-Ciuba B. Przystosowanie psychiczne do choroby nowotworowej a jakość życia uwarunkowana stanem zdrowia u chorych onkologicznych. Med Paliat. 2013;5(3):106-113
17. Mniszewska J, Chodkiewicz J, Zalewska-Jankowska A. Jakość życia w zdrowiu i chorobie – czym jest, jak i po co ją oceniać. Prz Lek. 2012;69:253-258.
18. Pawlik M, Kaczmarek-Borowska B. Akceptacja choroby nowotworowej u kobiet po mastektomii. Prz Med Uniw Rzesz Inst Lekow. 2013;2:204-205.
19. Szczepańska-Gieracha J, Malicka I, Figuła M, Rymaszewska J, Woźniewski M. Wpływ ośmiotygodniowego treningu nordic walking na jakość życia kobiet po mastektomii. Onkol Pol 2010;13:90-95.
20. Zegarski W, Głowacka I, Ostrowska Ż. Ocena jakości życia kobiet po mastektomii na podstawie standardowych kwestionariuszy: QLQ-C30 i QLQ-BR23. Nowotwory. 2010;60(6):532-532.

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ORIGINAL ARTICLE

MACROMICROSCOPIC ARGUMENTATION OF THE PATHOGENETIC SCENARIO OF BABESIOSIS IN THE COORDINATE SYSTEM «PATHOGEN-CARRIER-RESERVOIR»

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ABSTRACT

The aim is to get a thorough argument for the babesiosis pathogenetic scenario in the coordinate system «pathogen (Babesia spp.) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» with the emphasis on the epizootic/epidemic role of the carrier.

Materials and methods: The macromicroscopic method of research was used in order to maximize the clarification of the babesiosis scenario, its pathogenetic links, the connection of the latter with attacks of active stages of ixodes ticks, types of circulation of ontogenetic forms of *Babesia spp.* in the body of carriers and their inoculation of the pathogen into an organism susceptible to it. The use of this method helped to strengthen the diagnostic potential of the study, and increase the reliability of the results obtained. Taking this into consideration it was focused on the epizootological/epidemiological aspects of babesiosis, the role and significance of the most vulnerable epizootic link – Ixodes ticks on the body of the vertebrate provider (mammal), poikilomorphism, anisomorphy.

The study of the monolithic idiosome and ticks salivary glands were carried out on activated (capable of attack) female individuals aged 2-3 months after molting. Ticks were dissected in a cool (t=4ºC) Ringer's saline solution for arachnids. Ticks and prepared salivary glands were fixed in 12% formalin solution on 0.1 M phosphate buffer (pH=7.0-7.2) at t=4ºC for 3 hours, washed with the buffer, and fixed again for 1 hour (t=4ºC). To achieve tonicity, sucrose was added to the fixatives and the washing medium. Dehydration occurred due to a battery of alcohols of increasing concentration and absolute acetone. Microspecimens stained with hematoxylin and eosin were studied using an Olympus BX-41 microscope (Japan).

Results: Implementation of the leading stages of the babesiosis pathogenetic scenario is focused on the coordinate system «pathogen (*Babesia spp.*) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» in which carrier take the leading place.

The macromicroscopic specificity of the structure of the ticks (variability: ability to aniso-, poikilomorphism) is an evidence-based criterion for pathogens inoculation to the macroorganism of warm-blooded vertebrates. It determines the features of circulation and organ/cellular locations of *Babesia spp.* (intestines and its epithelium, hemolymph, gonads, salivary glands).

The species belonging of warm blooded vertebrates susceptible to babesiosis pathogens correlates with the species belonging of ticks and determines the tropicity of the latter. The simultaneous implementation of a complex of research procedures with the tick biological material samples is problematic taking into account the physical lack of material, which requires researchers to re-orient the diagnostic vector towards the use of additional methods for babesiosis diagnosing, including in vitro ones.

Conclusions: In the pathogenetic scenario of babesiosis, the carrier (Ixodes ticks) is the central figure in the epidemic/epizootic coordinate system.

KEY WORDS: babesiosis, pathogenetic scenario, epizootic/epidemic coordinate system, Ixodes ticks

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INTRODUCTION

Ontogenesis of *Babesia spp.* is impossible without the participation of biological vectors – Ixodes ticks [1]. Infection of the latter occurs in the case of ingestion of pathogens with the blood of an animal or a parasite carrier [2].

In the ticks organs there are separate stages of circulation of various ontogenetic forms of *Babesia spp.*, and it, in the end, leads to the entry of the latter into the gonads and salivary glands. Merogonia with the subsequent formation of merozoites, which are invasive to a susceptible vertebrate host, are extremely dangerous phenomena that objectively cause infection of the host with *Babesia spp.* pathogens by inoculation into the blood of merozoites with the tick saliva [3, 4]. However, the pathogenic effect of pathogens begins from the moment the latter enter a susceptible organism. Its skin is the first to face the attack of *Ixodes* and responds with a cascade of structural and functional changes. It was observed not only skin damage, but also affection the lymph nodes, internal organs lymphoid formations, liver, spleen etc. They are evidence-based criteria for inoculating pathogens into the body of susceptible vertebrates [5, 6].

The delayed observation period thoroughly demonstrates the presence of *Babesia spp.* in red blood cells [4, 7]. Intensive destruction of the latter, enhanced by the toxicity of accumulated metabolic products of protozoans, contributes to the launch of mechanisms for reproducing clinical prototypes of babesiosis. Their full comprehension, the introduction of effective therapeutic measures in relation to them, and

Table 1. The number of collected ticks (n=162) the Ixodidea family (in absolute and relative indicators, %) according to the species and groups of providers

Provider	**The number of ticks of the *Ixodidea* family (in absolute and relative indicators)**	
Species of animals	**Species of ticks: *Ixodes ricinus, Ixodes persulcatus* (n=34)**	**Species of ticks: *Dermacentor marginatus, Dermacentor pictus* (n=128)**
Cattle *(Bos taurus taurus)*	34 (20.99 %)	–
Horses (*Equus caballus*)	–	21(12.96 %)
Domestic dogs (*Canis familiaris*)	–	107 (66.05 %)

Table 2. The shape variability (poikilomorphism) of the monolithic idiosome of ticks of the Ixodidea family (n=162), the number of samples with the biological material (in absolute and relative indicators)

The number of ticks of the *Ixodidea* family	**Poikilomorphism of the monolithic idiosome of ticks of the *Ixodidea* family**					
Species of ticks	***Ixodes ricinus, Ixodes persulcatus* (n=34)**			***Dermacentor marginatus, Dermacentor pictus* (n=128)**		
The form type (by number)	1	2	3	1	2	3
Absolute and relative indicators	27 (79.41%)	5 (14.71%)	2 (5.88%)	23 (17.97%)	31 (24.22%)	74 (57.81%)

Table 3. The size variability (anisomorphy) of the monolithic idiosome of ticks of the Ixodidea family (n=162), the number of samples with the biological material (in absolute indicators)

The number of ticks, their taxonomy	**Anisomorphy of the monolithic idiosome of ticks of the *Ixodidea* family**					
Species of ticks	***Ixodes ricinus, Ixodes persulcatus* (n=34)**			***Dermacentor marginatus, Dermacentor pictus* (n=128)**		
The form type (by number)	I	II	III	I	II	III
Absolute indicator, $\times 10^{-3}$m	2-5	3-5	12-15	3-5	6-10	11-16

prevention are impossible without structural and functional grounding of the moments associated with the ontogenesis of babesiosis pathogens exactly in the tick's body [8].

Therefore, in view of all the above, the importance of the macromicroscopic argumentation of the babesiosis pathogenetic process through the coordinate system «pathogen (*Babesia spp.*) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» with the emphasis on the epizootic/epidemic significance of the carrier is an obvious fact.

THE AIM

The aim is to get a thorough argument for the babesiosis pathogenetic scenario in the coordinate system «pathogen (*Babesia* spp.) – carrier (ticks of the *Ixodoidea* superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» with the emphasis on the epizootic/epidemic role of the carrier.

MATERIALS AND METHODS

The macromicroscopic method of research was used in order to maximize the clarification of the babesiosis scenario, its pathogenetic links, the connection of the latter with attacks of active stages of Ixodes ticks, types of circulation of ontogenetic forms of *Babesia* spp. in the body of carriers and their inoculation of the pathogen into an organism susceptible to it. The use of this method helped to strengthen the diagnostic potential of the study, and increase the reliability of the results obtained. Taking this into consideration it was focused on the epizootical/epidemical aspects of babesiosis, the role and significance of the most vulnerable epizootic link – Ixodes ticks (registration of the number (table 1) on the body of the vertebrate provider (mammal), poikilomorphism (table 2), anisomorphy (table 2) caused by the state of blood saturation, location in the organs/cells of the tick body.

At the same time, morphological features of the monolithic idiosome structure, potentially tropical to *Babesia* spp. organs of the ticks' body were studied.

The study of the monolithic idiosome and ticks salivary glands were carried out on activated (capable of attack) female individuals aged 2-3 months after molting. Ticks were dissected in a cool (t=4°C) Ringer's saline solution for arachnids. Ticks and prepared salivary glands were fixed in 12% formalin solution on 0.1 M phosphate buffer (pH=7.0-7.2) at t=4°C for 3 hours, washed with the buffer, and fixed again for 1 hour (t=4°C). To achieve tonicity,

sucrose was added to the fixatives and the washing medium. Dehydration occurred due to a battery of alcohols of increasing concentration and absolute acetone.

Microspecimens stained with hematoxylin and eosin were studied using an Olympus BX-41 microscope (Japan).

RESULTS AND DISCUSSION

The analysis of poikilomorphism and anisomorphy (changes in the body shape and parameters of its size) of these invertebrate arthropods proved the heterogeneity of the latter. Depending on the phase of ontogenesis and blood saturation the body shape varied from flattened in the upper -lower direction to oval, rounded/spherical. The ticks with oval and rounded/spherical shapes had the quantitative advantage, which objectified the meaning of using these parameters as an undoubted macroscopic criterion for acarian danger. The size of the monolithic idiosome of bloodsuckers by groups varied from $(2\text{-}5)\times10^{-3}$m to $(11\text{-}16)\times10^{-3}$m. Mimicry phenomena were moderate. There were no abnormalities in the development of ticks, a clear delay in molting. In bloodsuckers, after successful blood saturation, there was a clear loss of activity followed by anfastenization.

Morphological examination of the tick's body showed that parasites had a complete monolithic idiosome without defects in the chitinous cover (scutum) and the loss of six-membered tarsi (four pairs), distinct sexual dimorphism. Sexual differentiation of individuals was accessible, understandable and focused on somatic parameters, location, and the chitinous cover size. The body size of females exceeded that of males which were protected by a denser (harder, thicker) chitinous cover. In males, the chitinous cover completely covered the body and genitals (located ventrally, behind). In the anterior part of the body of arachnid arthropods, a gnathosoma (proboscis) is visualized, with the basis, two pairs of palps and chelicerae, a hypostome. Proboscis (the ticks mouthparts) was a complex structure of a piercing-sucking type. The tick palps were covered with numerous, well-marked, somewhat misdirected bristles (sensory structures). Chelicerae armed with teeth were located between the palps. In the vast majority of our samples, a hypostome, which due to teeth fixed ticks on the animal/human skin, and clothing, was preserved. On the anterior part of the scutum the visual apparatus (eyes) of ticks was visualized.

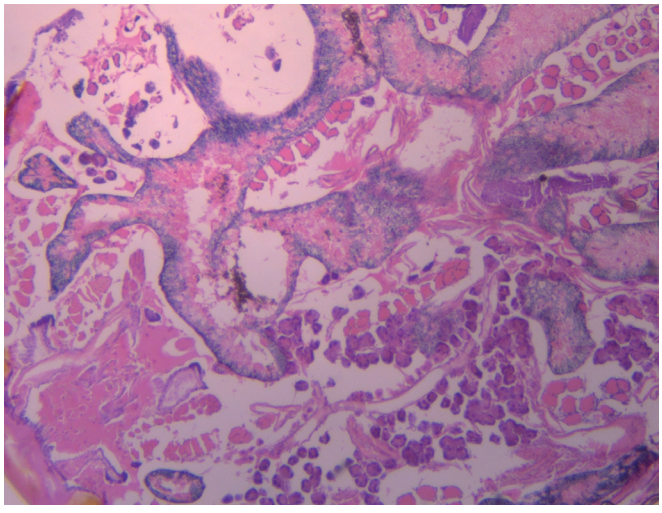

Fig. 1. A histological section of the monolithic idiosome of a sexually mature female tick of the Dermacentor genus of the Dermacentor marginatus species with a fragment of chelicera, racemose salivary glands. Stained with hematoxylin and eosin, × 100.

The ventral surface of the idiosome (less protected) was equipped with four pairs of six-membered tarsi, which last segment ended in a suction pad, two tarsal claws (the functional mobility and fixation of ticks). On the ventral side, at the level of the location of the second pair of cowies, a small genital pore covered with a chitinous plate was visualized. The genitals were in the lower (ventral) part of the tick's body, behind (a tool for internal/spermatoform fertilization of individuals). At the level of the fourth pair of cowies in the antero-posterior direction, the anal groove was located (a landmark of generic differentiation of ticks). The digestive system was developed, and it was a target locus (salivary glands, intestines) for babesiosis pathogens.

In the lateral areas of the monolithic idiosome from its anterior edge (the level of the posterior end of the pharynx) to the stigmas/spiracles, sometimes to the posterior end of the body, racemose paired salivary glands were localized (fig. 1). Each of the glands was formed by several hundred glandular vesicles – alveoli localized on clearly developed excretory ducts and their numerous primary, secondary, tertiary branches. Adult sexually mature mites were armed with two-lobed glands (corresponding to two primary branching of excretory ducts) and a large number of small lobes connecting alveoli associated with the tertiary, quaternary branches. The main excretory ducts were marked, directed to the salivary reservoir of the preoral cavity. The alveoli, in turn, opened directly into the excretory ducts of the salivary glands with their own short, well-contrasted ducts. The structure of the main, primary, secondary, and tertiary ducts was identical in each of the cases analyzed. The presence of cuticular expulsion was considered as a structurally specific aspect of the latter. A prognostically important fact was the detection of contacts (plexuses) between the salivary glands and numerous tracheae of ticks.

The traditional microscopic examination visualized type I alveoli. They were represented by pear-shaped, spherical structures (vesicles) localized on the walls of the main excretory ducts and primary branches. On histologic specimens stained with hematoxylin and eosin, type I alveoli were transparent, pink, pink-lilac in color, without inclusions, and secretory granules. These structures have the osmoregulatory function (secretion of hygroscopic hypertonic saline solution into the preoral cavity; it adsorbs water vapor from the atmosphere and replenish water loss by the tick's body in anabiosis and the absence of a nutrient substrate) [1, 10].

Type II-III alveoli contained heterostructural cells and heterogeneous secretory granules. It was obvious that the functional load of the alveoli, and their secretory activity

Table 4. Localization of ontogenetic forms of Babesia spp. in the organs/cells of the ticks body of the Ixodoidea superfamily of the Ixodidea family

Organs/cells of the tick's body	Ontogenetic forms of Babesia spp.	
	meronts	merozoites
intestines	+	+
intestinal epithelial cells	+	+
tick's body cavity	–	+
hemolymph	–	+
salivary glands	–	+

were determined by the specificity of cells. In view of the lack of basic structural criteria and ambiguity of conceptual approaches it was problematic to homologize these cell types among the ixodid species studied using the resources of available methodological approaches.

The content of secretory vacuoles and granules was observed in the alveolar cavities (saliva). The latter contributed to the infection of vertebrates with babesiosis pathogens under the conditions of inoculation of merozoites into their blood (since the appearance of a characteristic color of the inclusion was noted in the alveolar cells). A colorless hemolymph, intestinal epithelial cells, and the surface of the salivary gland ducts sometimes contained pairs of cells/single forms with a pink-lilac cytoplasm and blue inclusions marginally located. Detection of the later (full implementation of traditional histological procedures) in other organ structures of the ticks was complicated by a number of factors, including the state of blood saturation.

Ontogenetic stages of *Babesia* spp were characterized by certain tropism to the organs and cells of the body ticks (table 4). The trophism consisted in the predominant localization of parasites in the intestine and its epithelial layer, salivary glands. An intermediate stage of pathogen migration was hemolymph, which acted as a transport system for Babesia spp. It should be noted that the cells that played the role of substrate for the pathogens reproduction were of mesenchymal origin.

Ixodes ticks occupy a leading place in the babesiosis pathogenesis. Blood supply for them is a significant physiological moment [9, 10]. In the case of blood sucking by a tick (the *Ixodoidea* superfamily of the *Ixodidea* family) merozoites of the pathogen are inoculated into the blood, causing the fact of infection [3, 8]. Tick organs/cells (including a well-developed digestive system) are the known locations of individual stages of circulation of various ontogenetic forms of *Babesia* spp. In the intestines of arthropods, meronts are soon formed, in which merozoites are formed. The breakdown of meronts initiates the penetration of merozoites into the intestinal epithelium where the process repeats. Since then, merozoite inoculation is targeted at the host's body cavity and hemolymph. In the hemolymph the introduction of pathogens into the gonads and salivary glands takes place. The ontogenetic cycle of merozoites associated with the concentration in the salivary glands repeats. Transovarian transmission of the pathogen (merozoites) consistently accompanies the ongenetic stages of the tick's development [7-10].

In the wild, especially in pastures, animals and people can be attacked by ticks in all ontogenetic stages (larvae, nymphs, sexually mature individuals) [6]. Technically, they are armed with everything they need to do this. Three-four pairs of tarsi (larvae – nymphs – adult ticks) with a suction pad, two tarsal claws allow ticks to move easily and hold firmly on any surface. Compared to other representatives of the Ixodidea family the complex and prolonged mouthparts – gnathosome provides them with intensive unhindered nutrition (e.g., a female tick is able to suck up to 3 mL of blood, then changing somatometrically from 2-7 to 10-15 and even 35-40 mm) [3]. Attachment of the tick occurs painlessly due to a specific anesthetic secret that completely fills the resulting defect and localizes the pain from the bite. The effect is enhanced by simultaneous penetration of secretions with an anticoagulant and toxic substances into the wound [5]. The host's defense reaction (immunological reactivity manifestation) is triggered precisely at the moment when the tick wounds the skin and inserts the proboscis (oral apparatus) into the epidermal layer. The occurrence of local inflammatory and allergic phenomena is natural in this case.

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CONCLUSIONS

1. Implementation of the leading stages of the babesiosis pathogenetic scenario is focused on the coordinate system «pathogen (*Babesia* spp.) – carrier (ticks of the

Ixodoidea superfamily of the *Ixodidea* family) – reservoir (a susceptible organism)» in which carrier take the leading place.
2. The macromicroscopic specificity of the structure of the ticks (variability: ability to aniso-, poikilomorphism) is an evidence-based criterion for pathogens inoculation to the macroorganism of warm-blooded vertebrates. It determines the features of circulation and organ/cellular locations of *Babesia* spp. (intestines and its epithelium, hemolymph, gonads, salivary glands).
3. The species belonging of warm blooded vertebrates susceptible to babesiosis pathogens correlates with the species belonging of ticks and determines the tropicity of the latter.
4. The simultaneous implementation of a complex of research procedures with the tick biological material samples is problematic taking into account the physical lack of material, which requires researchers to re-orient the diagnostic vector towards the use of additional methods for babesiosis diagnosing, including in vitro ones.

REFERENCES

1. Gray JS, Estrada-Peña A, Zintl A. Vectors of Babesiosis. Annual Review of Entomology. 2019;64(1):149-165.
2. Parija SC, Kp D, Venugopal H. Diagnosis and management of human babesiosis. Tropical Parasitology.2015;5(2):88-93.
3. Gray JS. Identity of the causal agents of human babesiosis in Europe. International Journal of Medical Microbiology. 2006;296(40):131-136.
4. Chen Z, Li H, Gao X, Bian A, Yan H, Kong D, Liu X. Human babesiosis in China: a systematic review. Parasitology Research. 2019;118:1103-1112.
5. Zelya OP, Kukina IV. Babezioz cheloveka. Human babesiosis. Medical News of North Caucasus. 2020;15(3):449-455. (Ru).
6. Welc-Falęciak R, Bajer A, Paziewska-Harris A, Baumann-Popczyk A, Siński E. Diversity of Babesia in Ixodes ricinus ticks in Poland. Advances in Medical Sciences. 2012;2. doi: 10.2478/v10039-012-0023-9
7. Ozubek S, Bastos RG, Alzan HF, Inci A, Aktas M, Suarez CE. Bovine Babesiosis in Turkey: impact, current gaps, and opportunities for intervention. Pathogens. 2020;9:1041. doi:10.3390/pathogens9121041
8. Scott JD, Pascoe EL, Sajid MS, Foley JE. Detection of Babesia odocoilei in Ixodes scapularis ticks collected from songbirds in Ontario and Quebec, Canada. Pathogens.2020;9:781. doi:10.3390/pathogens9100781
9. Akimov IA, Nebogatkin IV. Ixodid ticks (Acari, Ixodidae) in urban landscapes: a review. Vestnik zoologii. 2016;50(2):155-162.
10. Negi T, Arunachalam K. Study on prevalence of ixodid tick infestation on bovines of Dehradun district, Uttarakhand. Biological Rhythm Research. 2020;51(8):1288-1297.

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ORIGINAL ARTICLE

CLINICAL AND MORPHOLOGICAL CORRELATION DEPENDENCIES AND THEIR SIGNIFICANCE IN PATIENTS WITH COMPLICATIONS OF LABORED ERUPTION OF THE LOWER THIRD MOLARS

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ABSTRACT

The aim is to determine the clinical and morphological dependencies, which are important for diagnostics, treatment and prediction of outcomes of pathological processes in the region of the LTM with complicated eruption, as based on the study of histopathological changes of paradental tissue (mucous membrane, walls of retromolar pocket, alveolar bone tissue).

Materials and methods: The materials of the study were 34 biopsy specimens of pathologically altered soft tissue and parodontium obtained as a result of pericoronectomy, extraction of the LTM and other surgical interferences performed based on the relevant indications in 28 patients in the region of the LTM with complicated eruption. Morphological and statistical research methods were used.

Results: The local pathological processes, which chronologically precede the destructive changes in the hard tissue of a tooth (caries), are developed in patients of both genders with complicated LTM eruption in soft tissue of parodontium and the adjacent bone tissue of the alveolar wall in the majority of cases. As per biopsy examinations, the frequency of the main pathological processes in paradental tissue in case of complicated LTM eruption varies from 25 to 60 % of the number of biopsy specimens and occurs in various combinations in patients with different values of clinical parameters. The correlation relationships between the patients' clinical data and the morphological parameters of damage to paradental tissue are weak, multidirectional and uncertain in the majority of combinations (considering the available number of biopsy specimens studied). The close certain positive dependence between the damage of the squamous epithelium and the inflammation activity in the lamina propria mucosae, covering the tooth: in the vast majority of cases, the presence of damaged epithelium (within the biopsy specimen) is associated with the inflammation of high activity, was established as based on correlation relationships between the morphological parameters of damage to paradental tissue.

Conclusions: The found pathological changes and the correlations justify surgical tactics on paradental soft and osseous tissues that are directed on the LTM sparing.

KEY WORDS: pathomorphological features, clinical and morphological correlation dependencies, labored eruption, lower third molars

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INTRODUCTION

The labored eruption of the LTM is one of the disturbances of the dentomaxillary system development, which is relatively frequently complicated by different pathological processes in the soft tissue and the parodontium of the third molar triggering the necessity of surgical interference [1-3].

There is no consensus in the related reference sources on the issue of the indications for the extraction of the intact LTM in case of complications of their eruption. A range of authors, who identified more frequent development of pathological changes in paradental tissue in the region of the LTM, support an idea of the relevance of «preventive» extraction of the intact LTM [4-9]. Other authors highlight the relatively low frequency of complications in the «restricted» LTM, which evidences in favor of an expectant treatment [10-13]. There are few research studies published on histological peculiarities of the separate pericoronal complications in the region of the LTM, and the correlation dependencies between the clinical data and histological peculiarities of the damage [14, 15].

THE AIM

The aim is to determine the clinical and morphological dependencies, which are important for diagnostics, treatment and prediction of outcomes of pathological processes in the region of the LTM with complicated eruption, as based on the study of histopathological changes of paradental tissue (mucous membrane, walls of retromolar pocket, alveolar bone tissue).

MATERIALS AND METHODS

The materials of the study were 34 biopsy specimens of pathologically altered soft tissue and parodontium obtained as a result of pericoronectomy, extraction of the

Table 1. The frequency of biopsy specimens distribution of various qualitative/semi-quantitative gradation of clinical parameters in patients with complicated eruption of the LTM

Major clinical signs of the patients who underwent biopsy of paradental tissue	Description of the clinical parameters gradation or alternative conditions	Number of biopsy specimens assigned to each gradation or alternative from the total number of informative biopsy specimens	Frequency of incidence of the specific gradation of clinical parameters, % of the number of informative biopsy specimens
Patient's age at the moment of biopsy performance	Up to and including 25 years of age	19	55.9
	Over 25 years of age	15	44.1
	Total number of biopsy cases considered	34	100.0
LTM condition	Intact	31	91.2
	Moderate or deep caries	3	8.8
	Total number of biopsy cases considered	34	100.0
Degree of the top of LTM crown overlapping by the mucous membrane	Less than half of the surface	14	41.2
	The half of the surface and more	20	58.8
	Total number of biopsy cases considered	34	100.0
Extent of antagonist tooth eruption	Absence of antagonist tooth	6	17.6
	Retention and semi-retention	11	32.4
	Complete tooth eruption	17	50.0
	Total number of biopsy cases considered	34	100.0
LTM position in a jaw	Vertical	24	70.6
	All others (medial oblique, dorsal oblique, buccal, glossal, horizontal)	10	29.4
	Total number of biopsy cases considered	34	100.0
The age of the onset of signs of the LTM complicated eruption	Less than 12 months	18	52.9
	12 months and over	16	47.1
	Total number of biopsy cases considered	34	100.0
Pericoronitis activity as of the moment of biopsy (as per clinical findings)	Chronic in the stage of remission	17	50.0
	Acute and chronic in the stage of exacerbation	17	50.0
	Total number of biopsy cases considered	34	100.0

LTM and other surgical interferences performed based on the relevant indications in 28 patients in the region of the LTM with complicated eruption. 8 biopsy specimens were obtained of patients 16-20 years old, 10 ones – of patients 21-25 years old, 11 ones – of patients 26-30 years old, 5 ones – of patients 31 years of age and older. The objects of the limited resection were as follows: soft tissue fold of the mucous membrane of the oral cavity (flap) covering the LTM with complicated eruption – 32 objects, the retromolar pocket wall – 8 objects, the periosteum and small (not more than 1×2 mm) bone fragments of the alveolar process margin – 13 objects.

The resected tissue was fixed in 10% formalin solution, processed into histological blocks, decalcinated in a 5% nitric acid solution if needed, embedded into celloidin after the relevant histological preparation. The sections, 10 μm in thickness, were obtained and stained with hematoxylin and eosin, hematoxylin-picrofuchsin by van Gieson technique.

As based on the results of the clinical, X-ray and histological examinations, the qualitative/semi-quantitative gradations of clinical and morphological parameters were determined (table 1, 2). The correlation analysis has been performed after gradation quantification of all biopsy specimens and frequency analysis of the received material, namely, the statistical contingency between the clinical and morphological parameters (clinical issues – morphology) and statistical contingency of separate morphological parameters between themselves (morphology – morphology), with calculation of tetrachoric correlation coefficient, its value and degree of certainty were determined (table 3).

RESULTS

The clinical characteristics, their gradation and frequency distribution of cases are provided in table 1.

The pathological changes of tissue, surrounding the LTM with complicated eruption, of various extensiveness and severity were found in all biopsy specimens (table 2). The examined resectates of the mucosa, covering the LTM (fig. 1), included the altered epithelium, the lamina propria mucosae, the muscle tissue, the end sections and ducts of minor

Table 2. The frequency of incidence of various qualitative/semi-quantitative gradation of morphological parameters in biopsy specimens of the patients with complicated eruption of the LTM

Major morphological parameters of the biopsy tissue condition	Description of the pathological changes gradation or alternative conditions	Number of biopsy specimens assigned to each gradation or alternative from the total number of informative biopsy specimens	Frequency of incidence of the specific gradation or pathological changes, % of the number of informative biopsy specimens
Damage to epithelium of the mucosa and/or retromolar pocket wall *	The epithelium is preserved within the extent of the biopsy specimen	15	48.4
	The necrosis and/or ulcers are found in the epithelium within the extent of the biopsy specimen	16	51.6
	Total number of biopsy specimens considered	31	100.0
Condition of the mucosal epithelium and/or retromolar pocket *	Multi-layered non-keratinized squamous epithelium	19	61.3
	Multi-layered squamous, with areas of keratinization manifested by orthokeratosis or parakeratosis	12	38.7
	Total number of biopsy specimens considered	31	100.0
Hyperplasia of basal epithelial layers *	Absent	8	26.7
	Present	22	73.3
	Total number of biopsy specimens considered	30	100.0
Discirculatory changes in the lamina propria mucosae and submucosa (microhemorrhage, fibronecrosis) *	Absent	16	51.6
	Present	15	48.4
	Total number of biopsy specimens considered	31	100.0
Activity of inflammation in the lamina propria mucosae and submucosa *	Completely absent inflammation or proliferative inflammation of low activity	15	48.4
	Proliferative infiltrative inflammation of high activity, including exudative, focal purulent or diffuse	16	51.6
	Total number of biopsy specimens considered	31	100.0
Pathological changes of biopsy specimen bone tissue **	Osteonecrosis (small focal interstitial, extended)	8	61.6
	Periostitis with periosteal regeneration	3	23.1
	Bone tissue remodeling	2	15.4
	Osteomyelitis, various forms	2	15.4
	Total number of biopsy specimens with osseous elements considered	13	– **

Notes: * – all informative biopsy specimens on alternative basis were considered; ** – the pathological changes of bone tissue were considered as supplementary, not alternative.

salivary glands. The deepithelialized regions of the mucosa, represented by ulcers, were found in squamous epithelium in many cases (fig. 2). The stringy anastomosing growths of the epithelium, penetrating the lamina propria mucosae, was found near those regions in the submucosa (fig. 3).

There was marked fibrosis of the lamina propria mucosae in almost all cases; the small foci of old hemorrhagic infiltration, microhematomas (fig. 2, 4), assembly of siderocytes and extracellular brown pigment were found in the fibrous tissue at various depth (1-3 mm from the epithelial surface), which can be collectively regarded as outcome of multiple traumas. In some cases, the areas typical for limited purulent inflammation (fig. 5) were observed in the lamina propria mucosae, it has been occasionally accompanied by the local

Table 3. The results of correlation analysis of the relationships between the clinical and morphological parameters of tissue damage in the area of the lower third molar with signs of complicated eruption (gradation of parameters – see Tables 1, 2)

First parameter*	Second parameter*	n, number of considered biopsy cases	Pearson's tetrachoric correlation coefficient (coefficient of association) and its confidence interval		
			r_a	t_ϕ	Confidence estimation r_a at k=n-1
Clinical issues – morphology					
Patient's age	Damage of epithelium	31	+0.163	0.92	UC
Patient's age	Condition of epithelium	31	+0.026	0.14	UC
Degree of the LTM crown overlapping by the mucosa	Damage of epithelium	31	+0.159	0.90	UC
Degree of the LTM crown overlapping by the mucosa	Condition of epithelium	31	+0.189	1.07	UC
Degree of the LTM crown overlapping by the mucosa	Hyperplasia of epithelium	31	–0.009	0.05	UC
Degree of the LTM crown overlapping by the mucosa	Dyscirculatory changes in the mucosa	31	–0.163	0.92	UC
Degree of the LTM crown overlapping by the mucosa	Activity of inflammation in the mucosa and submucosa	31	+0.029	0.16	UC
Extent of antagonist tooth eruption	Damage of epithelium	26**	–0.025	0.13	UC
Extent of antagonist tooth eruption	Condition of epithelium	26**	–0.090	0.46	UC
Extent of antagonist tooth eruption	Hyperplasia of epithelium	26**	–0.135	0.69	UC
Extent of antagonist tooth eruption	Dyscirculatory changes in the mucosa	26**	+0.061	0.31	UC
Extent of antagonist tooth eruption	Activity of inflammation in the mucosa and submucosa	26**	–0.028	0.15	UC
LTM position	Damage of epithelium	31	–0.335	1.98	<0.05
LTM position	Condition of epithelium	31	–0.075	0.42	UC
LTM position	Hyperplasia of epithelium	31	+0.061	0.34	UC
LTM position	Dyscirculatory changes in the mucosa	31	+0.051	0.28	UC
LTM position	Activity of inflammation in the mucosa and submucosa	31	–0.051	0.28	UC
The duration of the onset of signs of complicated eruption	Damage of epithelium	31	+0.100	0.56	UC
The duration of the onset of signs of complicated eruption	Condition of epithelium	31	–0.056	0.31	UC
The duration of the onset of signs of complicated eruption	Hyperplasia of epithelium	31	+0.009	0.05	UC
The duration of the onset of signs of complicated eruption	Dyscirculatory changes in the mucosa	31	–0.033	0.19	UC
The duration of the onset of signs of complicated eruption	Activity of inflammation in the mucosa and submucosa	31	+0.033	0.19	UC
Pericoronitis activity as per clinical findings	Damage of epithelium	31	+0.096	0.54	UC
Pericoronitis activity as per clinical findings	Hyperplasia of epithelium	31	+0.092	0.51	UC
Pericoronitis activity as per clinical findings	Dyscirculatory changes in the mucosa	31	+0.033	0.19	UC
Pericoronitis activity as per clinical findings	Activity of inflammation in the mucosa and submucosa	31	+0.225	1.29	UC
Morphology – morphology					
Damage of epithelium	Condition of epithelium	31	+0.239	1.37	UC
Damage of epithelium	Hyperplasia of epithelium	31	–0.050	0.28	UC
Damage of epithelium	Dyscirculatory changes in the mucosa	29	+0.105	0.57	UC
Damage of epithelium	Activity of inflammation in the mucosa and submucosa	30	+0.732	5.89	<0.001
Hyperplasia of epithelium	Dyscirculatory changes in the mucosa	30	–0.040	0.22	UC
Hyperplasia of epithelium	Activity of inflammation in the mucosa and submucosa	30	+0.191	1.07	UC
Dyscirculatory changes in the mucosa	Activity of inflammation in the mucosa and submucosa	31	–0.096	0.54	UC

Notes: * – full names and gradation of parameters – see Tables 1, 2; ** – cases with absent antagonist tooth were not taken into account during analysis; r_a – value of tetrachoric correlation coefficient; t_ϕ – measured value of the Student's criterion when evaluating the certainty of the relationship parameter r_a; p – error probability when determining the relationship parameter; UC – the differences are uncertain considering the available number of follow-ups.

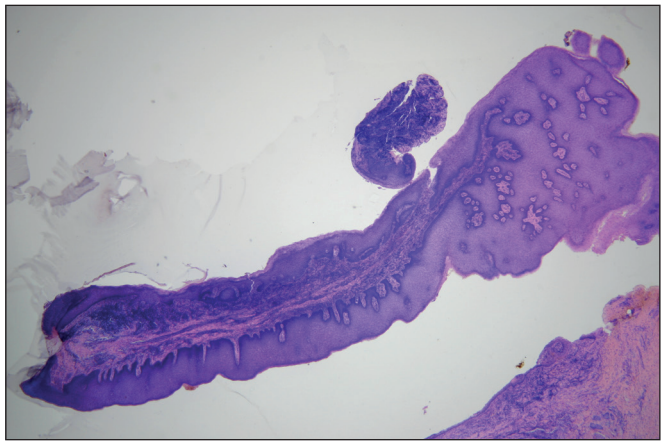

Fig. 1. The mucous membrane of the oral cavity («flap») covering the surface of the LTM with complicated eruption. Photo of a histological specimen of a 24 year old patient. Hematoxylin-eosin staining, × 15.

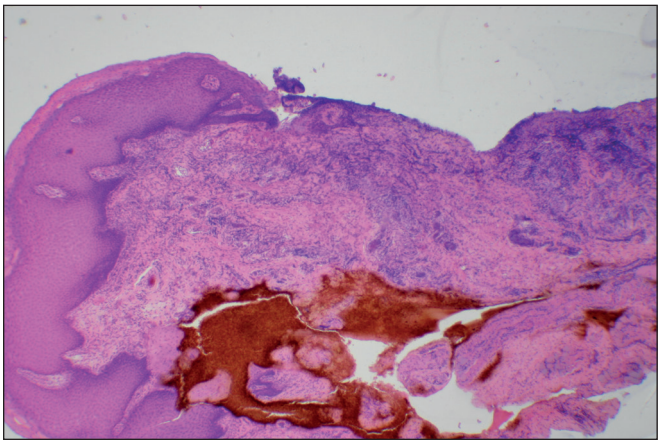

Fig. 2. The area of ulceration in the mucosa, covering the LTM. Photo of a histological specimen of a 27 year old patient. Hematoxylin-eosin staining, × 30.

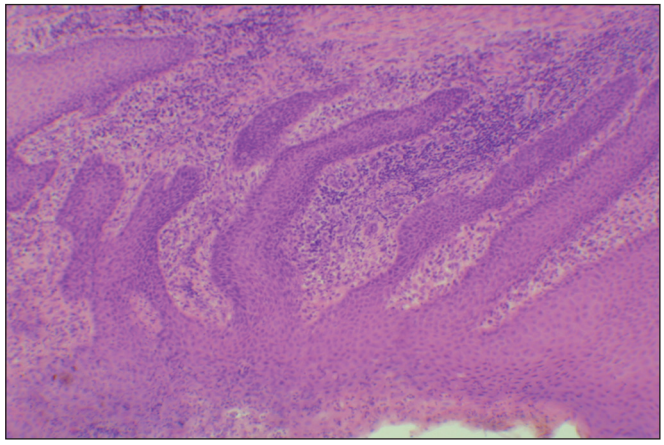

Fig. 3. Marked hyperplasia of multi-layered squamous epithelium in the mucosa, covering the LTM. Photo of a histological specimen of a 36 year old patient. Hematoxylin-eosin staining, × 50.

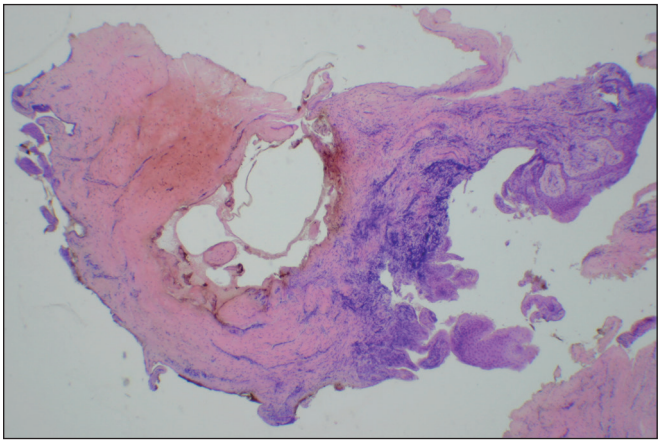

Fig. 4. Hematoma, epithelium defect and chronic proliferative inflammation in the mucosa covering the LTM. Photo of a histological specimen of a 26 year old patient. Hematoxylin-eosin staining, × 25.

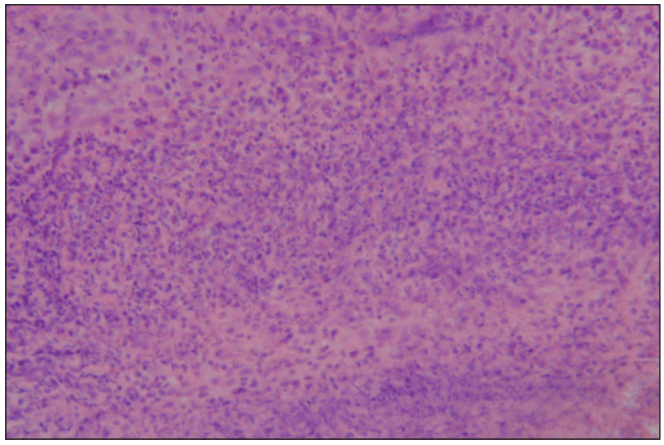

Fig. 5. Diffuse purulent inflammation of the lamina propria mucosae, covering the LTM. Photo of a histological specimen of a 36 year old patient. Hematoxylin-eosin staining, × 100.

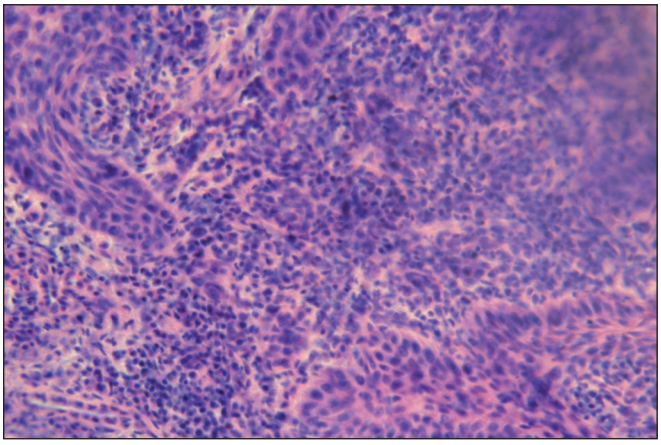

Fig. 6. Non-specific proliferative inflammation of high activity in the lamina propria mucosae, covering the LTM. Photo of a histological specimen of a 35 year old patient. Hematoxylin-eosin staining, × 160.

tissue destruction which presented the histological pattern of microabscesses. In many cases, the nonspecific inflammatory process did not include marked exudative component and corresponded to proliferative infiltrative inflammation of various activity. The lamina propria mucosae, predominantly papillary layer, was infiltrated by mononuclears, macrophages, plasmacytes with admixture of foreign body multinucleated giant cells and abundance of degenerative cells (fig. 6).

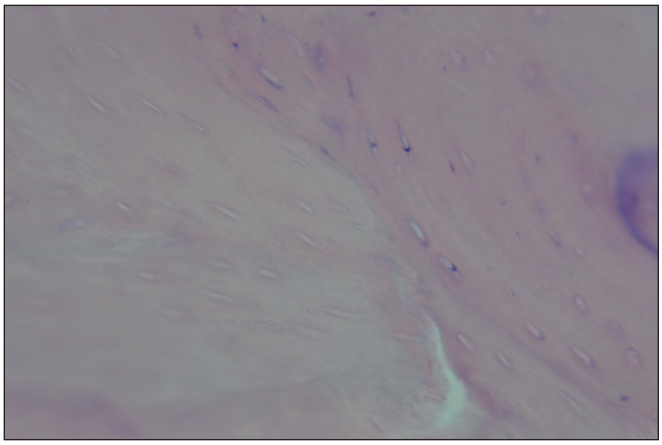

Fig. 7. Interstitial osteonecrosis in the bone tissue of the LTM alveolar margin. Photo of a histological specimen of a 33 year old patient. Hematoxylin-eosin staining, × 100.

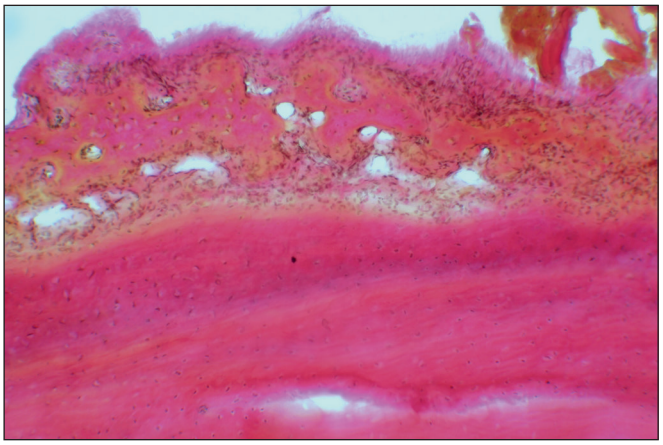

Fig. 8. Compacting periosteal regenerate on the surface of the bone tissue alveolar margin of the LTM. Photo of a histological specimen of a 27 year old patient. Staining with hematoxylin-picrofuchsin by van Gieson technique, × 50.

In case of bone tissue elements presence in biopsy specimen, the interstitial osteonecroses, determined by absence of osteocyte nuclei in groups of osseous lacunae, were most frequently registered (fig. 7). Occasionally, the areas of osteonecrosis constituted the major part of bone fragment. The signs of persistent remodeling, compacting periosteal regenerates were occasionally registered (fig. 8). The small foci of nonspecific inflammation of low activity, corresponding to histological pattern of nonspecific periostitis, were found in biopsy specimens in single cases [16].

The pathomorphological characteristics, their gradation and frequency distribution of cases are presented in table 2.

The correlation analysis findings of the relationship between clinical and morphological gradation parameters (table 3) evidence that only two of all studied pairs were characterized by absolute parameters of tetrachoric index higher than 0.2: «LTM position» – «damage of epithelium» and «pericoronitis activity as per clinical findings» – «activity of inflammation in the mucosa and submucosa». The meaning of the correlation relationship between the first pair of the parameters is that the vertical position of the LTM is more frequently associated with the lesser degree of mucosal epithelium damage (the parameter of tetrachoric index is certain); the meaning of the second pair is as follows – the high activity of pericoronitis as per clinical findings is more frequently associated with the high activity of inflammation in the mucosa and submucosa, determined during histological examination (the parameter of tetrachoric index is uncertain, considering the available number of follow-ups). However, the absolute values of tetrachoric index of both mentioned pairs correspond to weak relationship. The relationships in all other studied pairs «clinical signs – morphology» are even more weak and uncertain considering the available number of follow-ups.

The dependence between the «damage of the mucosal epithelium» and the «activity of inflammation in the mucosa» among the studied correlation pairs of the parameters «morphology – morphology» is strong positive, highly certain, which evidences the contingency of the damaged epithelium and the high activity of inflammation in the majority of informative cases of biopsy examinations of the patients with the LTM complicated eruption.

DISCUSSION

As compared to the results of biopsy examinations of the «restricted» LTM presented in the references, our studies are less extensive and do not include the tissue elements of the tooth follicle, since, the teeth were not extracted in most cases and the economic resection of paradental tissue was performed. Such an approach did not always ensure the adequate representativity of biopsy specimen in relation to major pathological changes, which finally could influence the results of the correlation analysis in groups of «Clinical issues – morphology» and «Morphology – morphology» pairs.

The main cohorts of age groups of our patients, who underwent pericoronectomy, were subjects of both genders aged 20-30 which correspond to the cohorts of age groups of operated patients in the majority of specialized studies [4, 17]. As opposed to some works, which determined the greater frequency of complications, related to the delayed eruption of the LTM in elder age groups [4, 5], our study did not confirm the strong correlation of age (groups of patients aged up to and included 25 and groups of patients aged over 25) and major pathological changes: damage of epithelium, discirculatory changes, activity of inflammation.

The range of pathological changes in paradental tissue, described in the related references, and their frequency are fairly well known, and their frequency is broadly variable [1, 2, 17-19]. Thus, the most frequent pathological processes in patients with «restricted» LTM were determined as follows: caries, paradental follicular cysts of a jaw; inflammatory processes in pericoronal tissue [9, 11, 17, 20, 21]. The incidence of the follicular cysts in the proximity of the LTM varies from 2.1% [1] to 51% [9]; the incidence of pericoronitis – from 4.8% [9] to 66.5% [1]. Some authors almost identify the retromolar pocket over 1 mm in width with epithelized walls and follicular cyst [18, 21].

In our study, there were no specific changes found in paradental soft tissue which would allow to differentiate the follicular cyst and the pathological retromolar pocket with epithelization in those cases where no X-ray signs of cyst were present: hyperplasia of basal layers and areas of keratinization of squamous epithelium, fibrosis of the lamina propria, inflammatory infiltration of various types and density – can be observed in the retromolar pocket wall as well as in the mucosal fold, partially covering the LTM crown.

A range of researchers report on relatively high incidence of carious changes in the «restricted» LTM, which reaches 31% [13, 18]. However, only about 9% of all biopsy specimens of paradental tissue in our material were obtained of the patients with the determined LTM carious changes of various extent: moderate or deep caries.

In some earlier published works the authors attempted to define the dependence between the specific pathological changes in the LTM and paradental tissue and the position of the tooth in the lower jaw. Thus, it has been determined that the most marked difference in the nature of pathological changes in paradental tissue is observed with the distal oblique tooth position. The incidence of the damaged paradental tissue in the area of the «restricted» LTM in all positions in a dental arch was higher than with the absence of the complicated eruption [9].

Some researchers determined the certain correlation relationship between the parameter characterizing the LTM position and such complications as formation of cysts and abscesses in a jaw, jaw angle fracture [19]. Our findings on studying the correlation relationships between the LTM position in a dental arch and damaged epithelium of the mucosa showed only weak negative, but certain dependence, other pairs of parameters «LTM position in a jaw» and morphological parameters of paradental tissue damage showed values corresponding to very weak relationship (table 3).

The correlation analysis of the relationships of gradation morphological parameters of paradental soft tissue damage showed strong, certain, positive dependence between the damaged epithelium and the activity of inflammation in the soft tissue (mucosal fold, wall of retromolar periodontal pocket). However, the analysis of frequency distribution of cases of damaged epithelium and inflammation of high activity in groups with various age of clinical signs showed no significant differences.

The fact of the significant predominance of the number of weak dependencies in the results of the correlation analysis of the relationships of gradation clinical and morphological parameters we explain by multivariance and multidirectionality of influence on the certain parameters of morphological changes, the impact of which was difficult or impossible to consider when analyzing data.

The discirculatory and inflammatory processes, collectively known as «pericoronitis», play the utmost and probably the major role in the pathology of complications of the delayed LTM eruption, which is the subject of works of almost all researchers [1, 2, 15, 18, 22]. However, the assessment of the frequency, types and activity of nonspecific inflammatory process in paradental tissue is widely varied, even in the works where the histological method was applied. Some authors do not pay great attention to inflammatory processes among the most frequent pathological changes of the tissue in the region of the LTM [5, 20, 21], others consider the inflammatory processes – acute and chronic – as the indications for therapeutic and surgical treatment of those complications, including the LTM extraction [9, 19].

However, the histological peculiarities of the inflammatory processes in paradental tissue are not presented in the mentioned works, due to which it is impossible to make a fair judgment of inflammation activity, its correlation with clinical parameters of complications of the LTM eruption in many cases. In our material of histological examinations of biopsy specimens of the paradental tissue, the inflammatory processes were found in the majority of biopsy specimens, moreover, the nonspecific inflammatory process of high activity was histologically confirmed in half of the cases: proliferative infiltrative, of high activity, or exudative, more often fibrinous or purulent. In separate follow-ups, where the biopsy material included fragments of bone tissue of the alveolar wall, the small areas of nonspecific proliferative inflammation of low activity were observed, this can be considered as a manifestation of small focal fibrotic osteomyelitis of low activity.

We did not encounter the descriptions and pathogenetic evaluation of local discirculatory changes in soft and bone tissue in the works related to the study of histological changes of paradental tissue with complications of the LTM eruption. The careful consideration of the characteristic signs of the pathology: the areas of ischemic fibronecrosis and osteonecrosis, microhematomas in the lamina propria mucosae and submucosa, showed that the similar changes are found approximately in the half of all biopsy specimens, however, no strong correlation of the pathology with any clinical or morphological parameters was established in our study. The relatively high frequency of incidence of the discirculatory changes in paradental tissue is most probably caused by frequent traumas of the mucosa, covering the LTM crown.

The main point of the issue of labored LTM eruption and its complications is which extent of pericoronitis in case of absence of the damage to the hard tissue of a tooth justifies the so called «preventive» LTM extraction. Many authors consider that the extraction of the LTM is reasonable in case of the developed pericoronitis, particularly in combination with deep and complicated caries, apical periodontitis, osteomyelitis, paradental cysts or abscesses [10, 17, 18, 22]. However, a range of researchers, including those who conducted histological examination of the resected tissue, insist on the «preventive» extraction of the intact LTM relying only on the probability of the complications development, which indeed are more frequently observed in this region rather than in other places of a dental arch [6-8].

The known published works with conducted metanalysis of the controlled comparative studies evidence of the

absence of current reliable data confirming the positive outcome and providing convincing arguments of the necessity of preventive extraction of the intact LTM [23, 24].

Our views on the issue are based on the results of clinical and morphological examinations presented in the paper. In our opinion, the probability of the possible pathological changes in the hard tissue of a tooth in the long-term is not a fundamental argument in favor of its extraction, but represents the complex of indications for the relevant therapeutic and surgical treatment.

CONCLUSIONS

1. The local pathological processes, which chronologically precede the destructive changes in the hard tissue of a tooth (caries), are developed in patients of both genders with complicated LTM eruption in soft tissue of parodontium and the adjacent bone tissue of the alveolar wall in the majority of cases.
2. As per biopsy examinations, the frequency of the main pathological processes in paradental tissue in case of complicated LTM eruption varies from 25 to 60 % of the number of biopsy specimens and occurs in various combinations in patients with different values of clinical parameters.
3. The correlation relationships between the patients' clinical data and the morphological parameters of damage to paradental tissue are weak, multidirectional and uncertain in the majority of combinations (considering the available number of biopsy specimens studied).
4. The close certain positive dependence between the damage of the squamous epithelium and the inflammation activity in the lamina propria mucosae, covering the tooth: in the vast majority of cases, the presence of damaged epithelium (within the biopsy specimen) is associated with the inflammation of high activity, was established as based on correlation relationships between the morphological parameters of damage to paradental tissue.

REFERENCES

1. Magid EA, Sheinberg VM, Zhitnitsky GD. Zatrudnennoe prorezyvanie nizhnih zubov mudrosti i svjazannye s nim oslozhnenija. Difficulty eruption of lower wisdom teeth and associated complications. Volgograd, 1970. 122 p. (Ru).
2. Adelsperger J, Campbell JH, Coates DB, Summerlin D-J, Tomich ChE. Early soft tissue pathosis associated with impacted third molars without pericoronal radiolucency. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology. 2000;89(4):402-406.
3. Volovar O, Topchii D, Logvynenko I, Dobryi-Vechir T, Oblap M. Internal changes of TMJ at young people with different somatotypes. International Journal of Pharmaceutical Research. 2020;1:1465-1469.
4. Chiapasco M, Crescentini M, Romanoni G. Germectomy or delayed removal of mandibular impacted third molars: the relationship between age and incidence of complications. Journal of Oral and Maxillofacial Surgery. 1995;53(4):418-422.
5. Curran AE, Damm DD, Drummond JF. Pathologically significant pericoronal lesions in adults: histopathologic evaluation. Journal of Oral and Maxillofacial Surgery. 2002;60(6):613-617.
6. Gröndahl H-G, Lekholm U. Influence of mandibular third molars on related supporting tissues. International Journal of Oral and Maxillofacial Surgery. 1973;2(4):137-142.
7. Hinds EC, Frey KF. Hazards of retained third molars in older persons: report of 15 cases. The Journal of the American Dental Association. 1980;101(2):246-250.
8. Marciani RD. Third molar removal: an overview of indications, smaging, evaluations, and assessment of risk. Oral and Maxillofacial Surgery Clinics of North America. 2007;19(1):1-13.
9. Rakprasitkul S. Pathologic changes in the pericoronal tissues of unerupted third molars. Quintessence International. 2001;32(8):633-638.
10. Adeyemo WL. Do pathologies associated with impacted lower third molars justify prophylactic removal? A critical review of the literature. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology. 2006;102(4):448-452.
11. Eliasson S., Heimdahl A., Nordenram A. Pathological changes related to long-term impaction of third molars/ a radiographic study. International Journal of Oral and Maxillofacial Surgery. 1989;18(4):210-212.
12. Hicks EP. Third molar management: a case against routine removal in adolescent and yong adult orthodontic patients. Journal of Oral and Maxillofacial Surgery. 1999;57(7):831-836.
13. Van der Linden W, Cleaton-Jones P, Lownie M. Diseases and lesions associated with third molars: review of 1001 cases. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology. 1995;79(2):142-145.
14. Almendros-Marqués N, Alajejos-Algarra E, Quinteros-Borgarello M, Berini-Aytés L, Gay-Escoda C. Factors influencing the prophylactic removal of asymptomatic impacted lower third molars. International Journal of Oral and Maxillofacial Surgery. 2008;37(1):29-35.
15. Baykul T, Salgam A, Aydin U, Basak K. Incidence of cystic changes in radiographically normal impacted lower third molar follicles. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology. 2007;99(5):542-545.
16. Ruzin GP, Tkachenko OV, Miroshnichenko MS, Pliten ON. Pathomorphological features of chronic toxic osteomyelitis in drug addicts taking amphetamine of a street drugs brand. Likars'ka sprava. 2013;4:87-93.
17. Stanley HR, Alattar M, Collett WK, Stringfellow HRJr, Spiegel EH. Pathological sequelae of "neglected" impacted third molars. Journal of Oral Pathology and Medicine.1988;12(3):113-117.
18. Al-Khateeb T, Bataineh A. Pathology associated with impacted mandibular third molars in a group of iordanians. Journal of Oral and Maxillofacial Surgery. 2007;64(11):1598-1602.
19. Werkmeister R, Fillies T, Joos U, Smolka K. Relationship between lower wisdom tooth position and cyst development, deep abscess formation and mandibular angle fracture. Journal of Cranio-Maxillofacial Surgery. 2005;33(3):164-168.
20. Glosser JW, Campbell JH. Pathologic change in soft tissues associated with radiographically "normal" third molar impactions. British Journal of Oral and Maxillofacial Surgery. 1999;37(4):259-260.
21. Manganaro AM. The likelihood of finding occult histopathology in routine third molar extractions. General Dentistry. 1998;46(2):200-202.
22. Badheri SC, Khan H.A. Extraction versus nonextraction management of third molars. Oral and Maxillofacial Surgery Clinics of North America. 2007;19(1):15-21.
23. Mettes TG, Nienhuijs ME, van den Sanden WJ, Verdonschot EH, Plasschaert AJ. Interventions for treating asymptomatic impacted wisdom teeth in adolescents and adults. Cochrane Database of Systemic Reviews. 2005;18(2):CD003879.

24. Song F, Landes DP, Glenny AM, Sheldon TA. Prophylactic removal of impacted third molars: an assessment of published reviews. British Dental Journal. 1997;182(9):339-346.

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ORIGINAL ARTICLE

EFFICACY AND SENSITIVITY OF PRENATAL AND POSTNATAL ULTRASOUND SCREENING OF CONGENITAL DEVELOPMENTAL ANOMALIES OF KIDNEYS IN SLOVAKIA

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ABSTRACT

The aim: To compare the effectiveness and sensitivity of prenatal and postnatal diagnostics in the diagnosis of congenital malformations of the urinary system in the Slovak Republic.

Materials and methods: Data of postnatal sonographic screening of congenital developmental malformations of the urinary system in Slovak Republic including 2017 were identified and updated using a questionnaire survey, 38,496 newborns were involved. Statistical data on the proportion of prenatal diagnosis for the years 1995, 2000, 2005, 2008 and 2013-2016 were provided from the National Register of Congenital Defects. The chi2-test and t-test were applied to assess the sensitivity differences.

Results: The study showed a low sensitivity of prenatal diagnosis with its maximum in 2016, reaching 32.3% and a minimum in 2005-2008 (8.0 – 8.4%). The sensitivity of postnatal diagnostics for selected years has always been a stable indicator and reaches 99.6%.

Conclusions: Available statistical data confirm that prenatal diagnostics of congenital developmental malformations of the urinary system in the Slovak Republic is not perfect. Our work underlines the importance, or we should rather say inevitability of postnatal ultrasound screening for congenital developmental anomalies in the kidneys.

KEY WORDS: kidney, postnatal diagnosis, prenatal diagnosis, screening, Slovak Republic, sonography

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INTRODUCTION

Congenital developmental anomalies of the urinary tract (CDAUTs) occur in 1-3% of all pregnancies and are diagnosed by prenatal and postnatal diagnostic ultrasound. The purpose of the diagnostic ultrasound is to identify the pathology before the onset of severe complications, such as urinary tract infection, formation of renal stones, renal dysfunction of insufficiency. In many cases, the ultrasound detection of uropoetic system dilation is a transient of physiological phenomenon with no clinical significance. However, in some cases the pathology presents serious and life-threatening conditions, such as posterior urethral valves and other types of obstructive uropathy. Such conditions have a significant morbidity and even mortality rates. In many cases, the etiology of uropoetic system dilation cannot be determined prior to birth and requires postnatal diagnostics using various types of imaging exams. In some developed countries, urinary tract dilations are preferably identified as part of prenatal diagnostics due high level of such diagnostics. In Slovakia, newborn screening takes the form of a well elaborated, country-wide preventive programme. With this programme, all monitored diseases are mostly captured in asymptomatic stage which improves the prognosis and quality of life of affected persons. Postnatal ultrasound kidney screening in newborns in the Slovak Republic is a recommended, but for now not mandatory exam. However, it is performed on a country-wide basis [1,2,3]. Severe congenital developmental abnormalities of the uropoetic system in Slovakia, requiring the care of a nephrologist and urologist and often urologic intervention in infants affect approximately 3.1-3.9/1,000 infants [1,4]. More marked regional differences have been recorded, namely between eastern and western part of Slovakia. While the incidence in the Preshov Region was 5.3/1,000 live-born infants, in the Bratislava Region it was 2.4/1,000 live-born infants which is a 2.2-fold difference [4].

THE AIM

The aim of our effort was to compare the effectiveness and sensitivity of prenatal and postnatal diagnostics in the diagnosis of congenital malformations of the urinary system in the Slovak Republic over the last decades, to underline the importance of postnatal sonographic screening.

MATERIALS AND METHODS

We have compared the sensitivity of prenatal and postnatal ultrasound diagnostics of congenital developmental anomalies of the uropoetic system in selected years in Slovakia. In cooperation with the National Health Information Center

Table I. Comparison of sensitivity of prenatal and postnatal diagnostics of CDAUTs in the kidneys in newborns in 2016 (N = 223).

Successful diagnosis of CDAUTs in the kidneys in the prenatal period in 2016		Screening		Total
		Prenatal	Postnatal	
No	N_P	151	1	152
	N_O	76,0	76,0	152,0
	N_{Pr}	67,7 %	0,4 %	34,1 %
	SR	8,6	-8,6	
Yes	N_P	72	222	294
	N_O	147,0	147,0	294,0
	N_{Pr}	32,3 %	99,6 %	65,9 %
	SR	-6,2	6,2	
Total	N_P	223	223	446
	N_O	223,0	223,0	446,0
	N_{Pr}	100,0 %	100,0 %	100,0 %
$\chi^2(1) = 224,557$				
$p < 0,001$				
Phi = 0,710				

Note: NP – frequency observed, NO – frequency expected, NPr – relative frequency observed, χ^2 – chi-square test of independence, SR – standardized residuals, Phi – indicator of the potency of association between observed variables, p – level of significance, $1.96 \leq SR < 2.58$ ($p < 0.05$); $2.58 \leq SR < 3.29$ ($p < 0.01$), $SR > 3.29$ ($p < 0.001$)
Source: Author's own elaboration

Table II. Comparison of sensitivity of prenatal and postnatal diagnostics of CDAUTs in the kidneys in newborns in 1995 to 2016 (N = 909).

Successful diagnosis of CDAUTs in the kidneys in the prenatal period in 1995 - 2016		Screening		Total
		Prenatal	Prenatal	
No	N_P	754	4	758
	N_O	379,0	379,0	758,0
	N_{Pr}	82,9 %	0,4 %	41,7 %
	SR	19,3	-19,3	
Yes	N_P	155	905	1060
	N_O	530,0	530,0	1060,0
	N_{Pr}	17,1 %	99,6 %	58,3 %
	SR	-16,3	16,3	
Total	N_P	909	909	1818
	N_O	909,0	909,0	1818,0
	N_{Pr}	100,0 %	100,0 %	100,0 %
$\chi^2(1) = 1272,745$				
$p < 0,001$				
Phi = 0,837				

Note: NP – frequency observed, NO – frequency expected, NPr – relative frequency observed, χ^2 – chi-square test of independence, SR – standardized residuals, Phi – indicator of the potency of association between observed variables, p – level of significance, $1.96 \leq SR < 2.58$ ($p < 0.05$); $2.58 \leq SR < 3.29$ ($p < 0.01$), $SR > 3.29$ ($p < 0.001$)
Source: Author's own elaboration

(NHIC), we have identified statistical data regarding the role that prenatal diagnostics played in the diagnostics of congenital developmental anomalies of the urinary tract in 1995, 2000, 2005, 2008 and 2013-2016. Data regarding the selected years have been selected and provided by the National Registry of Congenital Anomalies [5,6]. Data on postnatal screening have been collected using questionnaires that focused on the year 2017. The research involved 38,496 newborns which represents 66.5% of the entire population of 57,969 babies born in 2017. For the purpose of statistical analysis and processing of collected data we have used SPSS for Windows, version 21.0. On

Table III. Comparison of sensitivity of prenatal diagnostics of CDAUTs in the kidneys in newborns in 1995, 2000, 2005, 2008 and 2016 (N = 155).

Year		Successful diagnosis of CDAUTs in the kidneys in the prenatal period		Total
		Yes	No	
1995	N_P	91	14	105
	N_O	87,1	17,9	105,0
	N_{Pr}	86,7 %	**13,3 %**	100,0 %
	SR	0,4	-0,9	
2000	N_P	133	35	168
	N_O	139,4	28,6	168,0
	N_{Pr}	79,2 %	**20,8 %**	100,0 %
	SR	-0,5	1,2	
2005	N_P	172	15	187
	N_O	155,1	31,9	187,0
	N_{Pr}	92,0 %	**8,0 %**	100,0 %
	SR	1,4	**-3,0**	
2008	N_P	207	19	226
	N_O	187,5	38,5	226,0
	N_{Pr}	91,6 %	**8,4 %**	100,0 %
	SR	1,4	**-3,1**	
2016	N_P	151	72	223
	N_O	185,0	38,0	223,0
	N_{Pr}	67,7 %	**32,3 %**	100,0 %
	SR	-2,5	**5,5**	
Total	N_P	754	155	909
	N_O	754,0	155,0	909,0
	N_{Pr}	82,9 %	17,1 %	100,0 %
$\chi^2(4) = 62,043$				
$p < 0,001$				
Cramer V = 0,261				

Note: NP – frequency observed, NO – frequency expected, NPr – relative frequency observed, χ^2 – chi-square test of independence, SR – standardized residuals, Cramer V – indicator of the potency of association between observed variables, p – level of significance, $1.96 \leq SR < 2.58$ ($p < 0.05$); $2.58 \leq SR < 3.29$ ($p < 0.01$), $SR > 3.29$ ($p < 0.001$)
Source: Author's own elaboration

the level of statistical description, the collected data have been analysed using numbers (N), arithmetic mean (AM), standard deviation (SD), standard error of estimate (SE), median (Mdn), modus, selection variance, skewness and kurtosis coefficient and minimum and maximum values. Chi2-test and t-test have been used to assess differences in sensitivity of prenatal and postnatal diagnostic ultrasound.

RESULTS

Results of the analysis show that in 2016 there was a statistically significant difference of $\chi^2(1) = 224.557$, $p < 0.001$ between the sensitivity of prenatal and postnatal screening of CDAUTs in the kidneys. To put it more precisely, we have found out that the postnatal screening of CDAUTs in the kidneys showed a significantly higher sensitivity compared to the prenatal screening, SR = 6.2, $p< 0.001$. Prenatal screening only detected 32.3% of CDAUTs in the kidneys, while with the postnatal screening it was possible to diagnose CDAUTs in the kidneys in as many as 99.6% cases. Results were not only statistically significant, but had a high level of objective significance (Phi = 0.710), see. table I.

Results of the analysis for the years 1995 to 2016 show that there is a statistically significant difference of $\chi^2(1) = 1272.745$, $p < 0.001$ between the sensitivity of prenatal and postnatal screening of CDAUTs in the kidneys. To put it more precisely, we have found out that the postnatal screening of CDAUTs in the kidneys showed a significantly higher sensitivity compared to the prenatal screening, SR = 16.3, $p< 0.001$. Prenatal screening only detected 17.1% of CDAUTs in the kidneys, while with the postnatal screening

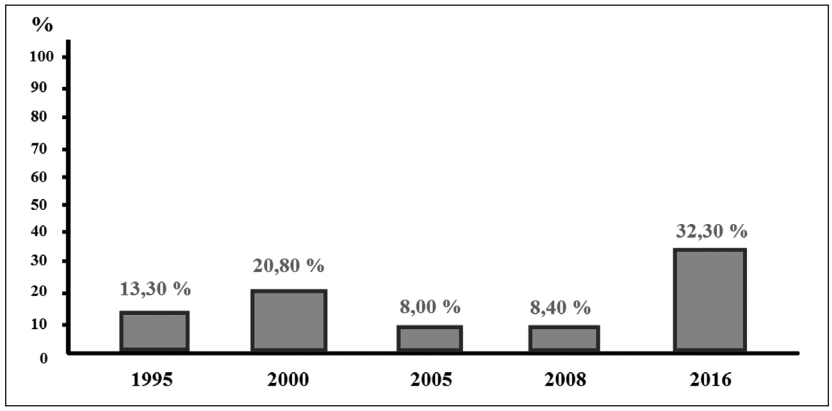

Fig 1. Comparison of sensitivity of prenatal diagnostics of CDAUTs in the kidneys in newborns in 1995, 2000, 2005, 2008 and 2016 (N = 155).
Source: Author's own elaboration and illustration

it was possible to diagnose CDAUTs in the kidneys in as many as 99.6% cases. Results were not only statistically significant, but had a high level of objective significance (Phi = 0.837), see. table II.

To obtain a more comprehensive view of the issue, we have also statistically analysed the differences in the sensitivity of prenatal screening of CDAUTs in the kidneys in newborns in 1995, 2000, 2005, 2008 and 2016. For this purpose, we have conducted the chi-square test of independence which was followed by the analysis of standardized residuals. This allowed us to compare the percentages of newborns with CDAUTs in the kidneys who in the specified years had their condition diagnosed as early as in the prenatal screening.

Results of the analysis showed that there are statistically significant differences in the sensitivity of prenatal screening of CDAUTs in the kidneys in the newborns conducted in 1995, 2000, 2005, 2008 and 2016, $\chi^2(4) = 62.043$, $p < 0.001$. To be more precise, we have found out that the highest number of CDAUTs in the kidneys was diagnosed in the prenatal screening in 2016, SR = 5.5, $p < 0.001$, when as many as 32.3% cases of such conditions were captured. On the contrary, the lowest number of CDAUTs in the kidneys was diagnosed in the prenatal screening in 2005, SR = -3.0, $p < 0.01$ and in 2008, SR = -3.1, $p < 0.01$, SR = 5.5, $p < 0.001$, when only 8%, respectively 8.4% of cases were captured. Results were not only statistically significant, but also had a nearly moderate level of objective significance (Cramer V = 0.261), see. table III, Fig. 1.

DISCUSSION

The first algorithms for the screening for congenital malformations of the kidneys were proposed in 1996 and in 2013 [7]. The author of the screening, docent, M.D. Oľga Červeňová, CSc., was awarded the Crystal Wing for the promotion of the nationwide neonatal screening program [3]. The algorithms have been modified and adapted to the current situation with the growing amount of data from practice and statistics.

Ultrasound screening of congenital developmental anomalies in the kidneys in the newborns is the first stage in the diagnostics of serious renal diseases, the treatment of which consequently requires multidisciplinary cooperation, in particular of paediatricians, nephrologists and urologists [1,2].

With neonatal sonographic screening of the uropoetic system we can detect defects that need to be addressed in the newborn or early infant age. Such anomalies include renal position anomalies, agenesis, dysplasia, obstructive uropathy – dilation of the hollow system according to Hofmann's classification (DHS) and cystic changes. Clinically significant are especially those abnormalities that are associated with impaired urinary outflow – they can lead to recurrent urinary tract infections and subsequently impair kidney function. The vast majority are **obstructive uropathies** [8].

The current situation in the diagnosis of congenital malformations in Slovakia has not been analyzed **for over ten years** therefore we carried out a new mapping throughout the territory of Slovakia to underline the importance of the screening for congenital malformations of kidneys, finding problems related to the screening and updating of data.

The proportion of prenatal diagnostics in the diagnosis of congenital urinary system disorders in the Slovak Republic has not yet been analyzed in more detail. We used data from the National Health Information Center to find statistics on the proportion of prenatal diagnostics, which were retrieved and provided from the National Register of Congenital Defects.

The level of prenatal screening in different regions of Slovakia varies to a large extent and there are differences among individual physicians as well [4,9]. Using the data from 2006 we know that prenatally, the suspicion of dilation was only raised in a fifth of all infants (18.5%) [1,3,5].

Our study has proven low sensitivity of prenatal diagnostics with maximum numbers achieved in 2016, when it reached 32.3% (mean: 24.4%) a minimum in 2005-2008 (8.0 – 8.4%), pointing to the fact that the sensitivity of postnatal diagnostics in the selected years has always been a stable indicator, reaching 99.6%. This is the very reason why we conduct ultrasound screening for CDAUTs in the kidneys of newborns on a nation-wide scale, with the primary objective to diagnose developmental anomalies which had not been detected prenatally. With the postnatal screening we are able to identify these anomalies with no clinical signs present yet. According to our findings, the structure of hollow system dilations does not differ

significantly, when compared with the data from 1996-2006. Serious congenital developmental anomalies of the uropoetic system requiring nephrologic and urologic care and often urologic intervention in early age (in particular significant dilation of the hollow system: stage 3 and 4 of Hofmann's classification) affect approximately 31-39 of 10,000 infants.

Also, to avoid any misunderstanding, it is advisable to include ultrasound screening for congenital developmental anomalies of the kidneys in the group of mandatory screening exams by means of an expert guidance of the Ministry of Health and thus remedy the situation caused by missing legislation. Once again we would like to draw your attention to the fact that in Slovakia, using diagnostic tools, CDAUTs of UPS are only identified in **one fourth of infants**. Nevertheless, it is a significant step, since it will reveal serious defects which must be dealt with urgently, immediately after birth. At present, the real and biggest advantage of prenatal diagnostics of CDAUTs of UPS in the Slovak Republic is the possibility to ensure early postnatal follow-up and initiate relevant treatment.

CONCLUSIONS

Available statistical data confirm that prenatal diagnostics of CDAUTs of UPS in the Slovak Republic is not perfect. Our work underlines the importance, or we should rather say inevitability of postnatal ultrasound screening for congenital developmental anomalies in the kidneys.

REFERENCES

1. Dobrovanov O., Kralinsky K., Kovalchuk VP., Molcan J. Relevance of ultrasound neonatal screening of the uropoietic system. Ros Vestn Perinatol Pediat. 2019;64(3):68-72. (In Russian).
2. Dobrovanov O., Kralinsky K., Babela R., Mamrilla R. The importance of screening obstructive uropathy in newborns. Pediatria (Bratisl.). 2018;13(1):17-20.
3. Dobrovanov O., Kralinsky K., Kovalchuk VP. Ultrasound screening of congenital uropoietic defects and its outlook in Slovakia. Sovremennaya pediatria. 2019; 1(97): 8-12; doi:10.15574/SP.2019.97.8
4. National Center for Health Information (NHIC). http://www.nczisk.sk/Documents/publikacie/analyticke/vrodene_chyby_2014.pdf.
5. Dobrovanov O., Kralinsky K. The role of prenatal diagnostics in the identification congenital malformations of urogenital system in Slovakia. Lek. Obzor. 2019;68(2):59-62.
6. National Center for Health Information (NHIC). National congenital malformation register (Slovakia).
7. Červeňová O., Payer J., Zatlukalová A. et al. Algoritmy vyšetrení obštrukčných uropatií v novorodeneckom veku. Slov. Radiol. 1996,3:129-131.
8. Vidiščák M., Huštavová L. a kol. Novorodenecká chirurgia I. M-SERVIS. 2008; 225 p.
9. Cisarik F. Current prenatal screening and possibilities of prenatal diagnostics. InVitro (Reprodukčná medicína). 2017;2:28-31.

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ORIGINAL ARTICLE

ANXIETY AND DEPRESSIVE DISORDERS IN PATIENTS WITH ARTERIAL HYPERTENSION

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ABSTRACT

The aim: To study the influence of anxiety and depressive disorders on life quality of patients with arterial hypertension.

Materials and methods: 55 patients with arterial hypertension (AH) of 2nd stage were examined to reach the goal. Age diapason was 25-73 years, the middle age was 53.56+10.28. There were 58.2% (32) of women and 41.8% (23) of men among the patients.

Results: Results analysis of the investigation of patients with arterial hypertension and anxiety and depressive disorders using the Spielberger-Khanin anxiety inventory showed moderate (30.9%) and high (69.1%) level of trait anxiety. As for the state anxiety the high level of it was confirmed in 74.6% of studied patients and moderate level of state anxiety in 25.5% of patients. During the analysis of gender-based distribution the trait anxiety level was significantly higher in women ($p<0.05$). Results of PHQ-9 showed subclinical depression level (12.7%), mild (49.1%) and moderate (16.4%). Moderate manifestations of depression were found in 12.7%, severe depression in 9.1% (5 individuals). As for the gender-based distribution, anxiety indicators were significantly higher in women ($p<0.01$). HADS method did not show significant difference of depression levels of gender-based distribution.

Conclusions: The majority of individuals with arterial hypertension and nonpsychotic mental disorders have a high level of trait and state anxiety. Direct correlation was found between the trait anxiety indicator and depression severity, which were defined according to HADS and PHQ-9 questionnaires. The level of anxiety and depressive episode severity were found to be reliably higher in women in gender-based distribution, that/which was accompanied by decrease in most of the life quality indicators.

KEY WORDS: Arterial hypertension, anxiety and depression disorders, life quality of patients

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INTRODUCTION

Multicenter investigations concluded that depressive persistence influences cardiovascular diseases (CVD) progression as well as obesity and hypercholesterolemia [1]. The frequency of anxiety (AN) and depressive (DEP) disorders in patients with cardiovascular system pathology is ranged between 13-28%. It is proved that existence of depression and anxiety worsens the prognosis, promotes the progression of main disease, complicates the selection of drug treatment and can be a trigger of life-threatening conditions [2]. It is defined that persistence of coronary artery disease, arrhythmia is more often accompanied by anxiety disorders and development of cognitive deficit [3]. Awareness of cardiovascular diseases risk factors in patients who suffers from anxiety/depression is reduced. When the level of anxiety increases, the part of smokers significantly grows up, less amount of individuals follow the diet recommendations and reduce alcohol intake, even after doctor's consultation [4, 5].

It is worth to remember, that depression correlates with life quality of patients: the relationship between psychosocial risk factors and cognitive disorders, which were combined with depression, anxiety, low psychosocial and socio-economic status in patients with CVD is proved [6, 7].

THE AIM

The goal was to study an influence of anxiety and depressive disorders on life quality in patients with arterial hypertension.

MATERIALS AND METHODS

55 patients with arterial hypertension (AH) of 2nd stage were examined to reach the goal. Age diapason was 25-73 years, the middle age was 53.56+10.28. There were 58.2% (32) of women and 41.8% (23) of men among the patients.

Diagnosis of arterial hypertension, anxiety and depression were verified relying on anamnesis data, physical examination, phsychodiagnostic examination using standard methods.

Questionnaires used for confirming and estimating of depressive severity are the following: HADS, PHQ-9 scale, Spielberger-Khanin anxiety inventory. SF-36 questionnaire was used to estimate quality of life.

The investigation was carried out following the main bioethics requirements for clinical researches. Statistical analysis of data was processed by using Microsoft Excel and Microsoft Access Soft. Distribution normality was checked

up by Kolmogorov-Smirnov test. Wilcoxon-Mann-Whitney test was used for comparison of samples, as distribution of most of them was different from normal. Correlation was estimated by Spearman's rank correlation coefficient.

RESULTS

Analysis of typical complaints showed following changes: heart pain was noticed in 96.4% cases, 56.4% of women and 40.0% of men were among them; headache was marked by 70.9% of patients, 49.0% of women and 21.8% of men among them. Headache was associated with elevation in blood pressure.

As for the character of heart pain, the major part of patients complained of pressing pain – 63.6%, 29.1% of patients noticed stabbing pain, the rest 7.4% marked aching pain. Patients associated appearance of pain as with physical activity – 65. 5%, among them 38.2% were women, as with emotional stress – 63.6%, among them 45. 5% were women.

Pain at rest was present in 18.2% of patients. Shortness of breath was noticed in 65.5% of patients, emotional stress provoked it in 36.4% of cases. Tachycardia harassed 65.5% of patients, dizziness was marked in 50.9% of cases, there were 47.3% of women. 50.9% of respondents noted arrhythmia.

30.9% of patients complained of decreased appetite, 21.8% of women were among of them. Mood decline was noted in 65.5% of patients (45.5% – women). 65.5% of patients complied on difficulty of falling asleep, frequent awakening disturbed 63,6% of patients, of which 40.0% were women, nightmares were noticed by 20.0% of patients. The major part of patients were married – 70.9%, those who lost husband/wife were 12.7%, 9.0% of patients were in civil marriage, 7.4% were single.

During the survey about occupational hazards 41.8% of patients noted excessive emotional strain, 23.6% of patients noted increased responsibility for result. 21.8% of patients talked about poor physical activity, associated with working process. Lack of physical strain and forced interruption of labor regime were marked in 10.90 and 5.5% of cases relatively. Family history of cardiovascular diseases was present in 58.2% of patients.

Gender-based distribution analysis showed that level of trait anxiety was significantly higher in women 51.75+1.17 points, than in men – 46.39+1.78 points ($p<0.05$). (fig.1). State anxiety as a reaction to external circumstances, was slightly different in gender groups of men and women – 46.57+1.90 points and 48.71+1.46 points relatively, but also reached a high level.

PHQ-9 results are the following: subclinical level depression in 12.7% (7 individuals), mild level depression – 49.1% (27 individuals), moderate level depression – 16.4% (9 individuals). Depression manifestations of moderate severity were found in 12.7% (7 individuals), severe depression – in 9.1% (5 individuals).

As for the gender distribution, anxiety and depression measures were significantly higher in women, than in men – 10.47+0.50 points та 8.57+0.77 points ($p<0.01$) respectively.

Study of depression levels using HADS method showed statistically unreliable difference in gender distribution: 8.75+0.81 points in women, 7.04+0.73 points in men. The level of depression was determined by PHQ-9 and was moderately higher in women compared with men – 8.94+0.87 points and 8.17+1.22 points respectively.

Stated indicators were considerably lower in the group with mild depressive episode compared with the group with moderate depressive episode. Correlation coefficient between measures of trait anxiety and depression severity was of 0.71 and 0.79 respectively, determined by results of HADS and PHQ-9 (fig. 2).

Absolute measures of anxiety levels in patients having mild depressive episode (DE) were 6.18+0.63 points compared with 8.5+0.87 points in patients with moderate DE ($p<0.05$). Trait anxiety level was significantly lower in mild DE than in moderate – 45+2.43 and 53.08+2.05 points ($p<0.05$) respectively. Absolute measures of state anxiety (SA) level were also significantly lower in patients having mild DE: 41.45+1.78 points compared with 54.5+2.58 points in moderate DE (fig. 3).

Role-emotional (RE) score was considerably lower in the group with mixed anxiety-depressive disorder (MADD) 26.56+6.34 points compared with 32.17+7.31 points in the group with depressive episode. Bodily pain (P) measures were also lower in mixed anxiety-depressive disorder than in DE – 51.94+4.10 points and 63.65+5.72 points respectively. It was determined that level of role-emotional and mental health decrease significantly with anxiety component present – 36.46+6.59 points and 55.00+2.85 points in MADD compared with 39.12+7.74 points and 59.74+4.39 points in DE. PF as a trait of physical activity, on the other hand, was of higher level in anxiety-depressive disorder compared with DE – 58.78+4.97 points and 53.26+5.35 points respectively, that most likely can be explained by restlessness of patients having ADD and quick switching of attention to extrinsic stimulus while being constantly fixated on their own bodily sensations.

Absolute measures of PF in women compared with men were 54,72+4,90 points – 58.91+5.50 points respectively (fig. 4).

Strong correlation was found between moderate DE and significant decrease in constituents of life quality in the process of analysis of life quality indicators and severity of DE, what most likely is associated with severity of non-psychotic mental disorder and absence of the vision for the future in this cohort. PF was significantly lower in patients having moderate DE compared with patients having mild DE, 37.08+5.41 points and 70.90+6.06 points respectively ($p<0.01$). Substantial difference was found between PF in patients having mild and moderate depressive episode, that shows fair limitations in usual role activities, physical activities due to the presence of depressive component. PF measures in mild DE accounted for 52.27+10.36 points and 13.75+7.13 points in moderate DE, ($p<0.01$).

Significant difference between RE scores in women and men was observed: 19.21+3.87 points and 42.39+7.9 points

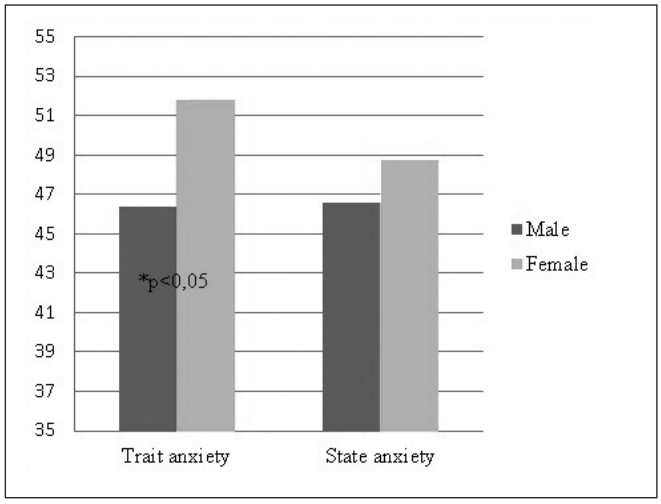

Fig. 1. Trait and state anxiety levels indicators in examined patients according to Spielberger-Khanin anxiety inventory results, gender distribution

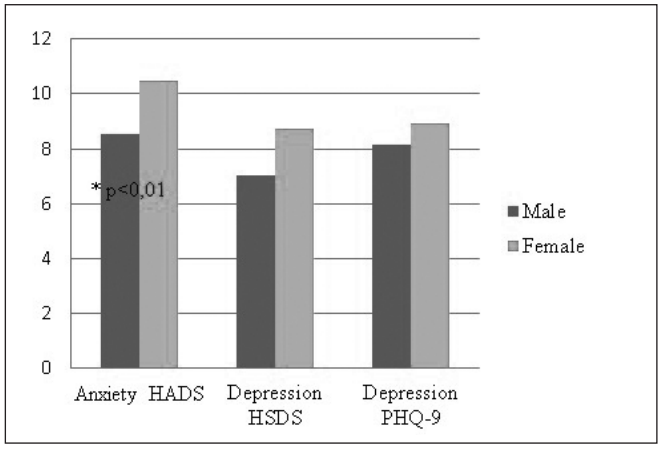

Fig. 2. Anxiety and depression levels measures in examined patients according to HADS and PHQ-9 results, gender distribution

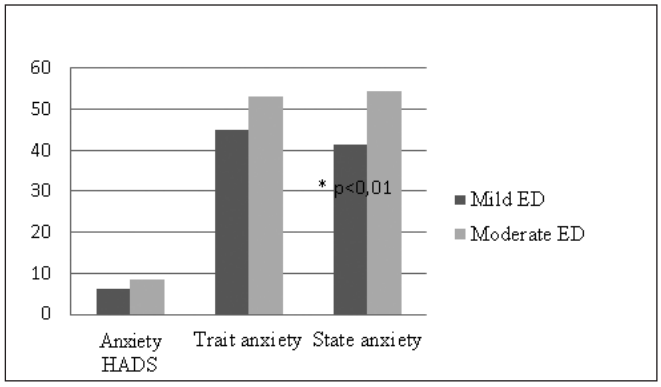

Fig. 3. Measures of anxiety level in examined patients according to HADS and Spielberger-Khanin anxiety inventory results depending on the severity of depression

respectively, what can be explained by the characteristics of women mentioned above. BP measures results were reliable, 47.97+3.87 points in women which is significantly lower than in men – 69.17+5.30 points, that states considerable limitations in physical activities in affected women compared with men. It is worthy of note that the character of pain also has gender specificities, 97.0% of women had complaints about pressing lingering pain that caused constant background anxiety. A substantial difference between general health (GH) and RE scores was not observed: GH – 50.81+2.8 points (women) and 49.26+3.94 points (men); RE – 36.46+6.59 points (women) and 39.11+7.75 points (men). The difference between vitality indicators (VI) in women and men was not significant – 44,37+3,46 points and 50.87+3.93 points respectively (fig. 5). Mental health (MH) measures results were reliably lower in women – 53.25+2.89 points than in men – 62.17+4.22 points, ($p<0.05$).

Significant decrease of GH level measure was found both in patients with moderate and mild depressive episode: 42.25+5.21 points and 59.18+5.99 points respectively, ($p<0.05$). Vitality indicator was significantly higher in patients having mild DE – 58.18+4.22 points, compared with patients having moderate DE – 36.67+5.88 points. RE scores were reliably lower in the group with moderate DE – 44.79+5.85 points and 22,19+6,26 points, compared with the measures of the group with mild DE – 79.55+5.40 points and 57.58+12.78 points, ($p<0.01$), ($p<0.05$). MH indicator in the groups with mild and moderate DE accounted for 69.64+4.73 and 50.67+6.27 points respectively ($p<0.05$), that can be associated with the mental status of patients.

DISCUSSION

The HADS and PHQ-9 are both rapid and reliable. The HADS has the advantage of evaluating both depression and anxiety, and the PHQ-9 of being strictly based upon the diagnostic and statistical manual of mental disorders. The correspondance between the scales at the suitable cut-off is proportional, however the identified prevalence was similar. This indicates that the scales do not identify similar cases complitely. This difference needs to be further explored.

Among patients with arterial hypertension there were distributed next nosologiacal forms: the biggest part was presented by patients with mixed anxiety and depressive disorder – 58.2%, less patients had mild depressive episode and moderate depressive episode – 20.0% and 21.8% relatively. Age depression is a significant public health problem and has an large effect on health when comorbid with a chronic medical condition. Coronary heart disease, hypertension, and diabetes are accompanied by a high incidence of depression and have substantial impact on the treatment and prognosis. Depression is a highly prevalent risk factor for incident of and is associated with morbidity and mortality of cardiovascular disease. In addition to the proactive and effective control of primary diseases, efforts should also be made to improve patients' psychological and social function. A better understanding of pathophysiological mechanisms underpinning depression and cardiovascular disease as well as the complex biological crosstalk of cardiovascular disease complicated with depression is particularly important for future therapeutic strategies.

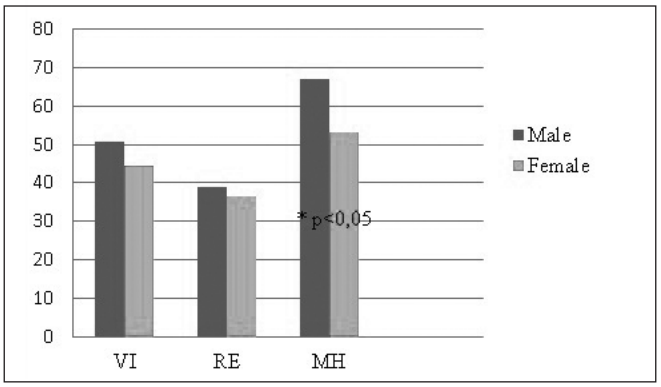

Fig. 4. Life quality measures in examined patients according to SF-36 questionnaire results, gender distribution

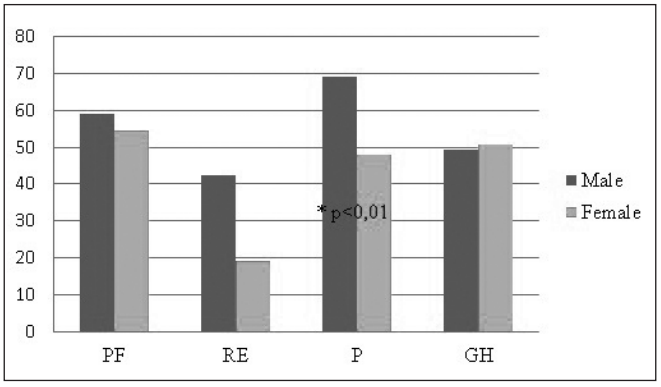

Fig. 5. Life quality measures in examined patients according to SF-36 questionnaire results, gender distribution

Spielberger-Khanin anxiety inventory results analysis in patients with AN, DEP and AH showed next regularities: moderate level of trait anxiety was defined in 30.9% of cases, high level of trait anxiety was defined in 69.1% of cases, mild level of trait anxiety was not defined at all. State anxiety of high level was noted in 74.5% of respondents, of moderate level – was noted in 25.5% of respondents.

In men and women there was diagnosed high level of trait anxiety. There were no differences between the study and control groups in psychological distress symptoms, including anxiety, depression, and hostility, or in anger expression. This can probably evidence about personal predisposition to AN, in patients with AH. In multivariate regression analyses, higher age, male gender, higher sodium intake, lower physical fitness, and alexithymia were independently and highly significantly associated with increased blood pressure, explaining altogether 39.5 % of the cross-sectional variation in mean arterial pressure [1].

Investigation of anxiety level and depressive episode type dependence showed reliable correlations between anxiety indicators measured by HADS and PHQ-9, trait and state anxiety and mild depressive episode.

Life quality level was assessed depending on anxiety-depressive disorders (ADD) in patients with AH. It was determined that anxiety component in mixed anxiety-depressive disorder decreases all measures except physical functioning (PF) and vitality as they are slightly increased. After the analysis of life quality measures in the distribution by gender, it can be argued that women get lower results than men for all indicators, which may be associated with personality traits of women's perceptions of social problems and tendency to overdramatize.

Our observation correlates with statement that dippers had not different levels of health-related quality of life (HRQOL) as compared with non-dippers. LV hypertrophy was associated with lower scores on bodily pain. Female gender, increased age were independently associated with lower physical and mental health scores. The stage of hypertension was not an independent predictor for any of the SF-36 dimensions. Dippers had not different levels of health-related quality of life (HRQOL) as compared with non-dippers. LV hypertrophy was associated with lower scores on bodily pain and kidney failure was associated with lower scores on general health perception [7].

The HADS and PHQ-9 are both rapid and reliable. The HADS has the advantage of evaluating both depression and anxiety, and the PHQ-9 of being strictly based upon the diagnostic and statistical manual of mental disorders. The correspondance between the scales at the suitable cut-off is proportional, however the identified prevalence was similar. This indicates that the scales do not identify similar cases complitely. This difference needs to be further explored.

CONCLUSIONS

1. The majority of individuals with arterial hypertension and nonpsychotic mental disorders have a high level of trait and state anxiety. Direct correlation was found between the trait anxiety indicator and depression severity, which were defined according to HADS and PHQ-9 questionnaires.
2. The level of anxiety and depressive episode severity were found to be reliably higher in women in gender-based distribution, that/which was accompanied by decrease in most of the life quality indicators.
3. Life quality indicators were significantly lower in case of mixed anxiety-depressive disorder, whereas increase of depression severity in depressive disorder caused reliable decrease in the indicators of both physical and mental status.

REFERENCES

1. Herrmann-Lingen C., Meyer T., Bosbach A. et al. Cross-Sectional and Longitudinal Associations of Systolic Blood Pressure With Quality of Life and Depressive Mood in Older Adults With Cardiovascular Risk Factors: Results From the Observational DIAST-CHF Study. Psychosom Med. 2018; 80(5):468-474. doi: 10.1097/PSY.0000000000000591.
2. Aragão J.A., de Andrade L.G.R., Neves O.M.G. et al. Anxiety and depression in patients with peripheral arterial disease admitted to a tertiary hospital. J Vasc Bras. 2019; 18. doi: 10.1590/1677-5449.190002.
3. Serpytis R., Navickaite A., Serpytiene E. et al. Impact of Atrial Fibrillation on Cognitive Function, Psychological Distress, Quality of Life, and Impulsiveness. Am J Med. 2018;131(6):703.e1-703.e5. doi: 10.1016/j.amjmed.2017.12.044.

4. Kotseva K., Wood D., De Bacquer D. Euroaspire investigators. Determinants of participation and risk factor control according to attendance in cardiac rehabilitation programmes in coronary patients in Europe: EUROASPIRE IV survey. Eur J Prev Cardiol. 2018;25(12):1242-1251. doi: 10.1177/2047487318781359.
5. Wändell P., Carlsson A.C., Li X. et al. Association Between Relevant Co-Morbidities and Dementia With Atrial Fibrillation-A National Study. Arch Med Res. 2019; 50(2):29-35. doi: 10.1016/j.arcmed.2019.05.007.
6. Albus C., Waller C., Fritzsche K. et al. Significance of psychosocial factors in cardiology: update 2018: Position paper of the German Cardiac Society. Clin Res Cardiol. 2019;108(11):1175-1196. doi: 10.1007/s00392-019-01488-w.
7. Figueiredo J.H.C., Oliveira G.M.M., Pereira B.B. et al. Synergistic effect of disease severity, anxiety symptoms and elderly age on the quality of life of outpatients with heart failure. Arq Bras Cardiol. 2019; 114(1):25-32. doi: 10.5935/abc.20190174.

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ORIGINAL ARTICLE

FEATURES OF VIOLATIONS OF THE STATE OF THE VAGINAL ECOSYSTEM IN PREGNANT WOMEN WITH BACTERIAL VAGINOSIS

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ABSTRACT

The aim: To assess the condition of the vaginal ecosystem in pregnant women with BV.

Materials and methods: The main group consisted of 60 pregnant women with BV in the II trimester. The bacterioscopic examination, of vaginal smears was carried out. DNA diagnostics of the microbial spectrum of vaginal contents was performed. Bacteria with biofilm were visualized by fluorescence hybridization in situ.

Results: Biofilms were found in 25 women (41.65%) of the main group, the main component of which was bacteria belonging to the Gardnerella cluster at a concentration of 7.9 ± 0.13 log CFU/ g. Atopobium vagine cluster bacteria gave positive hybridization signals in more than half of the patients and amounted to 6.8 ± 0.15 lg CFU / g. In addition, Snethia spp. was determined as a part of the biofilm at a concentration of 5.8 ± 0.3 lg CFU / g.

Conclusions: Thus, the use of the proposed treatment regimen for women with vaginal dysbiosis led to the elimination of pathogenic and conditionally pathogenic microflora. However, the effectiveness of treatment in 5 cases was lower than expected, which indicates the emergence of bacterial resistance.

KEY WORDS: dysbiosis, biofilms, pregnancy

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INTRODUCTION

Currently, the structure of genital infections in pregnant women is dominated by violations of the vaginal microbiocenosis. Pregnancy itself is a risk factor for the development of pathology caused by opportunistic microflora and microorganisms with weak virulence and aggression factors which leads to the occurrence of bacterial vaginosis (BV).BV in pregnant women is associated with an increase of obstetric complications and occurs with a frequency of up to 30%-50% [1].

The microecosystem of the vagina during a physiologically ongoing pregnancy is a dynamic self-regulating biological system characterized by colonial and nonspecific resistance, as well as biochemical parameters that reflect its metabolic activity [2, 3].There is still no single view on the problem of the influence of hormonal changes during pregnancy on the quantitative and qualitative composition of microorganisms. Under the influence of corpus luteum hormones, the vaginal mucosa thickens, the elasticity of the cells of the intermediate layer increases, the synthesis of glycogen increases in them, and favorable conditions for the vital activity of lactobacilli are created [4, 5].

According to other authors, during pregnancy, when hormonal status is rearranged towards the prevalence of gestagens and under their influence the secretion of cervical mucus changes the pH level increases, which leads to a change in the vaginal microflora and the propagation of opportunistic microflora, which is characteristic of the norm, but with an increase in the population it can cause all kinds of unpleasant phenomena and the development of inflammatory processes [6].

The main problem with bacterial vaginosis is that the vagina is easily infected with pathogenic microflora and BV passes into vulvovaginitis. Ascending infection of the genital tract provokes inflammation of the membranes, in the event of which the risk of complications increases several times [7].In this way, the high prevalence of BV in pregnant women make it an important public health problem. The etiopathogenesis of BV has not yet been fully studied and is still the subject of controversy due to the great diversity and complexity of the microorganisms involved; therefore, effective approaches to predicting outcomes and treating this infection are limited [8,9]. The standard regimens recommended for prophylaxis and treatment allow to achieve cure only in 60-70% of cases. This is due to the fact that BV is characterized not only by excessive bacterial growth, but also by the presence of a dense bacterial biofilm tightly attached to the mucous membrane of the vagina, which is an obligate sign of BV and is absent in healthy women [9,10]. Gardnerella vaginalis is known to be involved in the formation of biofilms on the vaginal epithelium. The primary attachment of Gardnerella to the vaginal epithelium serves as a framework for the attachment of other types of bacteria with a high degree of adhesion, cytotoxicity and the ability to form biofilms. Such biofilms contain bacteria enclosed in the intercellular matrix of polymer compounds that they synthesize immediately after adhesion –lipopolysaccharides, proteoglycans, endopolysaccharides, and have

altered physiological properties that betray them resistance to antibiotics and immune defense factors [11, 12].

Currently, there are various therapeutic strategies for combating biofilm infections. This is the use of antiseptics, natural antimicrobials, DNAse, plant compounds, prebiotics and probiotics, as well as acidification of the vagina to prevent recurrence of bacterial vaginosis [13].

There is no clear algorithm for treating bacterial vaginosis and its complications. Therefore, one should look for promising new methods of treating BV aimed at eliminating the pathogen, restoration of pH and colonization by strains of normal vaginal microflora [14].

It has been established that certain strains of Lactobacillus can act as probiotics, preventing the growth of bacteria associated with BV through two main mechanisms: inhibiting the adhesion of pathogens to the vaginal epithelium and the production of antimicrobial compounds such as hydrogen peroxide, lactic acid and bacteriocins [15, 16]. Antiseptics are also used to treat vaginal infections. They have antibacterial activity against a wide range of bacteria, acting by nonspecific destruction of their cell membrane [11].

THE AIM

The assessment of the state of the vaginal ecosystem in pregnant women with BV, as well as to study the clinical effectiveness of the therapy with the proposed method and to establish a possible cause of treatment failure.

MATERIALS AND METHODS

The main group consisted of 60 pregnant women with BV and 10 women with a physiological course of gestation and childbirth .When selecting patients, the totality of signs that determined the state of the vaginal microbiocenosis as a nosological form of BV using the Amsel criteria was taken into account: the presence of "key" epithelial cells, whites, vaginal pH> 4.6; A positive amine test was determined when a fishy odor appeared after applying a drop of a 10% potassium dioxide (KOH) solution to a sample of vaginal discharge [17]. Attention was drawn to the presence of a massive microflora content, the prevalence of morphotypes of Gardnerella vaginalis and obligate anaerobic bacteria (Eubacterium spp., Atopobium vaginae, Snethia spp., Megasphera spp., Peptostreptococcus spp., Lachnobacterium spp.)

The examination was carried out in the II trimester. The study of microbiocenosis included the determination of the qualitative and quantitative composition of vaginal bacteria. For bacterioscopic examination, the material from the vagina was taken with a sterile instrument and transferred to glass. Then we prepare smears subsequently stained by Gramand carried out microscopy using the immersion system. The presence of leukocytes and the degree of seeding by microorganisms were determined. For microbiological research, the material was taken from the vagina with a universal probe. The working part of the probe containing the test material was cut off and placed in a disposable Eppendorf tube with a preservative solution. The presence of bacteria in the test material was confirmed by real-time PCR on a DT-96 instrument (SPU«DNA-Technology», Moscow) with the «Femoflor-16» test system. DNA diagnostics of the microbial spectrum of the vaginal contents was carried out before and after the local correction of vaginal dysbiosis with the classification of the microbial spectrum according to Boldyreva M.N., 2010[18].

Bacteria from the biofilm were visualized using in situ fluorescence hybridization based on 16/23 S ribosomal DNA (rDNA). The material for the study was a biopsy from the lateral wall of the middle section of the vaginal fornix (1-3 mm in diameter) with its subsequent fixation and obtaining paraffin blocks. Microtome sections were examined by the FISH method using a variety of probes. Vaginal biopsies were studied before treatment and a week after. For each patient, bacterial biofilm diversification (relative microbial cluster content of different strains) and biofilm permeability (indicator of microbial activity) were evaluated using the ThermoBrite ™ system in combination with VP 2000 [19].

Pregnant women with moderate and severe dysbiosis were divided into I (n = 41) and II (n =19) clinical groups, respectively. In order to eliminate pathogenic microflora and normalize the condition of the vaginal ecosystem sanitation of the birth canal was carried out by irrigation of the vaginal mucosa with a broad-spectrum foaming antiseptic agent, which included chlorhexidine, hexamidine and chlorocresol 2 times a day with a diluted 1/10 solution within 7 days.

To update the vaginal microflora, a combination of the bacteria Lactobacillus acidophilus and estriol was used, 1 vaginal tablet daily, 12 days.

Efficiency assessment was carried out on the basis of the above research methods. Statistical processing was performed using the program Statistica for Windows (version 8.0). To compare groups by quantitative variables, parametric analysis of variance (ANOVA) was used, and by qualitative variables, Pearson $\chi 2$ criterion was used. Differences were considered statistically significant at $p < 0.05$.

Described in the publication research methods have been applied with respect to human rights in accordance with the existing ones in Ukraine legislation that meets international ethical requirements and does not violate ethical standards in science and standards for biomedical research, according to the conclusions of the ethics committee of Kharkiv National Medical University

RESULTS

When analyzing the data of bacteriological studies of the vaginal contents of women in the studied groups, it was found that the presence of an infectious process is accompanied by the formation of dysbiosis, the severity of which depends on the etiological factor and is characterized by varying degrees of decrease in lactobacilli, even to their complete absence against the background of the dominance of opportunistic microflora, both in the form of monocultures and microbial associations.

Table I. Microbial spectrum of vaginal microbiocenosis in women with bacterial vaginosis before and after treatment (Tab. I)

Microflora lg CFU / g	Studygroups					
	Igroup before treatment	I group after treatment	ρ	II group before treatment	II group after treatment	ρ
E.coli	3,2 ±0,12	2,2±0,02	<0,05	4,3±0,1	3,8±0,14	<0,05
Klebsiella spp.	2,8 ±0,13	2,1±0,04	<0,05	3,6±0,2	2,9±0,12	<0,05
Candida spp.	3,6 ±0,2	2,4 ±0,1	<0,05	4,3±0,1	2,1 ±0,05	<0,05
S.aureus	2,9±0,13	2,3±0,02	<0,05	3,3±0,01	2,9±0,02	<0,05
Streptococcus spp.	3,3±0,1	2,2±0,13	<0,05	3,8±0,03	2,4±0,12	<0,05
Gardnerella vaginalis	5,6 ±0,13	3,1 ±0,08	<0,05	6,9 ±0,13	3,5±0,02	<0,05
Atopobium vaginae	3,4 ±0,15	2,2 ±0,12	<0,05	5,4 ±0,15	2,8±0,05	<0,05
Mobiluncus spp.	2,6 ±0,2	1,3 ±0,2	<0,05	4,6 ±0,12	2,4±0,02	< 0,05
Snetia spp.	3,1 ±0,12	1,6 ±0,09	<0,05	4,9 ±0,05	3,1±0,2	< 0,05
Eubacterium spp.	4,9 ±0,14	2,4 ±0,13	<0,05	6,2±0,13	3,6±0,13	< 0,05
Megasphera spp.	4,0 ±0,2	2,7 ±0,05	<0,05	4,6±0,12	3,3±0,08	< 0,05
Lachnobacterium spp.	3,5 ±0,15	2,8 ±0,07	<0,05	4,8±0,21	2,3±0,16	< 0,05
Peptostreptococcus spp.	5,0 ±0,2	2,5 ±0,1	<0,05	6,3±0,13	3,4±0,12	< 0,05

A bacteriological study of the control group revealed that in 97% of women, the composition of the vaginal microflora corresponded to normal indicators – the average number of lactobacilli was (7.8 ± 0.3) lg CFU / g. In some women (3%), the concentration of lactobacilli was slightly reduced to (4.6 ± 0.2) lg CFU / g. In isolated cases, E. coli, opportunistic staphylococci and streptococci, as well as Candida, were founded.

In the I group of women with a moderate dysbiosis a decrease in the concentration of lactobacilli was noted below 10^7 CFU / g and amounted to an average of 6.5 ± 0.14 lg CFU / g. The main obligate anaerobic organisms turned out to be at a concentration of 5.6 ± 0.13 lg CFU /g and Atopobium vaginae at a concentration of 3.4 ± 0.15 lg CFU / g. Part of the women examined identified bacteria of the genus Mobiluncus – 2.6 ± 0.2 lg CFU / g, Eubacterium-4.9 ± 0.14 lg CFU / g, Snethia – 3.1 ± 0.12 lg CFU / g, Peptostreptococcus- 5.0 ± 0.2 lg CFU / g, Megasphera – 4.0 ± 0.2 lg CFU / g, Lachnobacterium spp. -3.5 ± 0.15 lg CFU / g. The vaginal microflora of women of group II with severe dysbiosis was characterized by a variety of opportunistic microorganisms, that formed microbial associations, among which the most frequent were Gardnerella vaginalis, Atopobium vaginae and Snethia spp. in concentration 6,9 ± 0,13lg CFU /g;5,4 ±0,15lg CFU /g;4,9 ±0,05lg CFU /g. respectively. At the same time lactobacilli were completely absent in 88,8% of the examined patients of this group and their concentration in the rest was sharply reduced to 10^2 CFU /g and averaged 1.3 ± 0.16 lg CFU / g. (Table I).

The biofilms were found in 25 (41,6 %) women of the main group: 14 pregnant women with moderate imbalance and 11 with severe dysbiosis. Gardnerella vaginalis was the main component of biofilms in a concentration (7.9 ± 0.13 lg CFU/g). Atopobium vaginae gave positive hybridization signals in more than half of the patients (6.8 ± 0.15 lg CFU/g). In addition, Snethia spp. was determined as part of the biofilm with concentration (5.8 ± 0.03 lg CFU/g). At the same time, the presence of the G. vaginalis and Megasphaera sp. community, which are usually identified during BV, was a criterion at for the absence of biofilms; therefore, the presence of Gadnerella vaginalis is not always accompanied by the formation of a biofilm with a violation of the vaginal microflora. Bacteria of the Lactobacilli and Bacteroides clusters were present in less than half of women and made up a small fraction of the biofilm.

After complex therapy of bacterial vaginosis, a bacteriological study of the vaginal contents in women of the studied groups was carried out.

In group I, after treatment, an increase in colonization with lactobacilli species was observed up to 10^4-10^5 CFU/ g. Initially, high colonization by bacteria of the genus Gardnerella vaginalis decreased to 3.1 ± 0.08 lg CFU / g, and Atolium vaginae to 2.2 ± 0.12 lg CFU / g, Snethiaspp.-1.6 ± 0.09 lg CFU / g, Mobiluncus -1.3 ± 0.2 lg CFU / g, Eubacterium-2.4 ± 0.13 lg CFU / g, Peptostreptococcus-2.5 ± 0.1lg CFU / g, Megasphera-2.7 ± 0.05 lg CFU / g, Lachnobacterium spp. -2.8 ± 0.07 lg CFU / g.

In group II, after treatment, the degree of colonization by Candida fungi decreased by more than half and amounted to 2.1 ± 0.05 lg CFU / g. A tendency towards the renewal of normobiocenosis due to increased colonization by lactic acid bacteria up to 10^3-10^4 CFU/ g (3.3± 0.07 lg CFU / g on the average), which in most cases were completely absent before the start of therapy was also identified. The number of opportunistic anaerobes such as Gadnerella vaginalis, bacteria of the genus Mobiluncus spp., Atopolium vaginae, Snethia spp. and their associations in some women also decreased significantly: 3.5±0.02 lg CFU / g, 2.4±0.02 lg CFU / g, 2.8±0.05 lg CFU / g, 3.1±0.2 lg CFU / g respectively. Biofilms were not detected when combining G. vaginalis and

Megasphaera. (Table I) amounted to an average

DISCUSSION

In more than half of the patients, the main component of the biofilm was bacteria belonging to the Gardnerella cluster and bacteria of the Atopobium cluster. In addition, Snethia spp. was determined in most cases as part of the biofilm. The presence of such a community of microorganisms can be considered a sign of biofilm [20]. According to the results of the study, Gadnerella vaginalis is not always accompanied by the formation of a biofilm with a violation of the vaginal microflora, since with the combination of G. vaginalis and Megasphaera no biofilms have been identified [21]. In addition, it should be noted that there was a tendency to increase the number of opportunistic microorganisms in the medium to 10^{5-7} CFU / g, which is also possibly one of the markers of biofilm formation [22]. Unfortunately, the treatment efficiency was lower than expected in some cases. We were not able to significantly improve the condition of the vaginal microbiota in 5 pregnant women, which indicates the persistence of vaginal ecosystem disturbances and the emergence of bacterial resistance (the presence of biofilms).

CONCLUSIONS

Thus, the use of the proposed treatment regimen for women with dysbiosis of the vaginal microflora led to the elimination of pathogenic and conditionally pathogenic microflora and promoted the colonization of the vaginal mucosa with lactobacilli. However, in some cases, the effect of the treatment was lower than expected. Therefore, the need to revise the standards for the diagnosis and treatment of bacterial vaginosis is obvious.

In our opinion, a promising method of treatment is the use of cavitated solutions (drug solutions treated with low-frequency ultrasound), the main advantage of which is an additional mechanical effect on the tissues, which contributes to the destruction of bacterial films, cleansing of the pathological substrate and trophic improvement. Our further research will be devoted to this topic.

REFERENCES

1. Nitsovych I.R., Semeniak A.V. Osoblyvosti perebihu ta likuvannia bakterialnoho vahinozu. [Features of the course and treatment of bacterial vaginosis]. Neonatology, surgery and perinatal medicine. 2016; 3:61- 64. (In Ukranian).
2. Lakhtin M.V., Lakhtin V.M., BajrakovaA.L. et al. Mul`tiuzlovaya konczepczi`ya mikrobioczenoza biotopa cheloveka. [Multinodular microbiocenosis concept of human biotope]. Clinical laboratory diagnostics. 2015; 9:83-85. (In Russian).
3. Sinyakova A.A. Sovremennyie predstavleniya o mikrobiotsenoze vlagalischa i ego vliyanii na ishodyi beremennosti. [Current understanding of vaginal microbiocenosis and its impact on pregnancy outcomes]. Journal of Obstetrics and Women's Diseases. 2017; 66(6):89-100. (In Russian).
4. Klymniuk S.Sh., Mykhailyshyn H.I., Malanchuk L.M. Mikrobiolohichni osoblyvosti bakterialnykh vahinoziv u zhinok riznykh vikovykh katehorii ta shliakhy yikh mikrobiolohichnoi korektsii [Microbiological features of bacterial vaginosis in women of different ages and ways of their microbiological correction]. Achievements of clinical and experimental medicine. 2019;3: 21-31. (In Ukranian) .
5. Rishhuk S.V. Disbioz vlagalishha: novy`j vzglyad na problemu. [Dysbiosis of the vagina: a new look at the problem]. Issues of gynecology, obstetrics and perinatology. 2016;3(15):54-63. (In Russian).
6. Mayorov M.V., Zhuperkova E.A., Zhuchenko S.I. Vaginalnyiy biotsenoz. Sovremennyie predstavleniya o norme i patologii [Vaginal biocenosis. Modern ideas about norm and pathology]. Medical aspects of women's health. 2017; 1:33 – 40. (In Russian).
7. Dubossarskaya Yu.A., Lebedyuk V.V. Optimizacziya taktiki vedeniya beremennosti s czel`yu profilaktiki prezhdevremenny`kh rodov.[Optimization of tactics of pregnancy management for the purpose of prevention of premature birth]. Woman's health. 2016; 2:20-24. (In Russian).
8. Machado A., Cerca N. Influence of Biofilm Formation by Gardnerella vaginalis and Other Anaerobes on Bacterial Vaginosis. J Infect Dis. 2015; 212 (12):1856-1861.
9. Shalepo K.V., Mikhajlenko T.G., Savicheva A.M. Rol` bakterial`ny`kh plenok v formirovanii khronicheskikh patologicheskikh proczessov vo vlagalishhe i e`ndometrii. [The role biofilms in the formation of chronic pathological processes in the vagina and endometrium]. Journal of Obstetrics and Women's Diseases. 2016; 4:70-75. (In Russian).
10. Alves P., Sousa C., Cereija T. Using an in vitro biofilm model to assess the virulence potential of Bacterial Vaginosis or non-Bacterial Vaginosis Gardnerella vaginalis isolates. Sci Rep. 2015; 5: 640-643.
11. Storchak A.V., Grishhenko O.V. Problemny`e voprosy` vosstanovleniya bioczenoza vlagalishha.[Problematic issues of restoration of the vaginal biocenosis]. Women's health. 2015; 1: 52-60. (In Russian).
12. Verstraelen H., Swidsinski A. The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment. Curr Opin Infect Dis. 2019; 32(1): 38-42.
13. Machado D., Castro J., Palmeira-de-Oliveira A. et al. Bacterial Vaginosis Biofilms: Challenges to Current Therapies and Emerging Solutions. Front Microbiol. 2015; 6:1528.
14. Karukkupalayam R., Bennur S., Nacimuthu G. Prenatal probiotics: The way forward in prevention of preterm birth. Journal of Clinical Gynecology and Obstetrics. 2019; 3:63 -69.
15. Hardy L., Jespers V., Abdellati S. A fruitful alliance: the synergy between Atopobium vaginae and Gardnerella vaginalis in bacterial vaginosis-associated biofilm. Sex Transm Infect. 2016; 92 (7): 487-491.
16. Tovstanovskaya V.A., Alatorskih A.E., Faranak Parsay Otsenka mikrofloryi vlagalischa s bakterialnyim vaginozom posle provedennogo lecheniya s tselyu opredeleniya ego effektivnosti [Assessment of vaginal microflora with bacterial vaginosis after treatment to determine its effectiveness]. Women's Health. 2017; 1:154–159. (In Russian).
17. Machado A., Cerca N. Influence of Biofilm Formation by Gardnerella vaginalis and Other Anaerobes on Bacterial Vaginosis. J Infect Dis. 2015; 212(12):1856-1861.
18. Nazarova V.V., Shipitsyina E.V., Shalepo K.V. Bakterialnyie soobschestva,formiruyuschie mikroekosistemu vlagalischa v norme i pri bakterialnom vaginoze [Bacterial Communities Forming the Vaginal Microecosystem in Health and Bacterial Vaginosis]. Journal of Obstetrics and Women's Diseases 2017; 66(6):30-42. (In Russian).
19. Machado D., Castro J., Cereija T. et al. Diagnosis of bacterial vaginosis by a new multiplex peptide nucleic acid fluorescence in situ hybridization method. Peer J. 2015; 3:780.
20. Mendling W., Palmeira-de-Oliveira A., Biber S. et al. An update on the role of Atopobium vaginae in bacterial vaginosis: what to consider when choosing a trearment? Arch Gynecol Obstet. 2019; 300(1): 1-6.

21. Vestby L.K., Torstein G., Roger S. et al. Bacterial Biofilm and its role in the pathogenesis of disease. Antibiotics. 2020; 9:59.
22. Pestrikova T.Yu., Yurasova E.A., Kotelnikova A.V. Sovremennyie podhodyi k verifikatsii diagnoza bakterialnogo vaginoza i taktike vedeniya [Modern approaches to verification of the diagnosis of bacterial vaginosis and management tactics]. Mother and child. 2018; 2: 48-53. (In Russian).

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ORIGINAL ARTICLE

STRUCTURAL AND FUCNTIONAL PARAMETERS OF THE CARDIOVASCULAR SYSTEM DURING ATRIAL FIBRILLATION IN PATIENTS, AFTER STROKE

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ABSTRACT

The aim: To evaluate the structural and functional parameters of the cardiovascular system during atrial fibrillation (AF) in patients after a stroke.

Materials and methods: In the main group, we selected 28 patients with non-valvular AF who had previously suffered an ischemic stroke (IS). The comparison group (30 people) included patients with AF without a stroke, comparable in age and gender.

Results: As a result of the study, we discovered an increase in the risk of stroke with an increase in the thickness of the intima-media complex>0.9mm. The thickness of the interventricular septum was 1.19 (1.1; 1.25) in the group of patients with IS, and in the group of patients without IS – 1.09 (1.0; 1.19) cm ($p = 0.019$), the thickness of the LV posterior wall is greater in the main group 1.14 (1.05; 1.24) and 1.09 (1.01; 1.18) cm in the comparison group ($p = 0.038$). The myocardial mass index is 123.3 in the main group and 107.4 g/m2 in the comparison group ($p = 0.41$), which indicates left ventricle (LV) hypertrophy in the main group.

Conclusions: thus, during AF in stroke patients, changes in the following structural and functional parameters of the cardiovascular system were discovered: an increase in the thickness of the interventricular septum, thickness of the posterior wall of the LV, and in the thickness of the intima-media complex.

KEY WORDS: ischemic stroke, non-valvular atrial fibrillation, echocardiography, heart

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INTRODUCTION

Atrial fibrillation (AF) is an important medical problem due to its widespread and increased mortality rate, increasing the risk of stroke by 5 times and increasing the risk of general mortality by 40 – 90% [1].

According to Hannon N. et al, with the development of stroke in a patient, a fatal situation arises not only for the brain, but for the whole body, which leads to a deterioration in the general somatic status [2].

One of the risk factors for ischemic stroke (IS) is a decrease in the peak blood flow velocity in the left atrial appendage (LAA) according to echocardiography (EchoCG). In this case, it is believed that the indicator is associated with a risk of atrial thrombosis, regardless of the form and duration of AF [3].

According to Golukhova E.Z. when conducting transesophageal echocardiography in patients with AF, it was found that the parameters of the hemodynamics of the heart, in particular: a decrease in blood flow velocity in the LAA less than 30 cm/s and the morphological type of LAA are predictors of intracardiac thrombogenesis [4].

There is evidence that a decrease in the ejection fraction of the left ventricle (LVEF) <50% and an increase in LA over 42mm increases the risk of LAA thrombosis in AF [5].

Basically, retrospective studies are conducted to study clinical or laboratory risk factors for thrombosis in AF patients who have had a cardioembolic stroke (CES). The anatomy and morphology of LAA describes the relationship between the formation of a thrombus in the LAA and complications of AF.

However, in recent years, many studies have proven that the most accurate criterion for the remodeling of the LA should be the volume index of the LA (index. LAV) [6]. It was proven that the volume of the LA correlates with the degree of atrial fibrosis [7]. There are data on the assessment of LV diastolic function (DF) in patients with AF as a predictor of recurrence of arrhythmia [8], however, there are still no accurate echocardiography parameters for stroke prediction. There are studies on the dysfunction of the LA (stanning), as a predictor of recurrence of arrhythmia and possible CES [6, 8]. «This phenomenon is based on several mechanisms: an excess of calcium in the cytoplasm of myofibrils, hibernation of the atrial myocardium due to tachycardia, as well as atrial fibrosis» [9].

In AF, diastolic dysfunction (DD) is difficult to assess with echocardiography due to the absence of atrial contraction (peak A), it is impossible to identify the type of left ventricular diastolic dysfunction LVDD. Also, there is a constant variation of indicators with each reduction, which makes repeated measurements necessary in order to obtain the average value and identify LVDF [1, 10, 11].

There is the possibility of assessing DD using tissue dopplerography (TD): the speed of movement of the

fibrous ring of the mitral valve (MV) is measured in the early filling phase of the left ventricle (E') and is an active component of diastole [12]. The ratio of the E' to the peak E of the transmitral blood flow (E / E' – i.e. the ratio of the rates of the early diastolic flow and the early diastolic movement of the myocardium) allows you to indirectly evaluate the pressure in the cavity of the LV. The ratio (E / E' > 15) which reflects the increase in pressure in the LV and the increase in the pressure of filling the LV, is one of the key parameters for assessing LVDF in patients with AF [13]. This indicator correlates with the «tau» coefficient, which reflects a decrease in LV pressure during the relaxation period.

Caputo M. et al. believe that the measurement of E / E', as a criterion for assessing LVDD, should be mandatory for the echocardiography protocol in individuals with AF, and treatment tactics for patients with LVDD and AF should include longer anticoagulant therapy [8]. However, till date, there are many questions regarding the accuracy of different methods for assessing LVDF in patients with AF.

Analyzing recent studies in patients with AF on echocardiography, it was revealed that the only risk factor for ischemic stroke (IS) is the presence of LV systolic dysfunction (LVSD) corresponding to LVEF <40%. When studying the data of trans esophageal echocardiography in ischemic stroke, evidence such as the presence of blood clots in the LAA, decrease in blood flow in the appendage less than 20 cm/s, the presence of spontaneous contrast, as well as atherosclerotic lesions of the aorta attest. [9].

It was found that in people with AF up to 60 years without structural heart disease, the risk of IS and systemic thromboembolic complications (TEC) is 1.3% over 15 years. Unlike patients older than 75 years, who, on the contrary, had a very high risk of stroke [14].

Thus, it seems important to us to evaluate the structural and functional parameters of the cardiovascular system for atrial fibrillation in stroke patients in order to identify echocardiography parameters that increase the risk of stroke and resulting from a stroke.

THE AIM

The purpose of our research was to evaluate the structural and functional parameters of the cardiovascular system during atrial fibrillation in patients after stroke.

MATERIALS AND METHODS

In connection with the aim, we selected 28 patients with non-valvular atrial fibrillation who had previously suffered an ischemic stroke, of which 18 (64.3%) were men and 10 (35.7%) were women, the average age of the patients was 69.6 (62; 74), body mass index (BMI) – 29.20 (24.6; 33.2) kg / m2. The selection was conducted at the «City Hospital No. 2 of Belgorod City State Budgetary Healthcare Institution» (from January 2018 to December 2019).

Written informed consent was obtained from patients to participate in the study, permission was also received from the Ethics Committee of the Belgorod State National Research University to conduct the study.

The comparison group (30 people) included patients with non-valvular AF without stroke and transient ischemic attacks (TIA), a history of comparable age: 19 (63.3%) men and 11 (36.7%) women. Inclusion criteria: presence of non-valvular AF; the presence of 2 points when calculating the risk of feasibility study on the scale of CHA2DS2-VASc in men and 3 in women. Patients with valvular AF (moderate to severe and severe mitral stenosis or mechanical prosthesis of heart valves), thyrotoxicosis, and a history of cancer were excluded from the study.

All the patients underwent laboratory diagnostics, electrocardiographic (ECG), echocardiographic (Echo) studies, ultrasound duplex scanning of the arteries of the brachiocephalic zone (ultrasound of the BCA), and also filled out an individual questionnaire, which indicated anamnesis with the date of onset of atrial fibrillation, accompanying stroke pathology and all medications that the patient was on.

Structural and functional parameters of the heart were evaluated on a GE VIVID 7 Vantage (Expert Class Digital Ultrasound System) ultrasound apparatus, a multi-frequency sensor 2.5-4 for cardiac research. Echocardiography was performed according to the standard technique in M and B modes and tissue dopplerography. Determination of LV ejection fraction (EF) using the Simpson method.

The assessment of the intima-media complex (IMC) and the percentage of stenosis of the carotid arteries (CA) was performed on the device in accordance with the Russian national recommendations for cardiovascular prophylaxis of 2017 [15]. The thickness of IMC CA (mm) was determined at three standard points. The increase was considered the thickness of the IMC > 0.9 < 1.3mm. Local thickenings of more than 1.3 were considered evidence of the presence of atherosclerotic plaque (ASP). The degree of stenosis of the carotid arteries was assessed using the criteria of the ECST method (European Carotid Surgery Method), in which the degree of stenosis of the bifurcation of the common carotid artery is calculated as the ratio of the difference between the maximum and free lumen of the vessel to the maximum diameter of the vessel, expressed in percentage [16].

Statistical processing of the material was carried out using the program "Statistica 6.0". The methods of descriptive statistics were used with the determination of the median (Me), and the interquartile range with the lower and upper quartiles (Q1 – Q3). Quality indicators were expressed in percentage. All indicators are given in the SI system. To assess the significance of differences between groups in quantitative terms, the nonparametric Mann-Whitney test was used. The analysis of differences in qualitative characteristics in two independent groups was carried out by constructing contingency tables with the subsequent calculation of the χ^2 Pearson's criterion and the Odd ratio, with the calculation of 95% CI. The normality of the distribution was checked using the Shapiro-Wilk test; an abnormal distribution was detected. Significant changes in the indicators were considered at which $p < 0.05$.

Table 1. The dynamics of the structural and functional parameters of the cardiovascular system during atrial fibrillation in patients after stroke.

Index	Main group (n = 28)	Comparison group (n = 30)	p
Thickness IVS, cm	1.19 (1.1; 1.25)	1.09 (1.0; 1.19)	0.019
Thickness LVPWd, cm	1.14 (1.05; 1.24)	1.09 (1.01; 1.18)	0.038
LVMI, g/m2	123.3 (117.1; 129.4)	107.4 (101.6; 115.4)	0.041
LVEDD, cm	4.81 (4.21; 5.48)	4.76 (4.1; 5.38)	0.28
LVESD, cm	3.42 (2.67; 4.12)	3.39 (2.62; 4.1)	0.19
LVEF Simpson, %	53.4 (44.8; 61.5)	55.12 (45.9; 63.4)	0.24
Width LAA, cm	4.26 (3.65; 4.81)	4.23 (3.69; 4.76)	0.31
Length LAA, cm	6.02 (5.08; 6.9)	5.86 (5.03; 6.61)	0.12
Volume LAA, ml	73.71 (49.6; 87.2)	71.2 (48.1; 86.3)	0.26
Volume index LAA, ml/m2	39.9 (34.8; 44.7)	37.6 (31.4; 43.5)	0.11
E/E', unit	14.6 (11.2; 17.4)	13.8 (10.1; 16.2)	0.14
IMC CA, mm	1.03 (0.97; 1.1)	0.91 (0.84; 1.05)	0.021

Table 2. Ratio of odds to stroke risk

Factor	OR	95 %CI
LVMI according to Echo	2.596	1.253 – 5.712
IM thickness CA	5.534	2.629 – 11.612

RESULTS

Among the examined patients, a history of concomitant diseases were identified: Hypertension (HTN), coronary heart disease (CHD), and chronic heart failure (CHF). The average duration of AF disease was 4.7 (2.6; 6.4) years in the study group and 3.8 (2.0; 5.1) years in the comparison group (p = 0.07). A stroke was suffered in the range of 4 to 19 months ago.

In the main group there were 9 (32.13%) patients with paroxysmal AF, 10 (35.7%) with persistent atrial fibrillation.

We identified a lack of commitment to taking anticoagulants. Only 12 (42.87%) patients took direct oral anticoagulants (DOAC), 5 (17.86%) took warfarin and 10 (35.7%) – indicated only the intake of acetylsalicylic acid (ASA) and / or clopidogrel. 1(3.57%) patient did not take antithrombotic drugs at all. In this case, the question about whether anticoagulants were prescribed, almost all patients (92.8%) answered positively, the remaining 2 could not remember the purpose of these drugs.

In the study of previous antithrombotic therapy, the following indicators were in the comparison group: ASA and / or clopidogrel were received by 15 (50%) people, warfarin – 8 (26.7%), novel oral anticoagulants (NOAC) – 7 (23.3%).

In patients in the main group, the average score on the CHA2DS2-VASc scale was 4.5 (3: 5) and in the comparison group 2.75 (2; 4) p = 0.068.

Currently, there is no single accurate echocardiographic parameter that is used to diagnose LV diastolic dysfunction in patients with AF.

It is recommended to use echocardiographic examinations, including a comprehensive analysis of two-dimensional echocardiography, Doppler echocardiography, as well as color tissue Doppler ultrasound. [10, 17].

From table 1, it shows that when comparing the main group and the comparison group, statistically significant differences were found in the following indicators: thickness of the interventricular septum (IVS) -1.19 (1.1; 1.25) in the group of patients with stroke, and not IS – 1.09 (1.0; 1.19) cm (p = 0.019), the thickness of the posterior wall of the left ventricle (LVPWd) is greater in the main group 1.14 (1.05; 1.24) compared to 1.09 (1.01; 1.18) cm in the comparison group (p = 0.038). The myocardial mass index (LVMI) is 123.3 (117.1; 129.4) g/m2 in the main group and 107.4 (101.6; 115.4) g/m2 in the comparison group (p = 0.041). This indicates LV hypertrophy in the main group.

LV sizes did not significantly differ: the end diastolic dimension of the left ventricle (LVEDD), the end systolic dimension of the left ventricle (LVESD). The ejection fraction of the left ventricle also did not have significant differences; in the group of patients with stroke, it was 53.4 (44.8; 61.5) % and 55.12 (45.9; 63.4) % in the group of patients without stroke (p = 0.24).

The width of the LV in the first group was 4.26 (3.65; 4.81) cm, and in the second group – 4.23 (3.69; 4.76) cm; without statistically significant differences. Similar results were obtained for the volume of the LV – 73.71 (49.6; 87.2) ml in the main group and 71.2 (48.1; 86.3) in the control group (p = 0.26).

The LV volume index was above norm in both groups, which indicates the presence of LV DD in both groups, (39.9 (34.8; 44.7) ml / m2 in the main group and 37.6 (31.4; 43.5) ml /m2 in the control group (p = 0.11)).

The ratio of the rates of early diastolic flow and early diastolic movement of the myocardium E / E' in the main group 14.6 (11.2; 17.4) units and in the control group 13.8 (10.1; 16.2) units did not differ significantly.

The thickness of the intima-media in the main group was statistically significantly different – 1.03 (0.97; 1.1) mm and 0.91 (0.84; 1.05) mm in the comparison group (p = 0.021), which indicates an increased thickness of the arterial walls in patients and in our opinion is not as a result of a stroke, but on the contrary, a stroke could be a result of the thickening of the intima-media of the arteries of the brachiocephalic zone. In the group of patients with IS, the thickness of the intima-media exceeded the norm in the carotid arteries more than 0.9 mm. In the main group, such patients were 19 (67.8%), and in the comparison group only 7 patients (23.3%); p = 0.01.

Analyzing the ECG data, it was found that signs of LV hypertrophy were observed in 16 patients (57.1%) in the main group and 12 patients (40%) in the comparison group, p = 0.043.

We evaluated the association of indicators with the risk of stroke. The results of calculating the odds ratio, when conducting a one-factor analysis are presented in table 2.

Table 2 shows that a significant association with the risk of stroke was identified by LVMI with echocardiography (evidence of left ventricular hypertrophy) and by the thickness of the intima-media of the CA.

DISCUSSION

We identified a lack of commitment to taking anticoagulants, despite the high risk of developing complications. The recommendation for the management of patients with atrial fibrillation and especially after ischemic stroke, the need for constant administration of anticoagulant therapy is clearly stated [1, 15].

Our data of previous antithrombotic therapy are comparable with the results of the study by A. Karpov, where ASA was received by 50.6% patients, 23.1% received warfarin, 8.3% – dabigatran, 19.4% rivaroxaban, and 4.3% received apixaban. [18].

The average score on the CHA2DS2-VASc obtained in our study is comparable with the data of other studies. There is a study of 281 patients with AF from Krasnoyarsk [19]. According to their register, in patients, the average score on the CHA2DS2-VASc scale was 3.6 (2.0 – 5.0), which is also comparable to the data obtained from the international registry GARFIELD, in which the average score is 3.2, and European (PREFER in AF) – 3.4 points [20, 21].

The LV volume index was above norm in both groups, which indicates the presence of LV DD in both groups. A study by Gupta S. et al. proved that the most accurate criterion for the remodeling of LV should be considered the index of LA volume [6]. It was also proven that the volume of the LA correlates with the degree of atrial fibrosis [7], however, there are still no exact echocardiography parameters for predicting stroke.

The ratio of the rates of early diastolic flow and early diastolic movement of the myocardium E / E' in the main group and in the control group did not differ significantly. In the presence of AF, LVDF certainly suffers, but its assessment during arrhythmia is difficult [21, 22].

Association with the risk of stroke was identified by LVMI with echocardiography (evidence of left ventricular hypertrophy) and by the thickness of the intima-media of the CA. Our data are consistent with the results of a study by Nikolin D.U. et al. In their opinion, left ventricular hypertrophy according to echocardiography is associated with an increased risk of ischemic stroke [23].

We realized statistically significant differences between the groups using the following echocardiographic parameters: thickness of the interventricular septum, thickness of the posterior wall of the left ventricle and the left ventricular myocardial mass index in patients who have had ischemic stroke. When studying the sources, we did not find such results.

In our study, a significantly higher frequency of occurrence was found in patients whose intima-media thickness exceeded the norm in the group with ischemic stroke. A number of studies have shown that an increase in the risk of stroke was shown with an increase in the thickness of the intima-media complex of the carotid arteries of more than 0.9 mm [24, 25].

The left atrial volume index was above norm in both groups in both groups, which indicates the presence of diastolic dysfunction of the left ventricle with atrial fibrillation. After all, it is the volume of the left atrium that correlates with the degree of atrial fibrosis and atrial fibrillation [6-8]. But, we were not able to determine the statistically significant differences in the left atrial volume index in patients with and without atrial fibrillation.

CONCLUSIONS

Thus, as a result of the study, we realized statistically significant differences between the groups using the following echocardiographic parameters: thickness of the interventricular septum, thickness of the posterior wall of the left ventricle and the left ventricular myocardial mass index in patients who have had ischemic stroke.

An increase in the risk of stroke was shown with an increase in the thickness of the intima-media complex of the carotid arteries of more than 0.9 mm and an increase in the left ventricular myocardial mass index. In our study, a significantly higher frequency of occurrence was found in patients whose intima-media thickness exceeded the norm in the group with ischemic stroke 67.8% and only 23.3 % in the comparison group.

The left atrial volume index is higher than normal in both groups, 39.9 (34.8; 44.7) ml / m2 in the main group and 37.6 (31.4; 43.5) in the control group), which indicates the presence of diastolic dysfunction of the left ventricle with atrial fibrillation. However, we were not able to determine the statistically significant differences in the left atrial volume index in patients with and without atrial fibrillation.

REFERENCES

1. Powers W.J., Rabinstein A.A., Ackerson T. et al. Guidelines for the early management of patients with acute ischemic stroke. A guideline for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2018; 49: e46–e110. Doi: 10.1161/ STR.0000000000000158.

2. Hannon N., Callaly E.L., Moore A.F. et al. Improved late survival and disability after stroke with therapeutic anticoagulation for atrial fibrillation: a population study. Stroke. 2011; 42:2503–2508. doi:10.1161/STROKEAHA.110.602235.
3. Grontkovskaya A.V., Borovkov N.N., Amineva N.V. Arterial'naya gipertenziya kak faktor riska vnutripredserdnogo tromboza u bol'nyh s fibrillyaciej predserdij neklapannoj etiologii. [Arterial hypertension as a risk factor of intra-atrial thrombosis in patients with atrial fibrillation of non-valvular etiology]. Klin. Med. 2016; 94 (6):433-438. (In Russian). Doi 10.18821/0023-2149-2016-94-6-433-438.
4. Golukhova E.Z., Gromova O.I., Arakelyan M.G. et al. Prediktory tromboza ushka levogo predserdiya i tromboembolicheskih oslozhnenij u bol'nyh s fibrillyaciej predserdij bez soputstvuyushchej klapannoj patologii i ishemicheskoj bolezni serdca. [Risk factors of left atrial thrombus and/or thromboembolism in patients with nonvalvular, nonishemic atrial fibrillation] Creative Cardiology. 2017; 11 (3):262–72. (In Russian). Doi: 10.24022/1997-3187-2017-11-3-262-272.
5. Karnialiuk I.U., Rabtsevich V.A., Karnialiuk A.M. Ekhokardiograficheskie prediktory tromboza ushka levogo predserdiya u pacientov s persistiruyushcheĭ fibrillyacieĭ predserdiĭ. [Echocardiography predictors of the left atrial appendage thrombus in patients with persistent atrial fibrillation]. Annaly aritmologii. 2014; 11 (3):170-176. (In Russian). Doi: 10.15275/annaritmol.2014.3.5.
6. Gupta S., Matulevicius S.A., Ayers C.R. et al. Left atrial structure and function and clinical outcomes in the general population. Eur Heart J. 2013; 34: 278-285. doi:10.1093/eurheartj/ehs188.
7. Lupu S., Mitre A., Dobreanu D. Left atrium function assessment by echocardiography – physiological and clinical implications. Med. Ultrason. 2014; 16(2):152-161. Doi: 10.11152/mu.201.3.2066.162.sl1am2.
8. Caputo M., Mondillo S. Echocardiography in the prediction of atrial fibrillation recurrence: a review. Journal of Atrial Fibrillation. 2012; 5(2):23-29. doi:10.4022/jafib.675.
9. Gromyko T.Y. Dinamika strukturno-funkcional'nyh harakteristik levogo predserdiya v prognozirovanii recidivov fibrillyacii predserdij. [The dynamics of the structural and functional characteristics of the left atrium in predicting the recurrence of atrial fibrillation] dis. ... Cand. Of Med. Sciences, 2018:1-28. (In Russian).
10. Nagueh S.F., Appleton C.P., Gillebert T.C. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr. 2009;10: 165-193.
11. Kamyshnikova L.A., Efremova O.A. Diastolicheskaya disfunkciya pri hronicheskoj serdechnoj nedostatochnosti – osnovnye diagnosticheskie parametry i kriterii tyazhesti. [Diastolic dysfunction in chronic heart failure – basic diagnostic parameters and criteria of severity]. Belgorod State University Scientific bulletin. Medicine Pharmacy. 2009; 4 (59):9-13. (In Russian).
12. Maurer M.S., Spevack D., Burkhoff D., Kronzon I. Diastolic dysfunction can it be diagnosed by Doppler echocardiography? J Am Coll Cardiol. 2004;44:1543-1549. Doi: 10.1016/j.jacc.2004.07.034.
13. Leong D.P., De Pasquale C.G., Selvanayagam J.B. Heart failure with normal ejection fraction: the complementary roles of echocardiography and CMR imaging. J Am Coll Cardiol Img. 2010; 3: 409-420. doi:10.1016/j.jcmg.2009.12.011.
14. Hughes M., Lip G.Y. Stroke and thromboembolism in atrial fibrillation: a systematic review of stroke risk factors, risk stratification schema and cost effectiveness data. Thromb Haemost. 2008; 99: 295-304. Doi: 10.1160/TH07-08-0508.
15. Kardiovaskulyarnaya profilaktika 2017. Rossijskie nacional'nye rekomendacii. [Cardiovascular prevention 2017. National guidelines]. Russian Journal of Cardiology. 2018; (6):7-122. (In Russian). Doi: 10.15829/1560-4071-2018-6-7-122.
16. European Carotid Surgery Trialists' Collaborative Group. MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70–99%) or with mild (0–29%) carotid stenosis. Lancet. 1991; 337: 1235-1243.
17. Gromyko T.Y., Sayganov S.A. Dinamika diastolicheskoj funkcii levogo zheludochka u pacientov s fibrillyaciej predserdij pri razlichnyh sposobah vosstanovleniya sinusovogo ritma. [The dynamics of diastolic function of the left ventricle at patients with atrial fibrillation at various]. Meditsinskiy sovet. 2017; 12: 202-208. (In Russian). Doi: 10.21518/2079-701X-2017-12-202-208.
18. Karpov Yu.A. Issledovanie bezopasnosti i effektivnosti apiksabana u pacientov s neklapannoj fibrillyaciej predserdij v real'noj klinicheskoj praktike v Rossii. [The Study of Safety and Efficacy of Apixaban in Patients with Nonvalvular Atrial Fibrillation in Real Clinical Practice in Russia]. Atmosphere. Cardiology News. 2018; 4: 3-12. (In Russian).
19. Tuchkov A.A., Gogolashvili N.G., Yaskevich R.A. Sostoyaniye i adekvatnost' antikoagulyantnoy terapii pri fibrillyatsii predserdiy v klinicheskoy praktike. [State and adequacy of anticoagulant therapy in fibrillation of precurities in clinical practice]. Lechashchiy vrach. 2018; 7: 7-10. (In Russian).
20. Kakkar A.K., Mueller I., Bassand J.P. et al. International longitudinal registry of patients with atrial fibrillation at risk of stroke: Global Anticoagulant Registry in the FIELD (GARFIELD). American Heart Journal. 2012; 163 (1):13-9.e1. Doi: 10.1016/j.ahj.2011.09.011.
21. Kirchhof P., Ammentorp B., Darius H. et al. Management of atrial fibrillation in seven European countries after the publication of the 2010 Guidelines on atrial fibrillation: primary results of the prevention of the thromboembolic events – European Registry in Atrial Fibrillation (PREFER in AF). Europace. 2014; 16 (1):6-14. Doi: 10.1093/europace/eut263.
22. Baymukhanov A.M., Khamnagadaev I.A., Gendlin G.E., Nikitin I.G. Narushenie diastolicheskoj funkcii serdca pri fibrillyacii predserdij. [The disorder of diastolic function of heart under fibrillation of atria]. Medical Journal of the Russian Federation. 2017; 23 (2):101-106. (In Russian). doi: 10.18821/0869-2106-2017-23-2-101-106.
23. Nicolin D.Y., Fokina E.G., Grachev V.G. et al. Dopolnitel'nye kriterii stratifikacii riska ishemicheskogo insul'ta u pacientov s fibrillyaciej predserdij i odnim ne svyazannym s polom ballom po shkale CHA2DS2-VASs obuslovlennym arterial'noj gipertoniej. [Additional criteria for stratifying the ischemic stroke risk in patients with atrial fibrillation and one non-sex-related score on the CHA2DS2-VASC scale due to arterial hypertension]. Ural Medical Journal. 2018; 3(159):5-11. (in Russian). Doi: 10.25694/URMJ.2018.03.030.
24. Lorenz M.W., Polak J.F., Kavousi M. et al. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet. 2012; 379(9831): 2053-62. Doi: 10.1016/S0140-6736(12)60441-3.
25. Willeit P., Tschiderer L., Allara E. et al. Carotid Intima-Media Thickness Progression as Surrogate Marker for Cardiovascular Risk: Meta-Analysis of 119 Clinical Trials Involving 100 667 Patients. Circulation. 2020; 142(7):621-642. Doi: 10.1161/CIRCULATIONAHA.120.046361.

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Conflict of interest
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN PRE- AND POST-THROMBOSIS FACTORS IN PATIENTS WITH STAGE VD CKD TREATED BY LONG-TERM HEMODIALYSIS

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ABSTRACT

The aim: To determine informative value of pre-thrombosis, post-thrombosis and anticoagulation factors as well as their correlations for assessment of hemostasis status in patients with stage VD CKD.

Materials and methods: Potential predictors of thrombophilia development as well as their relationships depending on the level of molecular markers of hemostasis were studied in 88 patients with stage VD CKD undergoing long-term hemodialysis with the view to determine their informative value.

Results: Accumulation of soluble fibrin (sF) was demonstrated to cause moderate reaction of D-dimer (D-d) being insufficient in the absence of reaction of anticoagulant component of hemostasis. Soluble fibrin levels were found to be associated with D-d concentration (r = 0.39) and functionally inactive prothrombin forms (FIPF) to some extent (r = -0.24). Accumulation of FIPF in individuals with high level of sF implies significant activation of blood coagulation system at the stage prior to thrombin formation. Absence of close relationship between pre- and post-thrombosis indices may be indicative of still preserved potential of anticoagulant component of hemostasis.

Conclusions: Accumulation of FIPF is an early marker of activation of blood coagulation and possible thrombosis. Levels of sF correlate with pre-thrombosis (fibrinogen, FIPF) and post-thrombosis (D-d) factors being associated with inhibition of anticoagulation processes. Comprehensive study of basic components of hemostasis in patients with VD stage of chronic kidney disease offer broader opportunities in arranging prophylactic measures to prevent thrombophilia.

KEY WORDS: VD CKD (VD stage of chronic kidney disease), hemodialysis, hemostasis, soluble fibrin, D-dimer, C protein, fibrinogen, functionally inactive prothrombin forms

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INTRODUCTION

Disturbance of homeostasis leading to thrombosis development is known to be one of the commonest complications in patients with stage V chronic kidney disease (CKD) treated by long-term hemodialysis [1]. Thrombophilia syndrome, as the process of intravascular thrombogenesis, is defined by systemic activation of blood coagulation in the absence of anticoagulant and fibrinolytic compensation [2, 3, 4]. Therefore, study of hemostasis is of great significance and should be based on comprehensive analysis of hemostatic parameters and their relationships for unbiased evaluation of blood coagulation processes. Soluble fibrin (sF), D-dimer (D-d), fibrinogen (Fg), protein C (pC), functionally inactive prothrombin forms (FIPF) as well as D-d/sF and pC/Fg ratios in patients with stage V CKD, treated by long-term hemodialysis, were studied to estimate informative value and associative relationships between thrombogenesis activators and its inhibitors as thrombophilia markers.

THE AIM

To determine informative value of pre-thrombosis, post-thrombosis and anticoagulation factors as well as their relationships for assessment of hemostasis status in patients with stage VD CKD.

MATERIALS AND METHODS

Blood sampling was taken in study patients and healthy volunteer donors (after receiving their informed consent) on empty stomach into vacutainer containing sodium citrate solution. Plasma was obtained after 20 minute centrifugation with relative centrifugal force 1200-1400 g.

Soluble fibrin was determined by two-site enzyme-linked immunosorbent assay [5]. D-dimer was estimated by enzyme immunoassay using monoclonal antibodies to D-dimer epitopes [5]. Protein C activity in blood plasma was estimated by its activation with copperhead snake venom, by spectrophotometry with wavelength of 405 and 492 nm [6]. Blood plasma fibrinogen content was determined by spectrophotometry using Antsistron-H (thrombin-like enzyme) [7]. Functionally inactive prothrombin forms (FIPF) were calculated by the ratio of ecamulin and prothrombin indices [8].

RESULTS

According to soluble fibrin level, study patients were divided into three groups: group 1 – sF less than or equal to 3.0 μg/ml (n = 28); group 2– sF 3.1-3.9 μg/ml (n = 37); group 3 – sF 4.0 μg/ml and over (n = 23).

Table I. Relationship between pre- and post-thrombosis factors in patients with stage VD CKD.

No	Groups of patients with stage VD CKD n=88	n	Hemostasis parameters				
			sF, µg/ml	D-dimer, pg/ml	Fg, mg/ml	pC, %	FIPF, %
1	sF <3.0 µg/ml	28	-	45.3±5.85	3.88±0.20	83.0±2.88	103.4±3.6
	sF 3.1-3.9 µg/ml	37	-	66.1±5.22*	4.44±0.19*	82.7±2.57	111.0±3.82
	sF >4.0 µg/ml	23	-	126.7±22.6*	4.03±0.25	78.9±3.41	113.8±3.87*
2	Fg < 3.3 mg/ml	17	-	68.5±7.52	-	83.1±3.64	106.9±4.75
	Fg 3.3-4.7 mg/ml	47	-	79.0±12.6	-	84.1±2.12	108.4±2.67
	Fg >4.7 mg/ml	24	-	73.1±9.36	-	76.5±3.54	113.2±5.33
3	pC >85%	38	-	60.6±5.75	3.95±0.16	-	105.2±3.30
	pC <85%	50	-	86.8±11.9*	4.31±0.17	-	112.3±2.91
4	FIPF <110%	48	-	69.8±8.94	-	84.5±2.20	-
	FIPF >110%	40	-	82.0±12.03	-	78.6±2.48	-
5	D-d pg/ml	88	-	-	-	-	-

Note: * - significant difference as compared to patients with low levels of D-d, Fg, pC and FIPF.

Table II. Correlation coefficients between indices of blood coagulation system in patients with stage VD CKD (n = 88).

No	Hemostasis indices	Hemostasis indices				
		sF	D-d	pC	Fg	FIPF
1	sF	-	-	-	-	-
2	D-d	0.39*	-	-	-	-
3	pC	-0.10	-0.21#	-	-	-
4	Fg	-0.12	-0.01	-0.19	-	-
5	FIPF	-0.24#	-0.15	-0.21#	0.02	-
6	D-d/sF	0.05	0.87*	-0.06	0.01	-
7	pC/Fg	0.06	-0.05	0.65*	-0.81*	-

Notes: * – significant correlation coefficient; # – moderate correlation relationship

Given that increased soluble fibrin concentration can serve as pre-thrombosis indicator, the decision was made to study all other indices of hemostasis and to grade the levels of other parameters (Fg, pC, FIPFs) as well as their correlations (Table I).

Analysis of sF concentrations in study groups, as well as their comparison with general group has shown normal fibrin values only in 28 patients (31.8%). The changes in other components of hemostasis were studied in the groups of patients with various sF levels as well. Sequential significant increase in D-dimer value reflecting post thrombosis process was detected in groups 1-3 ($p = 0.05 \div 0.01$). Such increase in D-dimer level along with considerable increase in soluble fibrin concentration should be regarded as the process of fibrinolysis, suggesting an increased risk of thrombosis in those patients. However, a tendency to pC decrease was found in group 3 (pC >4.0 µg/ml). It should be noted that patients of group 3 proved to have significant accumulation of early markers of pre-thrombosis – FIPF. Fibrinogen, being known as the source of fibrin, characterizes general potential of coagulation system, and its concentration is directly influenced by concentration of soluble fibrin and should be comparable with it. This statement was evidenced by generally high fibrinogen concentration in all three groups: blood plasma fibrinogen level was increased in 93.2% of patients. At the same time, high fibrinogen concentration along with normal sF values was observed in group 1(sF <3.0 µg/ml). Increased Fg concentration had no significant influence on post-thrombosis D-d values followed by the tendency of decrease in pC level.

It should be emphasized that values of natural anticoagulant – protein C – do not correspond to high potential risk of thrombosis. Table 1 shows low level of protein C in all the groups, and in 56.8% of patients it was lower the borderline values, being indicative of inadequate proportional anticoagulant reaction to procoagulant status in hemodialysis patients. It is well known that index of accumulation of functionally inactive prothrombin forms can serve as an additional marker in detection of thrombophilia [9], since the amount of FIPF increases in thrombosis development, as thrombin breaks down prothrombin to build up inactive form of pre-thrombin I [9]. FIPF is thought to be the first early marker of activation of blood coagulation system, since it is produced in all pathological conditions associated with thrombogenesis [9]. High soluble fibrin level is known to be associated with increased FIPF accumulation in 70% of cases, while the presence of FIPF was detected in 44% of individuals with decreased level of physiological blood clotting inhibitor pC [10], which was demonstrated in our study. Comparison of patients with the ratio of ecamulin

index to prothrombin index >110%, corresponding to FIPF level of 1.2 μg/ml and above [8], and those with FIPF level <110% showed no significant differences in those groups, except for the tendency to decreasing D-d level in the latter group. Those data demonstrate again the absence of clotting and fibrinolysis processes at the stage of FIPF accumulation.

Correlation relationships between soluble fibrin and D-dimer, fibrinogen, C protein and FIPF were assessed in general group, and the following data were obtained: medium direct relationship between soluble fibrin and D-dimer (r = 0,39; p = 0,02), absence of correlation with fibrinogen (r = -0.12), protein C (r = -0.10) as well as with D-d/sF and pC/Fg ratios (Table II). On the other hand, moderate negative relationship was observed between sF and FIPF (r = -0,24; p = 0,05). D-d showed high positive correlation relationship with D-d/sF ratio (r = 0,87; p = 0,001) and moderate negative – with pC (r = -0,21; p = 0,05). Moderate direct relationship between pC and pC/Fg ratio (r = 0,65; p = 0,01) as well as moderate negative relationship between pC and FIPF (r = -0,21; p = 0,05) were revealed. Besides, strong negative relationship between Fg and pC/Fg ratio (r = -0,81; p = 0,001) was observed.

DISCUSSION

According to the results of studies, two thirds of patients treated by program hemodialysis have high concentrations of soluble fibrin in blood plasma. This implies activation of blood coagulation system and the risk of thrombotic complications [4]. Since hemodialysis sessions are associated with constant damage to the formed elements of blood and entry of platelet coagulation factors into the bloodstream, plasma precursor of platelet state is activated triggering the activation of blood clotting processes through intrinsic pathway in hemostasis system with accumulation of soluble fibrin [5].

Thus, in response to accumulation of soluble fibrin, moderate reaction of D-dimer develops, though inadequate to our opinion, but the reaction of anticoagulant component of hemostasis is absent. However, strong correlation relationship was found between soluble fibrin level and D-d concentration (r = 0,39; p = 0,02) and FIPF to some extent (r = -0,24; p = 0,05). Accumulation of FIPF in individuals with high sF level by contrast to those with its low level suggests significant activation of blood coagulation system at the stage prior to thrombin formation [11]. The absence of close relationships between pre- and post-thrombosis indices may be indicative of preserved potential function of anticoagulant system in patients with VD CKD. Thus, those data confirm FIPF accumulation to be rather early marker of activation of coagulation system and possible thrombosis [8]. Stable moderate negative correlation between FIPF and C protein, which is known to be not only a physiological inhibitor of activation of blood coagulation system but also a regulator of fibrinolysis system, was observed as well [8, 12]. Such depressive state of anticoagulant component along with activation of coagulation factors may be one of conditions leading to thrombophilia.

CONCLUSIONS

1. There is close relationship between sF level and pre-thrombosis factors – Fg and FIPF, as well as between sF and post-thrombosis factor – D-d.
2. Increased sF concentration is associated with depressive state of anticoagulant component of hemostasis presented by pC.
3. Accumulation of FIPF along with decreased activity of natural anticoagulant – pC, may be one of predictors of thrombotic complications development in patients with VD CKD.

Directions for future research: Comprehensive study of major components of hemostasis in patients with VD stage of CKD provides opportunity of extending prophylactic measures to prevent thrombophilia.

REFERENCES

1. Melnik A. Sistema gemostaza i yeye regulyatsiya pri narushenii funktsional'noy sposobnosti pochek. [System of hemostasis and its regulation in renal dysfunction]. News of Medicine and Pharmacy in Ukraine. 2016;9(583):24-31. (in Russian).
2. Heit J. Thrombophilia: common questions on laboratory assessment and management. Hematology Am Soc Hematol Educ Program. 2007;1:127-135.
3. Bernakevich A., Vasiliev C., Eskin N. Sostoyaniye sistemy gemostaza u patsiyentov, podvergayushchikhsya endoprotezirovaniyu tazobedrennogo sustava [Status of hemostasis system in patients undergoing hip replacement]. Journal of Traumatology and Orthopedics. 2009;1:37-41. (in Russian).
4. Shevchuk S., Gornytska O., Chernyshenko T. et al. Kompleksna diahnostyka trombofiliyi za systemnoho chervonoho vovchaka [Comprehensive diagnostics of thrombophilia in systemic lupus erythematosus]. Laboratory Diagnostics. 2010;1:3-8. (in Ukrainian).
5. Lugovskoi E., Kolesnikova M., Lugovskaya N. et al. Kolichestvennoye opredeleniye D-dimera i rastvorimogo fibrina v plazme krovi cheloveka pri ishemicheskoy bolezni serdtsa i gipertonicheskoy bolezni [Quantitative determination of D-dimer and soluble fibrin in blood plasma of patients with ischemic heart disease and hypertension]. Ukrainian Biochemistry Journal. 2006;78(4):120-129. (in Russian).
6. Platonova T., Gornytska O., Moroz E. Zastosuvannya aktyvatoru proteyinu S z otruty shchytomordnyka zvychaynoho (Agkistrodon halys halys) dlya vyznachennya aktyvnosti proteyinu S u plazmi krovi za riznykh patolohiy [Use of protein C activator from copperhead snake venom (Agkistrodon halys halys) to determine the activity of protein C in blood plasma in various pathologies]. Laboratory Diagnostics. 2004;3:28-31. (in Ukrainian).
7. Sokolovska A., Platonova T., Grynenko T. et al. Porivnyal'na kharakterystyka metodiv vyznachennya vmistu fibrynohenu v plazmi krovi [Comparative characteristics of methods to determine blood plasma fibrinogen content]. Experimental and Clinical Physiology and Biochemistry. 2002;3:82-86. (in Ukrainian).
8. Storozhuk L., Shevchuk S., Storozhuk B. et al. Kliniko-laboratorna diahnostyka trombofiliy u khvorykh XXH VD stadiyi, shcho perebuvayut' na prohramnomu hemodializi [Clinical and laboratory diagnosis of thrombophilia in patients with stage VD CKD undergoing long-term hemodialysis]: Methodical recommendations. Vinnytsia: FOP Rogalska I.O. 2017. (in Ukrainian).

9. Storozhuk O., Selezneva I., Storozhuk L. et al. Funktsional'no neaktyvni formy protrombinu ta riven' fibrynohenu, yak markery hiperkoahulyatsiyi u khvorykh na khronichnu khvorobu nyrok VD stadiyi, yaki perebuvayut' na prohramnomu hemodializi [Functionally inactive forms of prothrombin and fibrinogen level as markers of hypercoagulation in patients with chronic kidney disease VD stage undergoing long-term hemodialysis]. Bulletin of Vinnytsia National Medical University. 2017;21(2):450-453. (in Ukrainian).
10. Mosnier L., Mejers J., Bouma B. The role of protein C in activation of thrombin activatable fibrinolysis inhibitor (TAFI) and regulation of fibrinolysis. Thrombosis and Haemostasis. 2001;86(4):1040-1046.
11. Dohlback B., Villoutreix B. Regulation of blood coagulation by the protein C anticoagulant pathway. Arteriosclerosis, Thrombosis and Vascular Biology. 2005;15:1311-1320.
12. Volkov G., Platonova T., Savchuk A. et al. Sovremennyye predstavleniya o sisteme gemostaza. [Modern ideas on hemostasis system]. Kyiv: Naukova dumka; 2005. (in Russian).

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ORIGINAL ARTICLE

GENOMIC AND EPIGENOMIC PREDICTORS FOR VARIOUS CLINICAL PHENOTYPES OF MYASTHENIA GRAVIS

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ABSTRACT

The aim: To evaluate the relationship of certain alleles of HLA class II leukocyte antigens and the profile of antibodies to various subunits of nicotinic acetylcholine receptors (nAChR), the level of Treg lymphocytes and the serum concentration of anti-inflammatory IL-10 for various clinical myasthenia gravis phenotypes.

Materials and methods: We examined 217 patients with thymus-independent myasthenia (n = 42) and thymus-dependent myasthenia, among them patients with thymus hyperplasia (n = 108) and thymoma (n = 67). We used the following methods: ELISA, flow cytometry, light and fluorescence microscopy.

Results: Certain genomic (polymorphism of leukocyte HLA-DR antigens) and epigenomic (antibodies to α1 and α7 nAChR subunits, expression of Treg lymphocytes and concentration of cytokines) predictors were identified for various myasthenia phenotypes. The presence of HLA haplotypes DR2 and DR7 in some young patients with M with disease progression led to the development of myasthenia gravis with thymoma (MT) at an older age. The presence of α7 nAChR subunit on thymocyte mitochondria was revealed, which is an additional autoimmune target for autoantibodies in patients with myasthenia gravis. An increase in the concentration of cytokines (IL-4, IL-8, IFN-γ) in all patients with myasthenia gravis was revealed.

Conclusions: Estimate the features of the formation of various variants of the immune response in thymus-independent and thymus-dependent myasthenia gravis is a necessary condition for targeted immunocorrection or surgery.

KEY WORDS: myasthenia gravis, leukocyte antigens HLA-DR, nAChR, Treg-lymphocytes

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INTRODUCTION

Recently, the frequency of autoimmune pathology, which includes myasthenia gravis, previously considered a rare disease, has increased many times. And now the frequency of the disease has increased 10-15 times. In myasthenia gravis, autoimmune processes are induced, the mechanisms of which affect various levels of the body from systemic to subcellular. In myasthenia gravis, autoimmune processes are induced, and their mechanisms affect various levels of the body from systemic to subcellular. Disorders of neurotransmitter reactions and progressive muscle weakness are formed in patients with myasthenia, which develop against the background of structural and functional disorders of the thymus [1]. Myasthenia gravis is clinically heterogeneous, synaptic disorders have different localization, and structural and functional changes of the thymus can manifest in the form of dysplasia, hyperplasia or thymoma [2,3].

Myasthenia gravis has a multifactorial nature, depending on various etiological factors, such as genetic and epigenetic. The debut of the disease in patients with myasthenia gravis, as a rule, corresponds to the irreversible phase of the process, when many links of the pathogenesis are formed [4]. The standard treatment for myasthenia gravis involves the use of drugs aimed at eliminating the effects of autoimmune aggression (anticholinesterase drugs, corticosteroids, plasmapheresis), which usually gives a short-term effect. Surgical removal of the thymus, as the target of autoimmune aggression, does not always allow avoiding anticholinesterase drugs, and sometimes leads to the development of myasthenic and cholinergic crises (in 20% of patients). And, as a rule, after a thymectomy, myasthenic disorders resume. The applied complex treatment of myasthenia gravis is effective only in 30–40% of patients [5, 6]. Further progress in the treatment of myasthenia gravis is not possible without understanding the fundamental mechanisms of the development of autoimmune pathology. There is a problem of differential diagnosis and the choice of individual treatment tactic for various types of myasthenia gravis.

THE AIM

To evaluate the relationship of certain alleles of HLA class II leukocyte antigens and the profile of antibodies to various subunits of nicotinic acetylcholine receptors (nAChR), the level of Treg lymphocytes and the serum concentration of anti-inflammatory IL-10 for various clinical myasthenia gravis phenotypes.

MATERIALS AND METHODS

We examined 217 patients with thymus-independent and thymus-dependent myasthenia (with the morphological and functional changes in the thymus – hyperplasia and thymoma), which were divided into groups depending on the clinical phenotype of this disease. The first group was consisted of 42 patients with thymus-independent myasthenia (M), the second group included patients with myasthenia with thymus hyperplasia (MH) – 108 patients, the third group – 67 patients with myasthenia and thymoma (MT).

We studied genomic (HLA-DR) and epigenomic (antibodies to α1 and α7 nAChR subunits, differentiation clusters CD45+ and CD4+CD25+, cytokine profile) factors in myasthenia gravis. The possible association of the presence of haplo- and diplotypes of leukocyte antigens of the second class HLA-DR with various clinical phenotypes of myasthenia gravis in a microlymphocytotoxic test was evaluated [7].

The determination of antibodies to the α1 and α7 subunits of nAChR was performed by enzyme-linked immunosorbent assay (ELISA) using the recombinant extracellular domains α1 (1-208) and α7 (1-208) as antigens [8].

The method of determining the presence of an additional autoimmune target of α7 subunits of nAChR in mitochondria was performed in thymus and thymoma preparations. A cell suspension was obtained by homogenizing the organ in Hanks solution and filtering through a nylon filter. The preparation of isolated mitochondria from the thymus and thymoma was obtained by differential centrifugation. To obtain a mitochondrial precipitate, the isolation medium of the following composition was used: 10 mM HEPES, 200 mM sucrose, 1 mM EDTA-1 (pH 7.4). Isolation was carried out at 2 °C. Mitochondria were suspended in an incubation medium of the following composition: 10 mM HEPES, 125 mM KCl, 25 mM NaCl, 0.1 mM P (as K-phosphate buffer; pH 7.4), 5 mM sodium succinate (22 °C). The protein concentration in mitochondria was determined by the Bradford method and was 0.20 mg/ml. The level of α7 mitochondrial nAChR subunits in thymus preparations was determined by ELISA [8].

The expression value of the total leukocyte antigen CD45 – tyrosine-specific phosphatase on lymphocytes was determined by indirect immunofluorescence method using monoclonal antibodies ("Sorbent", Russia) labeled with FITC-stain. The cells stained by corresponding antibodies were visualized by fluorescent microscope Olympus BX53.

The regulatory lymphocyte population (Treg) was analyzed by flow cytometry (Cytomics FC500, Beckman Coulter, USA) using monoclonal antibodies CD4-PE, CD25-FITC (Beckman Coulter, USA) [9].

Determination of the serum concentration of IL-4, IL-8, IL-10 and IFN-γ was carried out using a test system for ELISA using monoclonal antibodies adsorbed on polystyrene plates ("Vector-Best", Ukraine). The resulting antigen-antibody complex was detected using a conjugate whose peroxidase catalyzes the cleavage of the substrate (hydrogen peroxide), causing a change in the color of the indicator. The optical density was measured at a wavelength of 450 nm (StatFax 3200, USA) [10].

The results were analyzed using Student's t-test. Data were presented as mean ± standard deviation (m ± SD), using the software package "Statistika V.6".

RESULTS

High heterogeneity of the presence of class II HLA alleles in patients with myasthenia was revealed. The incidence of the DR5 phenotype in thymus-independent myasthenia gravis was 60% and it was 2 times higher than the occurrence of the DR1 and DR2 diplotypes, and 3 times more often than the DR3, DR7, DR52 phenotypes. In thymus-dependent myasthenia gravis phenotypes DR1 and DR5 were determined with a high frequency. The DR1 phenotype was found in 60% of cases, the DR5 phenotype was found in 70% and the DR2, DR3, DR7, DR52 phenotypes were much less common (Table 1). In the group of patients with thymus-dependent myasthenia it was revealed the higher frequency (89%) of a DR7 leukocyte antigen diplotype. In this group the DR2 haplotype was also detected – 38.5%.

Among patients with the DR1 phenotype 24% of them were patients with thymus-independent myasthenia gravis, 25% were patients with thymus hyperplasia and 19% – myasthenia gravis with thymoma. Among patients with the DR1 phenotype, there were no patients with myasthenic crisis, and only 9.5% of patients with myasthenic syndrome were detected. It can be assumed that this phenotype is more closely associated with pathomorphological changes in the thymus than with the severity of neuromuscular transmission disorders. Among patients with the DR2 phenotype 38% of them were patients with myasthenia without thymus lesions, 19% were patients with thymoma and myasthenia gravis.

Patients with myasthenia without thymus lesions were accounted for 28% of all patients with the DR5 phenotype and 21.8% with hyperplasia. The incidence of various clinical forms of myasthenia among carriers of the DR7 phenotype is less heterogeneous, however, carriers of this phenotype are more common in myasthenia gravis with thymoma. The identified sample of carriers of the DR52 phenotype in 33.3% of cases was presented by patients with neurotransmitter disorders without damage of the thymus. Approximately 16% were patients with hyperplasia and thymoma.

The concentration of autoimmune antibodies to α1 nAChR was higher than the control values in all of the examined groups; this indicator was highest in the group

Table I. Genomic markers in patients with thymus-independent (M) and thymus-dependent myasthenia (MH, MT)

The frequency of haplo- and diplotypes HLA-DR II class in myasthenia gravis		
Thymus-independent	**Thymus-dependent myasthenia**	
M	**MH**	**MT**
HLA DR2 – 35% HLA DR3 – 20% HLA DR5 – 60% HLA DR7 – 25%	HLA DR1 – 60% HLA DR5 – 70%	HLA DR2 – 38% HLA DR7 – 89%

Table II. The content of antibodies to α1 and α7 subunits of nAChR in patients with various clinical phenotypes of myasthenia gravis

	Control group	Thymus-independent myasthenia (M)	Thymus-dependent myasthenia	
			Thymus hyperplasia (MH)	Thymoma (MT)
Antibodies to α1 nAChR, units OD	0.180 ± 0.43	0.373 ± 0.052	0.389 ± 0.065	0.281 ± 0.027
Antibodies to α7 nAChR, units OD	0.180 ± 0.37	0.343 ± 0.089	0.268 ± 0.034	0.198 ± 0.021

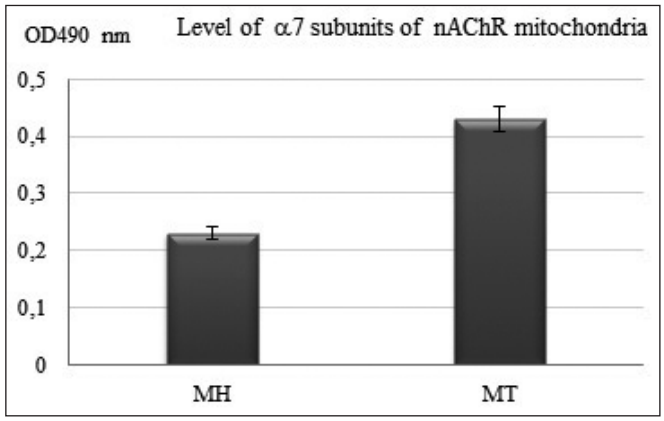

Fig. 1. Change in the level of α7-subunits of nAChR of mitochondria in thymus preparations of patients without (MH) and with thymoma (MT)

of patients with myasthenia gravis and thymus hyperplasia (0.389 ± 0.065 units OD). The content of antibodies to α7 nAChR was increased in groups of patients with myasthenia gravis without morphological and functional changes of the thymus and with myasthenia gravis on the background of thymic hyperplasia (Table 2).

The investigation of the characteristics of thymus cells, the central organ of immunity, will allow us to study some mechanisms of the central self-tolerance loss. As is known, nicotinic acetylcholine receptors (nAChR), related to the superfamily of ligand-dependent ion channels, are localized in synapses and ganglia of the central nervous system. These receptors containing the α7 subunit regulate apoptosis initiation processes by influencing voltage-dependent ion channels [11].

In patients with myasthenia gravis after thymectomy and thymomectomy (MH and MT), thymus and thymoma cells revealed the presence of α7 subunits of nAChR, which are expressed on the outer mitochondrial membrane. The level of α7 subunits of nAChR of mitochondria in the thymus in patients with thymus hyperplasia (MH) was minimal and amounted to 0.23 units E. The maximum level of α7 subunits of nAChR on mitochondria was detected in thymus preparations with thymomas (MT group) – 0.43 units E., which was in 1.9 times higher than in mitochondria of the thymus (Fig. 1).

The localization of the α7 subunit of nAChR in mitochondria may be an additional target in the development of the processes of central self-tolerance loss in the thymus.

Inhibition of CD45+ expression on immunocompetent cells showed a decrease in the expression of total leukocyte antigen in all patients with myasthenia gravis (Fig 2 A, B, C).

The minimum level (80.0 ± 3.4%) of expression of the CD45 transmembrane protein was revealed in the group with thymus-independent myasthenia M (Fig. 2A, 3), which, in turn, is associated with a decrease in the degree of signal transmission from antigenic lymphocyte receptors to the cell core for transcription induction.

In the regulation of immunity, in particular in the induction of immune suppression, the main role is played by regulatory CD4+CD25+ T lymphocytes. The insufficiency of the function of Treg cells is of great importance in the regulation of control during the development and progression of autoimmune diseases, in the process of oncogenesis, and in the regulation of immunity in infections [12], and also the function of CD4+CD25+ cells in autoimmune myasthenia is important. In this study, all patients showed a decrease in the expression of Treg cells. In the MH group, the level of cells with the CD4+CD25+ phenotype was on average 30% lower than in the control group. Probably, in the autoimmune myasthenia group with thymus hyperplasia (MH), CD4+ T cells with high expression of CD25 molecules prevailed to inhibit autoimmune processes, since such cells have suppressor activity (Fig. 4). In groups M and MT, a 4-fold decrease in the expression of CD25+ on CD4+ T cells was revealed in comparison with the control. These data indicate the presence of similar mechanisms of self-tolerance loss in these groups. Regulatory T lymphocytes CD4+CD25+ are a source of IL-10, which regulates autoimmune reactions. A decrease in the expression of Treg lymphocytes leads to a decrease in the synthesis of IL-10, which was noted in groups M and MT.

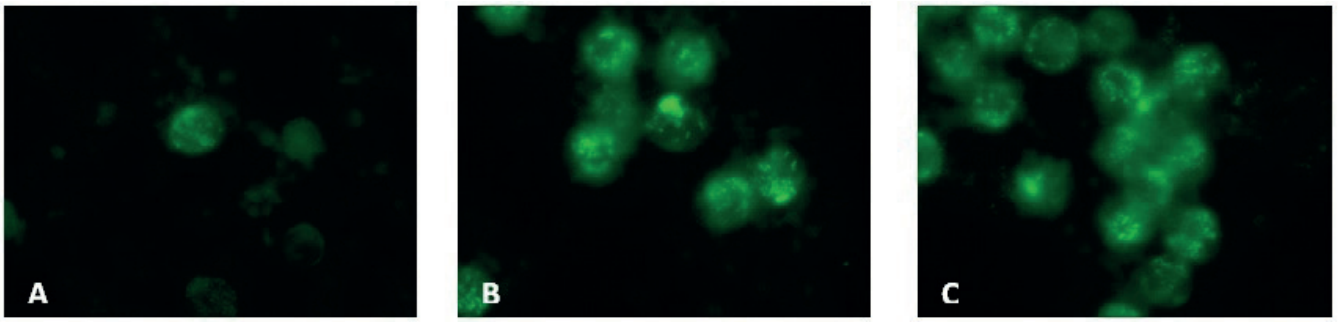

Fig. 2. Expression of CD45+ in patients with thymus-independent and thymus-dependent myasthenia gravis, stained with FITC, x1000: A – group M, 87%; B – group MH, 91%; C – group MT, 92%

In all examined patients with myasthenia, an increasing the levels of IL-4, IL-8, and IFN-γ relative to the control was revealed. The maximum value of IL-4 was found in patients with thymomas – (666.4 ± 44.5) mmol/l. With thymus hyperplasia against the background of myasthenia gravis, a multiple increasing the content of IL-4 was also revealed – (492.5 ± 52.8) mmol/l (Table 3). An increasing the level of serum IL-4 concentration accompanies the development of anti-infection immunity according to the type of Th2 dependent antibody formation, since activated IL-4 Th2 lymphocytes control the development of a humoral immune response.

The maximum increasing of IL-8 was detected in patients with myasthenia gravis, which was accompanied by a tumor of the thymus, since an increasing of this cytokine is often associated with the induction of oncogenesis, and it stimulates the release of active oxygen radicals and other bioaggressive substances. The content of IFN-γ was increased in all groups, and the maximum increase (15 times) was revealed in the MH group (Table 3). Therefore, in patients of the MH group, differentiation along the Th1 pathway prevails.

DISCUSSION

The relationship between the clinical phenotypes of myasthenia gravis and the variants of HLA leukocyte antigens, which are genetic factors that form various types of immune reactivity and determine resistance vectors, has been revealed. The characteristic associations of leukocyte antigens in M, MH and MT can be used as prognostic markers for the development of types of immune imbalance that develops during the latent period of the formation of autoimmune pathology or during remission. The presence of HLA haplotypes DR2 and DR7 in some young patients with M with disease progression led to the development of myasthenia gravis with thymoma (MT) in the elderly.

The maximum increasing of the concentration of antibodies to the α1 and α7 subunits of nAChR was found in patients with M and MH. In patients with thymoma the concentration of specific antibodies to different domains of nAChR did not significantly differ from the control values. Therefore, in patients with MT, the pathogenetic factor of the development of myasthenic syndrome and crisis may have other mechanisms that are not associated with the presence of antibodies to nAChR.

According to Hurst et al. (2013) the function of mitochondrial nAChR is to control the formation of a mitochondrial pore of transitional conductivity, which is a source of proapoptotic factors and reactive oxygen species released in the cytosol [11]. In additional, the level of α7 subunits of nAChR is higher in the thymus of patients with thymoma, therefore, tumor transformation is accompanied

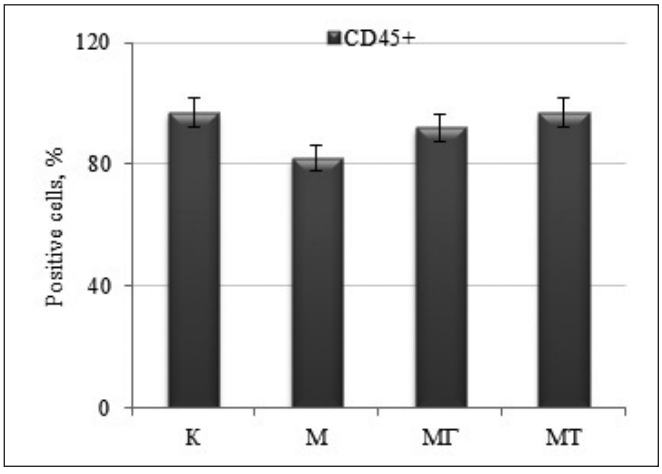

Fig. 3. Expression of CD45+ (%) in patients with various clinical phenotypes of myasthenia gravis

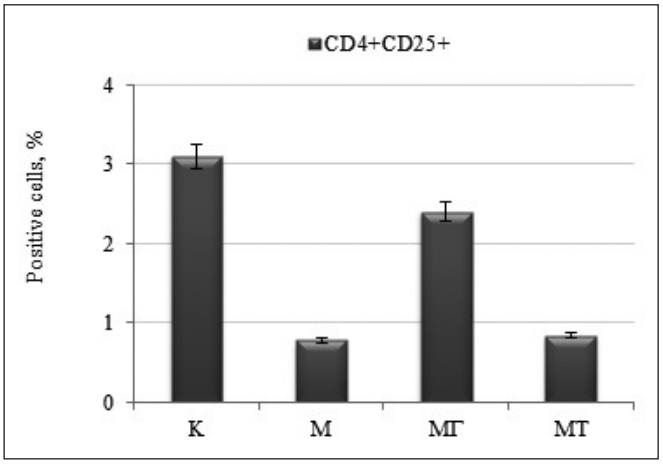

Fig. 4. The level of a subpopulation CD4+CD25+ Treg-lymphocytes in patients with various clinical phenotypes of myasthenia gravis

Table III. Change in the level of interleukins in patients with thymus-independent (M) and thymus-dependent myasthenia gravis (MH, MT)

Index	Control group	M	MH	MT
IL-4, mmol/l	70.0 ± 22.9	337.3 ± 59.4	492.5 ± 52.8	666.4 ± 44.5
IL-8, pg/ml	10.0 ± 8.4	72.3 ± 21.5	88.3 ± 34.2	590.9 ± 67.1
IFN-γ, pg/ml	9.4 ± 2.3	67.1 ± 10.4	147.0 ± 27.3	48.5 ± 5.3

by an increase in mitochondrial nAChR, which supports the viability of tumor cells.

It is known that the regulatory function of T lymphocytes is to control the strength and duration of the immune response by regulating the ratio of helper and cytotoxic T cells. Activation of T lymphocytes in patients with M, which was estimated by the expression of the main leukocyte antigen of the CD45+, was the lowest in comparison with the value of this indicator in MH and MT. Membrane-binding phosphatase CD45+ can act as a diagnostic criterion for the development of immune imbalance in the presence of acute infection.

The regulatory population of T cells plays an important role in suppressing immune hyperactive reactions and prevents autoimmune and allergic diseases, but at the same time they can reduce anti-infection and antitumor immunity. Treg-cells can play, on the one hand, a protective role, limiting the development of the inflammatory process, and on the other, an increase in the number of Treg lymphocytes can lead to an increased risk of concomitant infections as a result of a decrease in the proliferative and functional activity of effector cells, which was observed in patient with thymus hyperplasia (MH). That is, there is a change in the functional state of regulatory T lymphocytes, which, in turn, leads to a change in the body's resistance to infections. In the literature, there is a tendency for an increase in subpopulations of CD4+CD25+ cells as the disease progresses [13, 14]. In our studies, this tendency is observed in the MH group, where the level of CD4+CD25+ is 3 times higher than in the M and MT groups. At the same time, in patients with thymus-independent (M) and thymus-dependent (MT) myasthenia gravis, we did not reveal sufficient expression of CD4+CD25+. But, since autoimmune diseases are accompanied by impaired immunological tolerance, in the formation of which a special role belongs to the population of Treg cells, therefore, probably in the M and MT groups, the insufficiency of regulatory cells is primarily associated with the pathogenesis of these myasthenia phenotypes.

Regulatory cytokines can take part in the self-maintenance of the pathological process and determine the strategy for the formation of a specific metabolic pattern. A change in the concentration of cytokines, along with other lysing cytotoxic factors, can lead to the formation of autoimmune conditions. A subpopulation of Th2 lymphocytes produces IL-4, which is significantly increased (i.e., it is an indirect autoimmunization factor) in patients with MH and MT. Patients with MT revealed a sixty-fold increase in the concentration of IL-8, which is a marker of oncogenesis. Obviously, an increase in the concentration of IL-8 plays a pathogenetic role in the development of the pathology of synaptic transmission and morpho-structural changes in the thymus, and this leads to the formation of locally distributed thymus.

CONCLUSIONS

Certain genomic (polymorphism of leukocyte HLA-DR antigens) and epigenomic (antibodies to α1 and α7 subunits nAChR, expression of Treg lymphocytes and concentration of cytokines) predictors were identified for various myasthenia gravis phenotypes. Evaluation of the features of the formation of various variants of the immune response in thymus-independent (M) and thymus-dependent (MH, MT) myasthenia gravis is necessary for targeted correction of metabolic and immunological disorders. An analysis of changes in specific biomarkers, taking into account the genomic component, will allow for reasonable targeted immunocorrection or surgery.

REFERENCES

1. Priola A.M., Priola S.M. Imaging of thymus in myasthenia gravis: from thymic hyperplasia to thymic tumor. Clinical Radiology. 2014;69(5):e230-e245.
2. Berrih-Aknin S. Myasthenia Gravis: paradox versus paradigm in autoimmunity. Journal of Autoimmunity. 2014;52:1-28.
3. Klimova E.M., Lavinskaya E.V., Minukhin D.V. et al. On forming central and peripheral markers of self-tolerance loss in diverse clinical myasthenic phenotypes. Der Pharmacia Lettre. 2017; 9(6):8-17.
4. Bojko V.V., Klimova E.M., Kudrevich A.N. Lechenie miastenii s uchetom immunofiziologicheskih fenotipov. [Treatment of myasthenia gravis taking into account immunophysiological phenotypes]. Kharkov: Publishing House Sheinina E.V. 2008: 424. (in Russian).
5. Nazarbaghi S., Amiri-Nikpour M.R., Mahmodlou R. et al. Clinical outcomes of myasthenia gravis with thymoma and thymic hyperplasia undergoing extended transsternal thymectomy: a single-center experience. North American Journal of medical sciences. 2015;7(11):503-508.
6. Klimova E.M., Bozhkov A.I., Boyko V.V. et al. Endogenic cytotoxic compounds and formation of the clinic forms of myasthenia. Translational Biomedicine. 2016;7,3,84:1-16.
7. Schreuder G.M.Th. The HLA dictionary 2004: a summary of HLA-A, -B, -C, -DRB1/3/4/5 and –DQB1 alleles and their association with serologically defined HLA-A, -B,-C, -DR and –DQ antigens. International journal of immunogenetics. 2005;32(1).
8. Gergalova G.L., Lehmus O.J., Skok M.V. Possible effect of activation of α7-nicotinic acetylcholine receptors in the mitochondrial membrane on the development of apoptosis. Neurophysiology. 2011;43(3):195-197.
9. Parcs D.R., Lanier L., Herrengerg L.A. Flow cytometry and fluorescense activated cell sortins (Facs). Handbook of Experimental Immunology. 1986;302–324.
10. De La Rica R., Stevens M.M. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nature Nanotechnology. 2012;7(12),12:821.

11. Hurst R., Rollema H., Bertrand D. Nicotinic acetylcholine receptors: from basic science to therapeutics. Pharmacology therapy. 2013;137(1):22-54.
12. Sadeghifard N.T., Talaei M.H., Naser A. et al. Maleki regulatory cell and body tolerance. Der Pharmacia Lettre. 2017;9(5):23-28.
13. Fattorossi A., Battaglia A., Buzzonetti A. et al. Circulating and thymic CD4+CD25+ T regulatory cells in myasthenia gravis: effect of immunosuppressive treatment. Immunology. 2005;116:134–141.
14. Balandina A., Lecart S., Dartevelle P. et al. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005;105:735–741.

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ORIGINAL ARTICLE

APPLICATION OF INTELLECTUAL MONITORING INFORMATION TECHNOLOGY IN DETERMINING THE SEVERITY OF THE CONDITION OF PATIENTS WITH INFLAMMATORY BOWEL DISEASES

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ABSTRACT

The aim: Was to evaluate the effectiveness of the use of information technology of intelligent monitoring in solving the problems of assessing the morbidity of a patient with IBD during treatment.

Matherials and methods: 183 patients with IBD were observed. Among them 104(56.8%) patients suffered from Crohn's disease and 79(43.1%) patients had ulcerative colitis. For each patient and each disease, the formation of a list of signs, the extraction of information and knowledge will be carried out according to an individual method. At the lower level, tasks are performed: determination of the list of patient morbidity conditions, the formation of a list of indicators of the patient morbidity conditions, their identification as classes for machine learning models; formation of a list of signs, which identify the state of the patient's morbidity and whose characteristics obtained after results of medical tests.

Results: The number of correctly classified points reached 92%. An analysis of the conditions of patients characterized by incorrectly classified points revealed the information content of this fact. In those cases when the classification results did not coincide with the expert assessment of the patient's condition, additional factors were found that influenced his condition and whose characteristics were not taken into account in the structure of classifier models.

Conclusions: The results of the testing of classifier models indicate the effectiveness of the use of information technology of intelligent monitoring to assess the condition of patients with IBD.

KEY WORDS: Intellectual monitoring, information technology, inflammatory bowel diseases

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INTRODUCTION

One of the main problems in monitoring inflammatory bowel diseases (IBD) is the high level of uncertainty in the causes of these pathologies. This is due to the physician's limited ability to study the development processes of this pathology in various classes of patients and a significant level of individuality in the processes of disease formation and the course of the disease. The expansion of the doctor's capabilities is achieved today by applying data processing technologies, in particular modeling [1]. The simulation results largely depend on the understanding by the designer of the subject area, knowledge of the technologies for the formation of the initial description of the object and the formation of a database with the results of observations, the successful use of methods for constructing models, the correct application of the model testing process and the interpretation of modeling results.

It is also assumed that cost software is available that can adapt to changing the individual properties of each patient. A distinctive feature of this study is that its authors use intelligent monitoring technology as a whole, rather than modeling an individual element, to achieve their goals. In a single process of generating input data arrays (IDA), expert doctors, scientists, analysts, and fashion designers with intelligent monitoring technologies are combined. A monitoring intelligent system is also used – a software package that implements the process of obtaining information from observation results, turning it into a knowledge base and using this database as an algorithm for converting new data [2, 3].

The synthesis of individual models is carried out on the basis of the initial description obtained from the results of observations of a group of patients — determining the characteristics of the patient's condition by conducting medical tests at different stages of the disease. The primary description is formed by recording the numerous characteristics of significant factors, a list of which is formed by an expert doctor [4].

The indisputable advantage of ITIM is that the monitoring technology is built for each external order individually for each decision-making process. That is, the formation of a sequence of stages of observation is technologically

ensured to build a base of model knowledge about the disease process of each patient individually [5].

THE AIM

The aim of the study is to evaluate the effectiveness of the use of information technology of intelligent monitoring in solving the problems of assessing the morbidity of a patient with IBD during treatment.

It is required to solve the problem of classifying the patient's conditions when there is a classification of observation points for a patient with IBD in a multidimensional space of signs X results of medical tests. It is necessary to build the rules for the reflection of the set of signs of X to the set of classes P (determination of disease states)

The results of this task are used to create a non-invasive method in the diagnosis of the patient's morbidity by processing the results of standard medical tests and machine learning models, without painful procedures and complications after diagnosis.

MATERIALS AND METHODS

183 patients with IBD were observed. Among them 104(56.8%) patients suffered from Crohn's disease and 79(43.1%) patients had ulcerative colitis. All patients population was aged from 17 to 60 years. Duration of IBD were 1.2-9.4 years. 20 patients with a diagnosis of irritable bowel syndrome and 20 healthy persons were in control group.

In all patients complaints, clinical picture of the disease were analyzed before and after treatment, during 3 years of observations. Harvey Bradshaw CD Activity Index for Crohn's disease patients, Truelove-Witts criteria and Mayo Index for ulcerative colitis patients were used [6].

Laboratory parameters were studied. In all IBD patients and in control group levels of highly sensitive CRP, homocysteine, platelets, vitamin D, fecal calprotectin and albumin in the urine were observed (table I). During observation period all IBD patients obtained treatment according to protocols of European Crohn's and Colitis Organisation.

For each patient and each disease, the formation of a list of signs, the extraction of information and knowledge will be carried out according to an individual method.

According to the methodology for creating information technologies of multi-level intelligent monitoring [7], each local monitoring task is solved by constructing a separate model, a set of models of one level of information conversion are formed by strata, strata are combined into a global functional dependence. The combination of global functional dependencies forms the base of model knowledge [8]. The base of model knowledge is included in the structure of intelligent agents and multi-agent systems and determines their functioning as intended. Based on multi-agent systems, the information technology of multi-level intelligent monitoring is being built [2]. In accordance with the objectives of this work, two levels of information conversion were identified. At the lower level, the fulfillment of the complex of tasks is aimed at ensuring the reflection of the elements of the set of results of analyzes of X to the elements of the set of indicators of the state of the patient Y. At the upper level, it is necessary to ensure the grouping of the elements of the set of indicators of the conditions into classes of the patient's incidence. The constructed models solve the problems of transforming information of one level, form a stratum.

At the lower level, tasks are performed:

1) determination of the list of patient morbidity conditions, the formation of a list of indicators of the patient morbidity conditions of the set Y = {y 1, y 2, ..., y m}, their identification as classes for machine learning models;

2) the formation of a list of signs of the set X = {x 1, x 2, ..., x n}, which are sufficient to identify the state of the patient's morbidity and whose characteristics are planned to be obtained after tillage and the results of medical tests;

3) conducting research on patients. Standard procedures are carried out for the expert assessment of the patient's condition by the doctor using traditional methods of diagnosis, prescribing medical tests, processing their results, presenting the results in a standard form of a two-dimensional table of data for the initial description of the patient:

$$\begin{pmatrix} x_{11} & x_{12} & \cdots & x_{1n} & y_{11} & y_{12} & \cdots & y_{1m} \\ x_{21} & x_{22} & \cdots & x_{2n} & y_{21} & y_{22} & \cdots & y_{2m} \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ x_{k1} & x_{l2} & \cdots & x_{kn} & y_{k1} & y_{k2} & \cdots & y_{km} \end{pmatrix}, \quad (1)$$

where xij is the j-th indicator of the patient's analysis of the i-th observation, yij is the j-th indicator of the patient's condition of the i-th observation, k is the number of series of analysis of observations; n is the number of indicators planned to be obtained by the doctor according to the results of the analyzes, m is the number of characteristics of the patient's condition.

4) the construction of models that contain a solution to the problem of identifying the functional dependence of state indicators Y on the array of monitoring results (medical tests) X:

$$y_i = f(x_j),\ i = 1, m;\ j = 1, n, \quad (2)$$

where n is the number of indicators planned to be obtained by the doctor according to the results of the analyzes, m is the number of characteristics of the patient's conditions.

At the top level of information conversion, the following tasks are performed:

1) study of the output signals of the lower stratum models, the formation of an input data array and the synthesis of the upper start models [3];
2) the synthesis of classifier models for grouping points (monitoring the patient's condition) according to the morbidity classes;
3) model testing and interpretation of classification results. The formulated classification problem is poorly formalized. Signs of belonging of the observation point to the class of disease states according to the results of medical tests cannot be clearly set without additional diagnostic procedures.

Therefore, for each class of states, a classifier model is built. Models are built for each feature (there are rules for reflecting multiple features into multiple classes P). After that, decision rules are built (the second level of monitoring), which determine the patient's belonging to one of the classes (conditions) by:

1. Calculation of the value of one of the modeled indicators of the state of the set Y = {y1, y2, ..., ym} by substituting the results of medical tests from the set X = {x1, x2, ..., xn}. The vector of values of the results of the analysis is converted into the numerical value of the sign of the patient's condition.
2. The formation of an array of input data (MVD) for the stratum of the second level. Models of the second-level stratum contain solutions to the classification problem: construct a resolving rule a, which will correctly identify the new state of the patient (3):

$Z_i = f(y_j)$, $i = 1, k$; $j = 1, m$ (3)

to one of the described classes $P = \{p_1, p_2, \ldots p_l\}$:

a: $Z \rightarrow P$ (4)

A model is adequate if, according to the results of its use, a significant number of patient conditions are correctly classified. A significant number of correctly classified states that allow us to consider the model adequate is set by an expert method. The model is stable in the case when it is possible to provide the specified indicators of adequacy on the examination sequence of the patient's conditions, the characteristics of which were not used in the process of model building. If the model is adequate and stable, then this means that the structure of the patient's body is correctly reflected in its structure, that is, the model contains information. In this case, the results of the assessment of the influence of factors [3] will be reliable.

Adequacy and sustainability are characteristics of the utility of the model. A model is considered useful when, based on the results of its use, information about the patient is obtained, which allows the doctor to achieve the patient's recovery, or at least to improve his condition.

Simulation processes are used to transform data in monitoring intelligent systems. The monitoring system is built to provide information to decision-making processes in a given subject area. The decision maker determines the consequences of applying each of the available strategies.

In conditions of medical monitoring, the person making the decision is a doctor, strategies are treatment regimens, the object of monitoring is the patient's condition.

The purpose of medical monitoring is to provide the doctor with information about the patient's condition, that is, the diagnosis, about the properties of the body in the fight against the disease, about the mechanisms of disease formation and to predict the change in the patient's condition when applying one or another treatment regimen.

RESULTS

To accomplish the tasks, the following hypothesis was formulated: "Determining the condition of a patient with IBD is possible by constructing classifier models with an intelligent monitoring information system and expert

Table I. The list of features that were used to form the primary description

№ in order	sign	a comment	variable
1	Highly sensitive CRP, mg / L	C-reactive protein, highly sensitive method (h / sCRP) - a study that reveals a slight excess concentration of C-reactive protein. In general, an increase in the level of C-reactive protein (CRP) with high sensitivity indicates an inflammatory process that occurs in the body [9, 10].	x_1
2	Homocysteine, μmol / L	One of the markers of endothelial dysfunction is a change in the level of homocysteine, which is a replaceable cytotoxic and neurotoxic amino acid, which is present in all cells of the body and plasma in small quantities. data indicate that the level of homocysteinemia can serve as an independent marker of pathogenetic changes in IBD [6, 9, 10].	x_2
3	Platelets, thousand/mm ^{3}	Blood cell characteristics, but in this study are considered as a marker of inflammation activity. With their increase, the risk of blood clots increases against the background of the damaging effect of homocysteine on the inner shell, vascular intima, thrombosis of small vessels may develop with a decrease in oxygen supply to tissues, which is important in conditions of relative energy deficiency that occurs with inflammation	x_3
4	Vitamin D, ng / mL	A group of biologically active substances (including cholecalciferol and ergocalciferol). The main function of both cholecalciferol and ergocalciferol is to ensure the absorption of calcium and phosphorus from food in the small intestine. We can also assume the following additional functions of vitamin D: participation in the regulation of cell reproduction, metabolic processes, stimulation of the synthesis of a number of hormones [10]	x_4
5	Calprotectin fecal, μg / g	A protein produced by neutrophils in the intestinal mucosa. Its level (in feces) is increased in Crohn's disease and ulcerative colitis, in addition, this indicator is increased in infectious bowel infections, cancer, in remission phase [6, 10].	x_5
6	Albumin in the urine, mg/L	The main plasma protein, its purpose is the construction of new cells in the form of essential amino acids. Microalbuminuria - the allocation of albumin in the urine in an amount of 30-300 mg per day; refers to the early signs of impaired renal function and is one of the manifestations of target organ damage (an indicator of endothelial dysfunction, insulin resistance and hypercoagulation) [9].	x_6

interpretation of modeling results."

For each patient and each disease, the formation of a list of signs, the extraction of information and knowledge will be carried out according to an individual method. Modeling was carried out according to the results of observations obtained from August 2015 to December 2018 at the Therapy Department of the Azerbaijan State Institute of Advanced Medical Training named after A. Aliyev. We studied 183 patients with Crohn's disease (56.8%) and ulcerative colitis (43.1%) aged 17 to 60 years with a disease duration of 1.2-9.4 years. Verification of the results was provided by introducing 20 patients with a diagnosis of Irritable Bowel Syndrome and 20 people into the studies, who have no complaints.

To form an AID, the features described in Table I were expertly determined.

Characteristics of the characteristics presented in table I were obtained by conducting medical tests according to standard methods. If necessary, the tests were repeated, a total of 426 cases. Two groups of patients were formed (A and B), commensurate in gender and age. The feature vectors constructed from the results of group A observations formed an AID for training models. The results of observations of group B were used to test trained models.

The results of the expert classification of the patient's condition were used as a simulated indicator. It was suggested to distinguish four classes (states) of the patient – 0 (clinical and endoscopic remission); 1 (mild course of the disease); 2 (medium severity); 3 (serious condition of the patient). Table II presents the characteristics of the classes. Characteristics of the condition of the patients were analyzed by IBD activity indexes [6]. These characteristics fulfill the function of classification features. Clinical and endoscopic remission is characterized by the absence of clinical manifestations and macroscopic changes during endoscopy. The severity of the disease as a whole is determined by: the severity of the current attack, the presence of extraintestinal manifestations and complications, refractory treatment, in particular, the development of hormonal dependence and resistance [6]. The severity of the disease as a whole is determined by: the severity of the current attack, the presence of extraintestinal manifestations and complications, refractory treatment, in particular, the development of hormonal dependence and resistance [6].

The model was synthesized by a classifier, which was designed based on the agent approach [7]. Each agent used different methods for building the model. An agent was selected that provided the best classification results.

A group of the best models according to the quality criterion was chosen to build the classifier. The criterion for the quality of models is the number of correctly classified observation points on the examination sequence in a multidimensional space of features of the primary description. Each observation point describes an experimentally determined condition of the patient in accordance with table II.

The observation point in the multi-factorial space of signs is a vector that contains a numerical characteristic — the class value and the values of the numerous characteristics of the characteristics presented in Table I. The set of observation points forms AID in the form (1). Table III presents the test results of classifier models on a sequence of observation points B.

The number of correctly classified points reached 92%. An analysis of the conditions of patients characterized by incorrectly classified points revealed the information content of this fact. In those cases when the classification results did not coincide with the expert assessment of the patient's condition, additional factors were found that influenced his condition and whose characteristics were not taken into account in the structure of classifier models.

DISCUSSION

Information technology of multilevel intellectual monitoring [3] is used to study objects classified as complex systems in the conditions of low information content of their primary description. The object of study is reflected in the form of a complex system in the case when, in order to obtain its adequate model, it is necessary to use information that is not yet available [4]. Additional information about the properties of the monitoring object is acquired in the process of continuous observation, processing and conversion of their results [3]. One of the monitoring needs in the medical industry is to identify the mechanisms of disease formation and determine the condition of the patient according to the results of certain observations. The purpose of observations, as a rule, is to form an array of numerous characteristics that will allow, in the process of building the model, to reflect in its structure the properties of the patient's body to respond to control influences that are part of the treatment regimen. In [1], to obtain numerous characteristics, the surface of the colon was photographed during endoscopy. As a result of these observations, a number of images were obtained. According to the results of observations, the task of classifying the patient's disease was solved. Three classes have been described: 1) the patient has Crohn's disease; 2) the patient has ulcerative colitis 3) the patient is conditionally healthy and there are no signs of Crohn's disease and ulcerative colitis. The obtained images were segmented, a list of attributes of each segment was determined, an array of numerous characteristics of these signs was formed. To classify the images, a resolution rule was constructed in the form of artificial neural networks based on a multilayer perceptron. It was possible to obtain 84.2% of correctly classified images.

In [5], an array of input data was formed based on the results of observations carried out in the form of spectral analysis of the blood serum of patients. As in the previous case, three classes of patients were described: with Crohn's disease, with ulcerative colitis and conditionally healthy. The observation results were obtained in the form of spectrograms with characteristic peaks. The characteristics of the peaks and their placement on the spectrogram are used as numerical characteristics of the observation results. An array of numerous characteristics was subjected to multivariate entropy analysis using the DiaStat statistical

Table II. Description a class states of patients

Classes	Title	The value of the class for modeling
0	Clinical and endoscopic remission	0
1	Easy course of the disease	50
2	Medium severity condition	500
3	Severe condition of the patient	1000

Table III. Test results of classifier models

Patient Code	Patient Status	Patient prognosis	Result	Patient Code	Patient Status	Patient prognosis	Result	Patient Code	Patient Status	Patient prognosis	Result	Patient Code	Patient Status	Patient prognosis	Result
251	1000	999,61	1	220	50	50,85	1	189	500	390,26	1	158	1	1,077	1
250	1	0,36	1	219	500	499,96	1	188	500	387,20	1	157	1	0,939	1
249	1	6,80	1	218	50	72,09	1	187	50	100,75	1	156	1	9,56	1
248	1	1,58	1	217	50	50,04	1	186	1000	100,75	0	155	1	3,2	1
247	1	47,61	0	216	50	49,43	1	185	50	151,56	1	154	1	0,34	1
246	50	133,07	1	215	500	420,30	1	184	500	499,13	1	153	1	6,37	1
245	50	160,18	1	214	500	350,53	1	183	1	1,005	1	152	1	103,51	0
244	500	411,96	1	213	50	65,63	1	182	50	150,38	1	151	1000	933,28	1
243	50	143,03	1	212	1	0,007	1	181	50	71,77	1	150	1	57,18	0
242	500	497,25	1	211	50	50,48	1	180	50	52,73	1	149	1000	998,94	1
241	1	0,66	1	210	500	397,37	1	179	1	6,93	1	148	1	0,68	1
240	50	50,00	1	209	500	318,44	1	178	50	49,83	1	147	1	34,82	1
239	50	49,02	1	208	1000	626,10	0	177	1	2,66	1	146	1	0,58	1
238	50	65,80	1	207	1000	1035,35	1	176	500	499,87	1	145	1000	1001,57	1
237	500	290,48	1	206	1000	1006,36	1	175	500	494,14	1	144	1	0,67	1
236	50	50,00	1	205	500	361,09	1	174	1000	1000,3	1	143	1	147,14	0
235	1	0,23	1	204	500	333,27	1	173	1000	727,46	1	142	1	1,01	1
234	500	349,57	1	203	500	499,37	1	172	50	50,21	1	141	1000	998,07	1
233	1000	904,35	1	202	50	166,76	1	171	1	1,004	1	140	50	50,16	1
232	500	480,55	1	201	500	344,90	1	170	1	50,75	0	139	500	384,69	1
231	50	126,36	1	200	1	1,92	1	169	1	1,64	1	138	50	77,25	1
230	500	503,33	1	199	50	79,68	1	168	1	1,014	1	137	50	79,39	1
229	500	499,93	1	198	1	47,91	0	167	1000	1069,86	1	136	500	486,89	1
228	500	287,16	1	197	500	498,03	1	166	500	515,97	1	135	50	55,69	1
227	1	0,74	1	196	500	252,61	1	165	500	500,45	1	134	1	4,97	1
226	1	17,34	1	195	50	50,73	1	164	500	499,53	1	133	1	0,43	1
225	1	0,06	1	194	1	2,02	1	163	500	487,76	1	132	50	50,37	1
224	500	495,87	1	193	1	1,06	1	162	50	56,5	1	131	50	48,80	1
223	50	49,42	1	192	50	109,59	1	161	500	451,12	1	130	1	93,66	0
222	500	457,39	1	191	1	4,28	1	160	1	286,76	0				
221	1	12,10	1	190	50	50,31	1	159	1	1,029	1				

analysis software package [7]. According to the authors, they managed to get 93-95% of correctly classified objects. It was not possible to detect methods for non-invasive assessment of the dynamics of the pathology in a patient with IBD, classification of his condition.

Today, the task of creating a reliable methodology for non-invasive diagnosis of a condition in IBD remains relevant. The results of the classification of diseases described in the literature require improvement, bringing the process of obtaining individual scientific results to the level of technology that is available to the ordinary doctor. The process of using the simulation results in selecting a treatment regimen requires additional research. It is also necessary to predict the individual response of the patient to the standard treatment regimen.

CONCLUSIONS

The results of the testing of classifier models indicate the effectiveness of the use of information technology of intelligent monitoring to assess the condition of patients with IBD. According to the results of standard medical tests, presented in the form of a two-dimensional array of numerous characteristics, the doctor has the opportunity to assess the individual response of the patient to the treatment regimen and identify the need for its correction.

REFERENCES

1. Bakulin I., Skalinskaya M., Skazyvaeva E. et al. Artificial neural network as an assistant in the differential diagnosis between ulcerative colitis and Crohn's disease. United European Gastroenterology Journal. 2019;7:336-337
2. Holub S., Kunytska S. The concept of multi-agent intellectual monitoring systems Projekt interdyscyplinarny projektem XXI wieku. Processing, transmission and security of information. 2019: 183-188.
3. Holub S.V. Multilevel modeling in environmental monitoring technologies. Bohdan Khmelnytsky National University. 2007: 220.
4. Peregudov F.I., Tarasenko F.P. Introduction to systems analysis. Higher school. 1989: 367.
5. Fedulova E.N., Gordetsov A.S., Fedorova A.V. et al. Using a mathematical model of infrared spectroscopy of blood serum in the differential diagnosis of ulcerative colitis and Crohn's disease in children. Bulletin of the Russian Academy of Medical Sciences. 2013;12: 44-48.
6. Chen J.H., Andrews J.M., Kariyawasam V., Moran N. IBD Sydney Organisation and the Australian Inflammatory Bowel Diseases Consensus Working Group. Review article: acute severe ulcerative colitis – evidence-based consensus statements. Aliment Pharmacol Ther. 2016;44(2):127-44.
7. Kunytska S., Holub S. Multi-agent Monitoring Information Systems . Mathematical Modeling and Simulation of Systems . MODS 2019 Advances in Intelligent Systems and Computing, Springer , Cham. 2019; 1019: 164-171.
8. Zhiryakova I.A., Holub S.V. New approach to conceptual knowledge. Technical science and technology, 2015; 2; 78-82.
9. Babayeva G. H., Babayev Z. M. A new approach to assessing the clinical condition of patients with ulcerative colitis and Crohn's disease. Experimental and clinical gastroenterology. 2019; 162 (2): 19-23.
10. Babayeva G.H., Babayev Z.M. The frequency of detection of some markers of endothelial dysfunction in patients with inflammatory bowel disease. Therapeutic archive. 2018; 4: 12-16.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

^{13}C-METHACETIN BREATHE TEST IN EARLY DIAGNOSTICS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

The aim: Of the study was to evaluate the early utility changes of the ^{13}C methacetin breath test parameters in patients with NAFLD.

Materials and methods: There were included 50 subjects in the study, among them 35 patients had steatosis and 15 patients had steatohepatitis, including 35 (70.0%) male subjects and 15 (30.0%) female subjects. The control group included 17 apparently healthy volunteers, among them 10 (58.8%) subjects were male and 7 (41.2%) subjects were female.

Results: It was determined that metabolism kinetics in case of liver steatosis was significant decreased more than 30% compared to the control group ($p = 0.0001$) and in case of steatohepatitis that decrease was more than 65% ($p = 0.00001$) compared with normal values. It resulted in less cumulative dose accumulation in steatosis ($p = 0.00001$) and steatohepatitis ($p = 0.00001$). Among the reasons for the decrease in the kinetics of metabolism in steatosis, there were insufficient response of hepatocytes on 10 minutes (<10 dose/h,%) in 40% of cases and reduction of metabolism rate amplitude at 20-40 minutes following methacetin administration.

Conclusions: The results of ^{13}C-methacetin breath test demonstrate that in patients with NAFLD there is a gradual slowing of metabolism rate in hepatocytes, which leads to a decrease in cumulative dose.

KEY WORDS: non-alcoholic fatty liver disease (NAFLD), ^{13}C-methacetin breathe test, steatosis, steatohepatitis

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INTRODUCTION

With the implementation of breath tests in the clinical practice to determine the liver function, it became possible to quantify its functional capacity. It is mainly associated with the need for early diagnosis of non-alcoholic fatty liver disease (NAFLD), which is one of the predictors of cardiovascular diseases and type 2 diabetes mellitus [1,2].

Systematic intake of fatty food and physical inactivity are leading to critical increases in obesity and metabolic syndrome frequency in industrialized countries. As a result, NAFLD has become an important public health issue [3,4].

The intense accumulation of fat in the liver eventually leads to the occurrence of steatosis, which is found in 80-90% of cases in patients with obesity, and subsequently it leads to steatohepatitis, which is diagnosed in 10-20% of patients [5,6].

In majority of cases, obese patients consider themselves to be healthy individuals, although they may report fatigue and sensation of heaviness in the right hypochondriac region. It is found hepatomegaly in 75% of cases based on results of direct examination. Laboratory data indicate the transaminases levels elevation only in 30% of cases; however, ultrasonography examination of the internal organs reveals hyperechogenicity of the liver, as well as computed tomography data demonstrate homogeneous decrease in the density of the liver structure [5].

In general, ultrasound diagnostic methods allow to detect NAFLD in the cases of at least 30% fat accumulation in hepatocytes [7].

Therefore, the results of static biochemical markers analysis and instrumental examination allow to make only indirect conclusions regarding the liver function and extent of the liver damage, whereas the needle biopsy is associated with significant risk of complications [8].

In this aspect, the use of breath tests based on the metabolism of orally administered substrates labeled with a stable carbon isotope ^{13}C makes it possible to evaluate the functional status of the liver in normal and pathological conditions [2,9].

Currently available breath tests allow to determine microsomal (methacetin, phenacetin, aminopyrine, caffeine), mitochondrial (methionine) and cytosolic (galactose, phenylalanine) liver function [1].

In clinical practice, the ^{13}C methacetin breath test (^{13}C-MBT) is used most widely and cumulative dose and metabolic rate are analysed for determination of the liver functional capacity. In apparently healthy subjects, the metabolic capacity of the liver presented as a percentage of the accumulated dose during 120 minutes ranges from 20% to 35% that corresponds to 100% of functioning hepatocytes and is the main constant for the evaluation of the results of methacetin test. The second parameter for analysis of the metabolism kinetics of

^{13}C labeled methacetin is the time from the substrate intake to a peak of $^{13}CO_2$ elimination in exhaled air. A shift in the peak of the metabolism rate from 10 to 50 minutes indicates the development of fatty liver infiltration [10,11,12].

In general, a decrease in ^{13}C-MDT parameters relative to normal values evidences of impaired liver microsomal function, which may be characteristic of steatosis and fibrosis [13,14].

However, the diagnosis of the early stages of NAFLD is not yet fully understood. The proposed criteria for identifying the liver steatosis, steatohepatitis and cirrhosis need clarification.

THE AIM

The aim of the study was to perform a comprehensive comparison of changes in ^{13}C-MDT, biochemical and ultrasonographic features in patients with steatosis and steatohepatitis.

MATERIALS AND METHODS

There were included 45 subjects in the study, among them 30 patients had steatosis and 15 patients had steatohepatitis, including 32 (71.1%) male subjects and 13 (28.9%) female subjects. The control group included 17 apparently healthy volunteers, among them 10 (58.82%) subjects were male and 7 (41.18%) subjects were female.

The diagnosis of NAFLD was based on clinical examination data (overweight/obesity, ultrasound parameters, and hepatic transaminases). Subjects with history of viral hepatitis, hemochromatosis, autoimmune hepatitis, Wilson's disease and subjects who consumed alcohol at a daily dose of 30 mg for men and 20 mg for women or received hepatotoxic drugs were excluded from the study. There were also excluded from the study patients who took drugs that affect CYP1A2 activity and anticoagulants.

The complete clinical examination included anthropometric characteristics, such as height, body weight, body mass index, waist and hip circumferences. Hepatic transaminases, blood lipids, glucose and insulin were also determined with the calculation of insulin resistance index.

The methacetin breath test was performed in the morning after 12 hours of fasting and 30 minutes of resting in sitting position. Patients were advised that pineapple, kiwi fruit or mineral water should be avoided 48 hours before the test. The test breakfast consisted of 75 mg of ^{13}C-methacetin dissolved in unsweetened fruit tea. Breath samples were collected in aluminum bags before the administration of methacetin and then every 10 minutes during the first hour of the test and every 20 minutes

Table I. Characteristics of study groups

		Control group (Group 1), (n=17)	Steatosis (Group 2), (n=30)	Steatohepatitis (Group 3), (n=15)	p-value
Gender	Males	10 (58.82 %)	19 (67.86 %)	12 (75 %)	–
	Females	7 (41.18 %)	9 (32.14 %)	4 (25 %)	
Age (years)		58.24±2.31	57.14±2.04	53.75±2.85	–
BMI (kg/m^2)		26.88±1.60	31.61±0.76	30.00±1.02	p_{1-2}=0.00007 p_{1-3}=0.0039 p_{2-3}=0.3307
Total cholesterol (µmol/L)		4.91±0.19	5.56±0.26	5.63±0.21	p_{1-2}=0.1749 p_{1-3}=0.0425 p_{2-3}=0.5646
LDL (µmol/L)		2.90±0.20	3.51±0.23	3.28±0.26	p_{1-2}=0.123 p_{1-3}=0.2907 p_{2-3}=0.595
Triglycerides (µmol/L)		1.84±0.11	2.18±0.22	2.53±0.31	p_{1-2}=0.6039 p_{1-3}=0.064 p_{2-3}=0.1478
HDL (µmol/L)		1.13±0.05	1.14±0.05	1.02±0.05	p_{1-2}=0.9715 p_{1-3}=0.0945 p_{2-3}=0.1362
ALT (IU/L)		32.11±2.30	38.93±1.09	50.62±2.95	p_{1-2}=0.0013 p_{1-3}=0.0002 p_{2-3}=0.00001
AST (IU/L)		31.23±2.35	41.28±2.07	53.94±3.09	p_{1-2}=0.0024 p_{1-3}=0.00002 p_{2-3}=0.0004

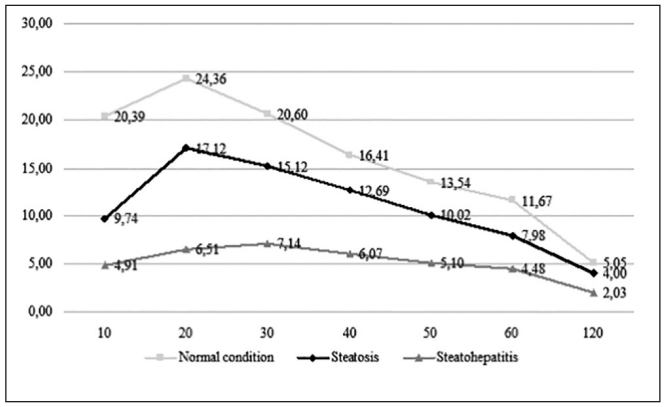

Fig. 1. Metabolism rate in normal condition, steatosis, and steatohepatitis

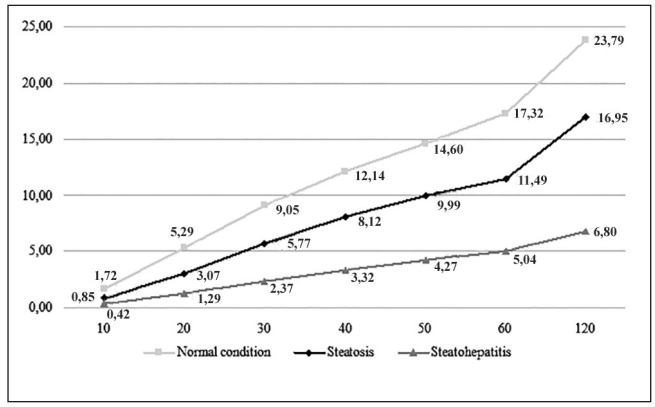

Fig. 2. Cumulative dose in normal condition, steatosis, and steatohepatitis

during the second hour of the test. No adverse events were reported by patients.

Measurement of ^{13}C-labeled CO_2 in exhaled air was performed using the infrared spectrometer IRIS (manufactured by Wagner Analysen Technik GmbH). The following parameters were analyzed: cumulative dose at 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, and 120 minutes of the test period. The metabolism rate was also measured at the same time points.

Statistical analysis of the received data was performed on a personal computer using software Microsoft Excel and Statistica v. 10.0, StatSoft Inc., USA. The reliability of difference between average values was estimated using the Student's paired t-test. The mean value (M) and standard error of the mean (m) also were evaluated. When investigating the relationship of normally distributed quantitative parameters, Pearson's correlation analysis (r) was used to assess their orientation and strength. The difference between the parameters was considered statistically significant at p-value < 0.05.

RESULTS

Following the examination of patients with NAFLD, the most common signs of metabolic syndrome are: overweight or obesity, dyslipidemia, increased over time the concentration of ALT and AST, depending on the presence of steatosis or steatohepatitis (Table I).

According to ultrasound examination data, hepatomegaly, hyperechogenicity of the liver parenchyma and heterogeneity of the liver structure due to steatosis were observed in 80% of cases. In addition, diameter enlargement of the portal vein and of the splenic vein was found in case of steatohepatitis.

During the ^{13}C-MBT, the features of the hepatocytes response were revealed depending on the stage of the course of NAFLD in steatosis and steatohepatitis.

In apparently healthy subjects, the metabolism rate interval after methacetin administration was between 24.36 ± 1.18 and 11.67 ± 0.82 dose/h,% during 60 minutes of the examination and was not lower than 10 dose/h,% (Figure 1).

In case of steatosis, the metabolism rate after methacetin administration was significantly decreased compared to the control group (p=0.0001). Among the features of the decrease in the metabolism rate in steatosis, there were insufficient response of hepatocytes to the intake of methacetin on 10 minutes (<10 dose/h,%) in 40% of cases and reduction of amplitude at 20-40 minutes in the kinetics of metabolism at a sufficient level instead of 60 minutes compared to normal.

In patients with steatohepatitis compared with the control group and liver steatosis group, there was observed a further decrease in the rate of metabolism, which did not reach 10.0 dose/h,% during the first 60 minutes of the examination.

Accumulation of the cumulative dose was significantly reduced in the case of steatosis and steatohepatitis compared to the control group for the first 10 minutes of the methacetin breath test. Thus, in apparently healthy individuals, the values at 10 minutes (Cum10) ranged from 1.4 to 2.8 dose/h,% with the mean value 1.72 ± 0.10 dose/h,%. However, the methacetin accumulation level at 10 minutes was lower than 1.0% in 64.3% of cases in the presence of steatosis, and it was more than 4 times lower than in the control group in the presence of steatohepatitis (Figure 2).

The results of the study showed that there is a decrease in the rate of metabolism resulting in a correspondingly reduced cumulative dose in steatosis of the liver. In the case of steatohepatitis, such changes are more pronounced throughout the examination. While the rate of metabolism was 68.5% in steatosis compared to the control values (p = 0.00001), then in steatohepatitis it was decreased in 32% compared to normal (p = 0.00001).

DISCUSSION

Despite the wide use of common methods of examination for the detection of NAFLD, none of them provide reliable diagnosis confirmation, including anthropometric parameters for the determination of the obesity stage, liver tests, blood lipid spectrum, liver echolocation and elastography [5]. Only in the presence of fibrosis, the best results are obtained by

liver elastometry. However, indices of static biochemical markers allow only indirect conclusions to be drawn on the liver function in early stages of NAFLD [1,13]. Therefore, the use of breath tests to determine the metabolism of orally administered substrates, in particular methacetin, makes it possible to evaluate the functional status of the liver in dynamics in the normal condition and in NAFLD [1,12].

It is well known that there is a gradual decrease in the rate of metabolism and in the cumulative dose in steatosis and steatohepatitis [2,11,12]. Thus, in the presence of steatosis, it is observed the shift of the peak metabolism rate from 10 minutes to 20-30-40-50 minutes of the test [3,6]. The progression of the pathological process leads to a decrease in the rate of metabolism and in the cumulative dose that characterizes the "capacity" of the liver [9]. However, for early diagnosis of NAFLD, it is important to evaluate the cumulative dose accumulation during the examination [3,9]. Thus, the points for difference between normal condition and pathology are metabolism levels of 1.05 at 10 minutes, 4.15 at 20 minutes, 7.15 at 30 minutes, 9.00 at 40 minutes, and 20.00 at 120 minutes [6]. Among the generally parameters of metabolism assessment at 40 and 120 minutes, attention should be also drawn to Cum 10, as ^{13}C-dose/h,% at 10 minutes and 60 minutes coincide and have similar accuracy in the diagnosis of steatohepatitis [1].

This study showed the comparable significance of the obtained parameters of the metabolism rate and the accumulation of cumulative dose, which are specific in the normal condition and in steatosis or steatohepatitis. In apparently healthy subjects, the rate of metabolism is maintained at a high level, which provides sufficient cumulative dose accumulation and corresponds to approximately 100% of functioning hepatocytes [5]. In the presence of steatosis, decreased metabolism rate and accordingly significant decrease in the cumulative dose are caused by the reduction of functional activity of hepatocytes to 30-40 minutes instead of 60 minutes compared with apparently healthy individuals.

In the case of steatohepatitis, there is a sharp decrease in the rate of metabolism and accordingly the cumulative dose, which amounts to only a quarter of the functional capacity of hepatocytes in healthy persons.

CONCLUSIONS

1. Methacetin breath test (^{13}C-MBT) allows to detect impaired metabolic rate and accordingly changes in the metabolic capacity of the liver in dynamics at the early stages of NAFLD.
2. Steatosis of the liver is characterized both by decrease in the metabolism rate and by a reduction of the metabolism kinetics at a sufficient level to 20-40 minutes instead of 60 minutes in apparently healthy individuals.
3. The presence of steatohepatitis is evidenced by a sharp decrease in the rate of metabolism and metabolic capacity of the liver (less than 10 dose/h,%) compared with apparently healthy individuals and patients with steatosis of the liver.

REFERENCES

1. Rapoport S.I., Shubina N.A. Respiratory tests in diagnostics of liver disease. Clin med. 2016;94(12):885-892. doi:10.18821/0023-2149-2016-94-12-885-892.
2. Miele L., Marrone G., Cefalo C. et al. Potentian use of liver function breath tests in the clinical practice. Eur Rev Med Pharmacol Sci. 2013;17(2):82-89.
3. Stepanov Yu.M. Results of the observational cross-over PRELID 2 study (2015–2016). Part 1. The prevalence of non-alcoholic fatty liver disease, the characteristics of concomitant pathology, metabolic syndrome and its individual criteria in patients seeking general practitioners' and gastroenterologists' help in Ukraine. Gastroenterol. 2019;53(1):26-33. doi:10.22141/2308-2097.53.1.2019.163454.
4. Williams C.D., Stengel J., Asike M.I. et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterol. 2011;140(1):124-131. doi:10.1053/j.gastro.2010.09.038.
5. Gerok V., Blyum H.E. Zabolevanija pecheni i zhelchevydelitel`noi sistemy [Diseases of the liver and biliary system]. Moscow: MEDpress-inform;2009.199 p.(In Russian).
6. Razlan H., Marzuki N.M., Tai M.L. et al. Diagnostic value of the C methacetin breath test in various stages of chronic liver disease. Gastroenterol Res Pract. 2011;2011:235796. doi:10.1155/2011/235796.
7. Chalasani N., Younoss Z., Lavine J.E. et al. The diagnosis and Management of non-alcoholic fatty liver disease: practice guideline by the American gastroenterological association, American association for the study of liver disease, and American college of gastroenterology. Gastroenterol. 2012;55(6):2005-2023. doi:10.1002/hep.25762.
8. Lock J.F., Taheri P., Bauer S. et al. Interpretation of non-invasive breath tests using (13)C-labeled substrates – a preliminary report with (13) C-methacetin. Eur J Med Res. 2009;14:547-550.
9. Musialik J., Jonderko K., Kasicka-Jonderko A. et al. (13)CO2 breath tests in non-invasive hepatological diagnosis. Prz Gastroenterol. 2015;10(1):1-6. doi:10.5114/pg.2014.47501.
10. Gorowska-Kowolik K., Chobot A., Kwiecien J. 13C methacetin breath test for assessment of microsomal liver function: methodology and clinical application. Gastroenterol Res Pract. 2017;7397840. doi:10.1155/2017/7397840.
11. Grattagliano I., Lauterburg B.H., Palasciano G. et al. 13C-breath tests for clinical investigation of liver mitochondrial function. Eur J Clin Invest. 2010;40(9):843-850.
12. Kempiński R., Neubauer K., Wieczorek S. et al. 13C-Methacetin Breath Testing in Patients with Non-Alcoholic Fatty Liver Disease. Adv Clin Exp Med. 2016;25(1):77-81. doi:10.17219/acem/60878.
13. Aksentiychuk K., Kurlyak N., Sklyarov E. Monitoring of liver function in patients with fatty liver disease in combination with coronary heart disease and type 2 diabetes mellitus. Curr Iss Pharm Med Sci. 2013;26(4):376-379. doi:10.12923/j.2084-980X/26.4/a.04.
14. Moran S., Mina A., Duque X. et al. The utility of the 13C-methacetin breath test in predicting the long-term survival of patients with decompensated cirrhosis. J Breath Res. 2017;11:036011. doi:10.1088/1752-7163/aa7b99.

This article is a fragment of scientific research work "Features of the respiratory, cardiovascular, digestive system in patients with diabetes mellitus and obesity: features of pathogenesis, clinic, diagnostics".

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The Author declare no conflict of interest.

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ORIGINAL ARTICLE

NUTRITION PECULIARITIES OF UKRAINIAN ADOLESCENTS WITH METABOLIC SYNDROME

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ABSTRACT

The aim: To study the dietary peculiarities of Ukrainian adolescents with obesity and to identify the eating disorders on the background of metabolic syndrome.

Materials and methods: A survey on the nutrition of Ukrainian adolescents was conducted at O. Yaremenko Ukrainian Institute for Social Research within the framework of the international project Health and Behavioral Orientations of Student Youth (HBSC), 2018. As a result, a sample of 200 adolescents with obesity (age 14-18 years: 100 boys and 100 girls) was formed, with the following examination by the Institute of Children and Adolescents Health Care of the National Academy of Medical Sciences of Ukraine.

Results: A survey conducted revealed the problem of irrational and unbalanced diet in Ukrainian adolescents. Thus, irregular and unbalanced diet naturally causes digestive disorders and contributes to the formation of comorbid pathologies such as obesity and metabolic syndrome. The general analysis of the DEBQ survey results revealed that patients with metabolic syndrome were twice as likely to have abnormalities in eating behavior (71.8 ± 3.7)% than in patients without them (39.4 ± 4.1)%, ($\varphi < 0.05$). Adolescents with obesity experienced significant zinc and magnesium deficiency in the body ($\varphi < 0.05$).

Conclusions: Modification of eating behaviour through healthy balanced nutrition and psychological support is one of the most important tasks in the treatment of patients with obesity and metabolic syndrome.

KEY WORDS: adolescents, metabolic syndrome, eating disorders

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INTRODUCTION

According to WHO definition, nutrition is an organized and timely supply of nutritious and tasty food to the human body, which contains the optimal amount of nutrients needed to support life, develop and increase productivity. Nutrition is a leading fact in preserving the body, especially when it concerns a growing organism. Such a demand in childhood and adolescence is caused by the increase of the basic metabolism rate (1.5 times higher than in adults), high neuropsychiatric loads, puberty, acceleration, sports activities, etc. [1]. A teenager's daily nutritional diet depends on many reasons: family well-being, state of health, school training and other pressures, the time of year. Individual advice in this case can be provided by a nutritionist. Unfortunately, the diet of today's Ukrainian youth remains unbalanced, with high-calorie foods, sometimes harmful to health, which over time leads to overweight and obesity.

Childhood obesity is a pressing medical and social problem in Ukraine and beyond, due to the progressive increase in its prevalence, a significant spectrum of comorbid disorders [2, 3, 4], the risks of metabolic syndrome (MS), the main criteria of which in childhood are abdominal obesity, insulin resistance (IR), dyslipidemia, increased blood pressure (IDF, 2007) [5, 6]. It is wrongly believed that MS is the problem of middle aged and elderly people, as evidenced by recent studies on its prevalence in adolescents [7, 8]. Childhood obesity is rooted in genetic susceptibility, which is influenced by the environmental factors, primarily irrational nutrition during infancy and adolescence. According to Fonseca H, 2009, particular attention should be paid to overweight young people who are at most risk of disordered eating, since overweight (or just the idea of being overweight) can be a motivation to adhere to rigid diets and other eating disorders [9, 10]. In addition, such unbalanced diets lead to the imbalance of microelements in the body, which are essential and perform many functions. These microelements, at low concentrations, are involved in all metabolic processes, including intracellular, the provision of reproductive function and immunity, the development of the cognitive sphere and behavioral responses [11]. There is evidence of zinc (Zn) involvement in energy metabolism, pancreatic insulin synthesis, antioxidant protection, cell membrane stabilization, etc. Therefore, zinc deficiency is likely to affect carbohydrate and lipid metabolism, as it is an integral part of insulin, which substantiates its role in the development of IR. In particular, magnesium deficiency (Mg) leads to disorders of the nervous system in the form of a syndrome

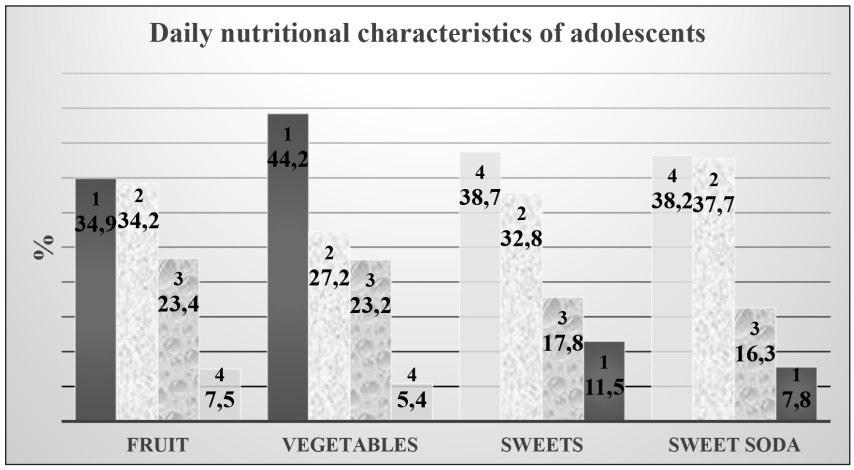

Fig. 1. O. Yaremenko Ukrainian Institute for Social Research within the framework of the international project Health and Behavioral Orientations of Student Youth (HBSC), 2018
1 – never, less than once a week; 2 – once a week, 2-4 times a week; 3 – 5-6 times a week; 4 – daily, more than once a day.

of 'chronic fatigue', cardiovascular system (increased blood pressure, impaired cardiac rhythm) and metabolism. Most often, the deficiency or imbalance of these microelements may be due to an unbalanced diet [12, 13]. Unfortunately, the studies of nutrition of Ukrainian children and adolescents, psychological predictors of childhood obesity, their relation to metabolic disorders, imbalances of biological substances that regulate eating behavior (EB) did not clarify the essence of the problem [14]. Therefore, a survey conducted by *O. Yaremenko Ukrainian Institute for Social Research* within the framework of the international project *Health and Behavioral Orientations of Student Youth* (HBSC), 2018, which aimed to study the daily nutrition of Ukrainian adolescents with subsequent identification of EB disorders and metabolic disorders like metabolic syndrome in adolescents with obesity and possibilities of modifying them to improve the effectiveness of treatment and prevention has undoubtedly become relevant.

THE AIM

To study the dietary peculiarities of Ukrainian adolescents with obesity and to identify the eating disorders on the background of metabolic syndrome.

MATERIALS AND METHODS

A survey on the nutrition of Ukrainian adolescents was conducted at *O. Yaremenko Ukrainian Institute for Social Research* within the framework of the international project *Health and Behavioral Orientations of Student Youth* (HBSC), 2018. As a result, a sample of 200 adolescents with obesity (age 14-18 years: 100 boys and 100 girls) was formed, with the following examination by the *Institute of Children and Adolescents Health Care of the National Academy of Medical Sciences of Ukraine*. To confirm the diagnosis of obesity in all patients, the body mass index (BMI) was calculated according to the formula (body weight (kg)/height^2 (m^2)) with the estimation of data on the percentile tables recommended by WHO. All BMIs surveyed exceeded 95 percentiles. The type of obesity was analyzed by the waist-to-growth ratio (WC/height); waist circumference to hip circumference ratio (WC/HC). The abdominal type of obesity in adolescents was diagnosed at the value of ≥ 0.9 for boys, ≥ 0.8 for girls [15]. The control group consisted of 30 healthy children of the same age category. All patients underwent a comprehensive clinical and laboratory examination: anthropometric measurements, biochemical blood testing with the determination of lipid and protein metabolism, liver enzyme levels and bilirubin. The criterion for IR was the homeostatic model HOMA – IR (Homeostasis model assessment of Insulin Resistance, Matthews D.R., 1985). The calculation was carried out according to the formula: HOMA = (G_0 • Ins_0) / 22.5; (G_0 – fasting glucose level of the blood serum, mmol/l; Ins_0 – the content of insulin in blood serum, mkU/ml. The result of more than 3.5 units testified to IR presence. On the basis of the studies conducted, the obese subjects were divided into the main and the control groups. The main consisted of two subgroups: 1 – with signs of MS and 2 – without signs of MS, each of which included 100 patients. Criteria used for diagnosing MS in children were presented by the International Diabetes Federation. The main ones in childhood are abdominal obesity, IR, dyslipidemia, high blood pressure [International Diabetes Federation, 2007]. A study of EB in obese adolescents was conducted using a Dutch questionnaire (DEBQ, The Dutch Eating Behaviour Questionnaire). The Dutch questionnaire allows you to identify the causes of overeating and, accordingly, to establish the type of eating behaviour (restrictive, emotional, external). It should be noted that according to this test, it is possible to have several EB disorders in one patient at a time. Also, the levels of zinc and magnesium trace elements indispensable for the life of the organism were analyzed using *Cormay* standard apparatus and kits.

Table 1. Characteristics of complaints in patients with obesity, (%)

Complaints	MS + (n= 100)	MS - (n= 100)	Total (n= 200)
increased fatigue	62.8 ± 4.5	61.1 ± 4.5	61.9 ± 3,2
irritability	27.4 ± 4.2	25.7 ± 4.1	26.5 ± 2.9
weakness	17.7 ± 3.6	17.7 ± 3.6	17.7 ± 2.5
headache	85.0 ± 3.3	80.5 ± 3.7	82.7 ± 2.5
epigastric pain	55.8 ± 4.6*	45.1 ± 4.7	50.4 ± 3.3
pain in the region of liver	62.8 ± 4.5*	18.6 ± 3.6	40,7 ± 3.2
pain in the pyloric duodenal area	61.1 ± 4.6*	32.7 ± 4.4	46.9 ± 3.3
increased appetite	76.1 ± 4.0*	55.8 ± 4.6	65.9 ± 3.1
heartburn	61.9 ± 4.5*	38.9 ± 4.6	50.44 ± 3.3
nausea	52.2 ± 4.7*	31 ± 4.3	41.6 ± 3.2

* - Difference between patients from group 1 (MS +) and 2 (MS -) ($p < 0.05$)

The database creation and the statistical processing of the results were performed on IBM-Pentium III using application packages 'Stadia-6' (serial number of license certificate 1218 May 24, 2000, version 'Prof'), Microsoft 'Access', 'Excel'. The t-criterion of the Student (p), Fisher (φ), Mann-Whitney test were used to assess the likelihood of differences, as well as the correlation analysis. The critical significance level for checking statistical hypotheses when comparing groups was assumed to be 0.05. Ethical norms at all stages of the survey were observed. The work was conducted taking into account the requirements of the European Convention (Strasbourg, 1986), the provisions of the ICH GCP (2008), GLP (2002). The studies did not cause psychological discomfort in patients. Patients and their parents were provided with the information on the methods and scope of the research, signed informed consent to participate in the study.

RESULTS

A survey conducted by *O. Yaremenko Ukrainian Institute for Social Research* within the framework of the international project *Health and Behavioral Orientations of Student Youth* (HBSC), 2018 demonstrated the adolescent consumption of fruit, vegetables, sweets and sodas, containing sugar by time: daily, more than once a day, once a week, 2-4 days a week, never (Figure 1).

It was also found that about 7.8% of 14-years and 15.6% of 16-years do not have breakfast on weekdays. Every day, only half of adolescents consume fruit and vegetables 42.2% (boys) and 51.2% (girls). 17% of adolescents in all sex-age groups regularly ('daily') consume drinks (soda and sweets). One third (27.9%) consume food daily or 5-6 days a week while watching TV.

It should be recognized that the proportion of students who regularly ('daily') consume both healthy (vegetables, fruit) and potentially harmful food (carbonated drinks and sweets) remains stable across all age groups, indicating that food preferences and eating behaviour are formed in adolescence. In addition, the sugary beverage intake is high, more than once a week (42.4% of respondents), especially among boys (48.7% versus 37.5% among girls).

A comprehensive clinical and laboratory-instrumental study of 200 adolescents with obesity allowed dividing patients into 2 groups: I – with the main features of MS (50.0% of patients with the presence of IR, abdominal type of obesity, dyslipidemia), II – patients with obesity and without IR, borderline changes in lipidogram (also 50.0%). In patients of group I, when determining BMI, its values were statistically significantly higher than those of group II (36.25 ± 4.45 kg/m^2 and 28.0 ± 3.73 kg/m^2, respectively, $p < 0.01$). Group I was characterized by statistically significantly higher WC/height ratios compared with patients in group II (0.69 ± 0.07 U and 0.59 ± 0.04 U, respectively, $p < 0.05$). The WC/HC ratio in obese adolescents also indicated an abdominal type of obesity, but did not differ significantly in the groups ($p > 0.05$). Thus, the WC/height index was more sensitive to establishing the type of adipose tissue distribution, which is consistent with the world literature [15]. That is, half of the patients surveyed had MS by the main criteria. A comparative analysis of the degree of obesity in adolescents was also performed depending on the presence of MS signs by use of the Mann-Whitney U test. As shown in the analysis, patients in group 1 had, on average, a higher degree of obesity than patients in group 2 ($p = 0.009$). Moreover, this trend persisted regardless of gender and age.

In patients with obesity the following complaints were noted (Table 1). Most often, children complained of headaches – (82.7 ± 2.5)% and fatigue – (61.9 ± 3.2)%. There were less frequent complaints of being irritable (26.5 ± 2.9)% and feeling weak (17.7 ± 2.5)%. Patients also complained of abdominal pain and had manifestations of dyspeptic syndrome.

Thus, the signs of asthenovegetative syndrome were almost identical in both groups of patients ($p > 0.05$). As to the digestive system, about half of adolescents complained of epigastric pain (50.4 ± 3.3%), pyloric duodenal (46.9 ± 3.3%) and liver projection (40.7 ± 3.2), and it occurred significantly more frequently in the group of patients with

Table 2. Deviant forms of eating behavior in adolescents with obesity according to the data DEBQ, (%)

Type of EB	MS +		MS -		Total	Control group
	Boys	Girls	Boys	Girls		
Restrictive	44.18 ± 2.3 **	52.5 ± 2.8**	24.0 ± 2.0	28.8 ± 3.0	73.9 ± 3.2 *	16.73 ± 2.4
External	17.0 ± 2.0	28.8 ± 3.0	19.3 ± 2.1	24.7 ± 2.7	43.8 ± 3.1 *	13.67 ± 2.1
Emotional	14.7 ± 1.9	17.5± 2.9	11.6 ± 1.9	24.7 ± 2.7	32.3 ± 3.1 *	10.86 ± 1.9

* Difference between the patients from the main group and the control group (φ < 0.05)
** Difference between the patients from group1 (MS +) and 2 (MS -) (φ < 0.05)

Table 3. Zinc and magnesium levels in adolescents with obesity, depending on the type of EB, (M ± m)

Type of EB	Zinc, mkmol/l (N – 10-15)	Magnesium, mmol/l (N – 0,7-1,1)
Restrictive	7.31 ± 0.5 *, **	0.34 ± 0.4 *, **
External	8.87 ± 0.3 *	0.67 ± 0.2 *
Emotional	8.92 ± 0.7 *	0.73± 0.2 *
Control group	12.7 ± 0.3	0.93 ± 0.03

* Difference between the patients from the main group and the control group (φ < 0.05)
** Difference between the patients from group 1 (MS +) and 2 (MS -) (φ < 0.05)

obesity and signs of MS (p < 0.05). The characteristics of dyspeptic syndrome complaints also indicate their high prevalence in the patients surveyed, with a likely predominance of complaints of increased appetite, heartburn, nausea in group1of patients compared with group 2 of adolescents (p < 0.05).

On objective examination, an increase in liver size was found in (37.8 ± 3.2)% of adolescents with obesity, which was more commonly reported in individuals with MS (50.4 ± 4.7)%, compared with individuals without MS (24.8 ± 4.0)%, (p <0.001). According to ultrasound, an increase in liver size was found in (38.0 ± 4.0)% of patients, statistically significantly more often in individuals with MS (49.4 ± 5.4)%, in contrast to individuals without MS (23.5 ± 5.1)%, (p < 0.01). Increased liver echogenicity was also more prevalent in patients with MS (24.7 ± 4.6)% than in patients without MS (13.2 ± 4.1)%, (p < 0.01), which indicated unfavorable dynamics of pathological liver changes against the background of IR. Sealing of liver vessels was observed at almost the same frequency in the groups compared (36.5 ± 5.2)% and (33.8 ± 5.7)%, respectively (p > 0.05).

The relationship between MS and IR as a leading symptom of this syndrome and the eating behaviours of the main groups examined were further analyzed by correlation analysis. Therefore, correlation analysis revealed a weak, statistically significant correlation between EB and HOMA index (r = 0.31; p < 0.001). That is, it confirms the possible pathogenetic role of IR in the progression of obesity and the development of EB abnormalities.

The analysis of the Dutch questionnaire data (Table 2) showed that most adolescents with obesity had a restrictive type of EB – (73.9 ± 3.2)%, whereas in the control group only (16.73 ± 2.4) % of children have a specified EB disorder (φ < 0.05). This disorder of EB is characterized by excess food self-restraint and rigid diets that alternate with the episodes of overeating. In the presence of signs of MS, a statistically significant difference was found in both groups boys and girls (φ <0.05).

A fairly common variant of eating disorders was the external one – in (43.8 ± 3.1)% of children with obesity, against (13.67 ± 2.1)% in the control group (φ < 0,05), characterized by the increased reaction to external stimuli (food advertising, etc.) rather than the internal stimuli for eating, such as hunger. No significant difference by gender and in the MS + and MS – groups was found (φ > 0.05).

More than a third of patients with obesity had an emotional type of EB – (32.3 ± 3.1)%, against (10.86 ± 1.9)% in children in the control group (φ < 0,05), without significant difference by gender and MS (φ > 0.05). In case of emotional type of EB, the stimulus for eating becomes not hunger but emotional discomfort: a person eats not because of being hungry, but because of feeling anxious, insulted, irritated, and so on.

It was also found that in general, patients with MS + group had statistically significantly more frequent disorders of EB (71.8 ± 3.7%) than patients without signs of MS (39.4 ± 4.1%), (p <0.05). That is, disorders of EB, as a clinical feature, are more common in individuals with metabolic disorders in the background of IR.

The next step was the study of serum magnesium and zinc levels with an assessment of these indicators depending on the type of EB disorder (Table 3).

According to the results of studies, in adolescents with obesity there was a significant deficiency of zinc and magnesium in the body, in contrast to the control group surveyed (φ < 0.05). It was observed that the lack of these trace elements was most pronounced and statistically significant (φ < 0.05) in the group of patients with restrictive type of EB. Thus, haphazard, rigid and unbalanced self-prescribed diets are very harmful and, at times, dangerous.

DISCUSSION

Eating disorders lead to somatic disorders, psychological discomfort of the patient, even to depressive states [4]. The lack of trace elements in the blood of adolescents with obesity and metabolic syndrome may be important in the formation of these conditions due to the huge range of their physiological functions, perhaps closing the wrong circle of these metabolic disorders [3, 6]. Therefore, drugs containing zinc and magnesium may be recommended to these patients in rehabilitation programs. It is also advisable to modify eating behaviours through healthy balanced eating and psychological counseling to improve the effectiveness of the treatment of obesity and the prevention of metabolic syndrome. The daily requirement of zinc for adolescents 14-17 years is 15 mg (boys) and 13 mg (girls), magnesium – 300 mg [1, 10].

These results confirm the current ideas about obesity as a psychosomatic disease, in the origin of which both psychological and somatic factors play a role [16, 17, 18]. The psychological effects of childhood obesity on the patient and his or her family require screening for mental health and counseling if needed. The prevention of childhood obesity by promoting healthy eating habits appropriate to the age of physical activity and a healthy environment should be the primary goal of pediatricians and general practitioners. Unfortunately, there are few studies of psychological predictors of childhood obesity, including EB, their connection with metabolic disorders, and trace element imbalances caused by unbalanced nutrition [3, 4, 19, 21]. Therefore, a research with a detailed study of the nature of the daily diet of Ukrainian adolescents, namely children with obesity and signs of metabolic syndrome, eating disorders, levels of essential trace elements, is certainly relevant. In the long run, this will make possible to correct at early stages eating behaviour by lifestyle modification, to prevent obesity and metabolic syndrome progression, as well as to correct the microelement imbalances.

CONCLUSIONS

1. A survey conducted revealed the problem of irrational and unbalanced diet in Ukrainian adolescents. Thus, irregular and unbalanced diet naturally causes digestive disorders and contributes to the formation of comorbid pathologies such as obesity and MS.
2. A comprehensive clinical and laboratory-instrumental study proved that 50% of patients with obesity have classic signs of metabolic syndrome (abdominal obesity, insulin resistance, dyslipidemia, etc.).
3. The general analysis of the DEBQ survey results revealed that patients with MS were twice as likely to have abnormalities in EB (71.8 ± 3.7)% than in patients without them (39.4 ± 4.1)%, ($\varphi < 0.05$). Among the eating disorders, the most widespread was the restrictive type – 73.9% of all surveyed, with less frequent occurrence external (43.8%) and emotional (32.3%).
4. Restrictive type of EB was twice as common in patients with metabolic syndrome, correlation analysis determined a positive association of these disorders with the index HOMA ($r = 0{,}311$; $p < 0{,}001$).
5. Adolescents with obesity experienced significant zinc and magnesium deficiency in the body ($\varphi < 0.05$). The most pronounced, statistically significant ($\varphi < 0.05$) deficiency of these trace elements was observed in the group of patients with restrictive type of EB, that is, haphazard, rigid and unbalanced diets are harmful and, at times, dangerous.

Thus, the modification of eating behaviour through healthy balanced nutrition and psychological support is one of the most important tasks in the treatment of patients with obesity, which will make possible to normalize body weight, psychological and general health, improve the quality of life and take preventive measures to avoid metabolic syndrome and the development of trace elements imbalance.

REFERENCES

1. Marty L., Chambaron S., Nicklaus S., Monnery-Patris S. Learned pleasure from eating: An opportunity to promote healthy eating in children? Appetite. 2018; 1(120): 265-274. https://doi.org/10.1016/j.appet.2017.09.006.
2. Huang J.Y., Qi S.J. Childhood obesity and food intake. World J Pediatr. 2015; 11(2): 101-107. https://doi.org/10.1007/s12519-015-0018-2.
3. Hemmingsson E. Early childhood obesity risk factors: socioeconomic adversity, family dysfunction, offspring distress, and junk food self-medication. Curr. Obes. Rep. 2018; 7(2): 204-209. https://doi.org/10.1007/s13679-018-0310-2.
4. Schoentgen B., Lancelot C., Le Gall D. Eating behavior in pediatric obesity: Of the advantages of combining the neurobiological and neuropsychological approaches. Arch Pediatr. 2017; 24(3): 273-279. https://doi.org/10.1016/j.arcped.2016.12.004.
5. Kawada T. Socioeconomic status and childhood metabolic syndrome. Int J Cardiol. 2019; 283: 189-193. http://dx.doi: 10.1016/j.ijcard.2019.01.106.
6. O'Neill S., O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. Obesity Reviews. 2015; 16(1): 1-12. http://dx.doi.org/10.1111/obr.12229.
7. Al-Hamad D., Raman V. Metabolic syndrome in children and adolescents. Translational Pediatrics. 2017; 6(4): 397–407. https://doi.org/10.21037/tp.2017.10.02.
8. Gromnatska N., Cherkas A., Lemishko B., Kulya O. The pattern of metabolic syndrome in children with abdominal obesity. Georgian Med News. 2019; 289: 68-72.
9. Nicklaus S. The role of dietary experience in the development of Eating Behavior during the first years of life. Ann Nutr Metab. 2017; 70(3): 241-245. https://doi.org/10.1159/000465532.
10. Campbell M.K. Biological, environmental, and social influences on childhood obesity. Pediatr Res. 2016; 79(1-2): 205-211. https://doi.org/10.1038/pr.2015.208.
11. Daniels L.A., Mallan K.M., Battistutta D., Nicholson J.M. et al. Child eating behavior outcomes of an early feeding intervention to reduce risk indicators for child obesity: the NOURISH RCT. Obesity (Silver Spring). 2014; 22(5): 104-11. https://doi.org/10.1002/oby.20693.
12. Isachenkova O. Nutritional behavior as an important factor in the development of obesity. J. Obesity and metabolism. 2015; 12(4): 23-29. https://doi.org/10.14341/omet2015414-17.
13. Barnes M., Caltabiano M. The interrelationship between orthorexia nervosa, perfectionism, body image and attachment style, Eat Weight Disord. 2016; 2(1): 33-65. https://doi.org/10.1007/s40519-016-0280-x.

14. Seo S.H., Shim Y.S. Association of Sleep Duration with Obesity and Cardiometabolic Risk Factors in Children and Adolescents: A Population-Based Study. Sci Rep. 2019; 91: 9463. doi: 10.1038/s41598-019-45951-0.
15. Barclay L., Desiree L. Waist-to-height ratio may predict cardiometabolic risk in normal-weiht children CME. BMC Pediatr. 2010; 10: 73-78.
16. Fang Y., Ma Y., Mo D. et al. Methodology of an exercise intervention program using social incentives and gamification for obese children. BMC Public Health. 2019; 19(1): 686. doi: 10.1186/s12889-019-6992-x.
17. Tagi V.M., Giannini C., Chiarelli F. Insulin Resistance in Children. Front Endocrinol (Lausanne). 2019; 10: 342. doi: 10.3389/fendo.2019.00342.
18. Spreghini N., Cianfarani S., Spreghini M.R. et al. Oral glucose effectiveness and metabolic risk in obese children and adolescents. Acta Diabetol. 2019; 56(8): 955-962. doi: 10.1007/s00592-019-01303-y.
19. Gibbs B.G., Forste R. Socioeconomic status, infant feeding practices and early childhood obesity. Pediatr Obes. 2014; 9(2): 135-146. https://doi.org/10.1111/j.2047-6310.2013.00155.x.
20. Lewellyn C.H., Fildes A. Behavioural Susceptibility Theory: the Role of appetite in genetic risk of obesity. Curr. Obes. Rep. 2017; 6(1): 38-45. https://doi.org/10.1007/s13679-017-0247-x.
21. Strashok L., Buznytska O. Study of eating behavior in adolescents with obesity and signs of metabolic syndrome. AML. 2019; 25(2-3): 69-75.

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ORIGINAL ARTICLE

ANALYSIS OF THE VITAMIN D RECEPTOR BSMI GENE POLYMORPHISM IN CHILDREN WITH GROWTH HORMONE DEFICIENCY

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ABSTRACT

The aim: The objective of the study was to investigate the polymorphism of the vitamin D receptor (VDR) BsmI gene in children with growth hormone deficiency and the level of their vitamin D supply.

Materials and methods: Sixteen children diagnosed with of growth hormone deficiency who were treated at the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine» were examined. The patient's gender and age, the anthropometric data, the vitamin D level in the blood, the bone age, the GH level, the IGF-1 levels, the level of calcium in the blood and VDR gene polymorphism were taken into account.

Results: It was shown that in the presence of the G/A genotype, the risk of growth hormone deficiency development was increased OR = 1,096 (95% CI 0.39-3.02; p = 0.86). For BsmI, mean values of height, body mass, height SDS, serum 25(OH)D, in the studied population (16 children) were 123.49 ± 19.62 cm, 26.96 ± 11.11 kg, -2.25 ± 0.85, 48.86 ± 16.71 nmol/l, respectively; total calcium level consisted of 2.40 ± 0.12 mmol/l, serum phosphorus – 1.43 ± 0.11 mmol/l.

Conclusions: The allele frequency of the VDR BsmI polymorphism was 62.5% for the G allele (n = 20) and 37.5% for the allele A (n = 12). The G allele carrier of the polymorphic locus BsmI rs1544410 of the VDR gene (rs11568820) is associated with an increased risk of growth hormone deficiency development OR = 1.31 (95% CI 0.62-2.75; p = 0.47).

KEY WORDS: VDR gene, children, growth hormone deficiency, polymorphism

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INTRODUCTION

As is generally known the vitamin D and its active metabolites play a key role in phosphorus-calcium homeostasis and bone metabolism, regulate the cell growth and differentiation in different target organs [1,2].

The vitamin D is a ligand for the nuclear receptor which is encoded by the VDR gene and is a regulator of the activity of many target genes by interacting with specific DNA sequences in the promoter regions of these genes [3].

The vitamin D levels are generally lower in patients with growth hormone (GH) deficiency than in the control group with different prevalence of insufficiency or deficiency, and this status may make worse the already known cardiovascular and metabolic risks of growth hormone deficiency, although this statement (affirmation) is not generalized for all the studies. In addition, data on the effect of growth hormone treatment on vitamin D levels in patients with GH deficiency are quite controversial. On the contrary, in active acromegaly, a condition characterized by chronic growth hormone excess, both increased and decreased levels of vitamin D have been observed, and the interaction between vitamin D and the growth hormone / IGF-1 axis becomes even more complex when considering acromegaly treatment [4].

Hamza R.T. et al. (2018) evaluated the vitamin D status in prepubertal children with idiopathic growth hormone deficiency and the effect of treatment on the vitamin D level. The vitamin D deficiency was revealed in 40 % of children with idiopathic growth hormone deficiency; a deficiency was found in 44 %, a sufficient level of the vitamin D was only observed in 16 %. A positive correlation between the vitamin D and peak GH levels was noted. The GH peak was a significant predictor of the vitamin D levels. After 1 year growth hormone therapy, the level of vitamin D was significantly increased. Overall, the level of vitamin D remained insufficient in 22 % of cases and the vitamin D deficiency was found in 24 % of children. It was proved that Vitamin D negatively correlated with parathyroid hormone (PTH). Therefore, hypovitaminosis D is common in children with idiopathic growth hormone deficiency and was significantly reduced 1 year after GH therapy [5].

P. Ameri, et al. [6] and F. Bogazzi, et al. [7] show that the vitamin D levels affect the function of the GH / IGF-1 axis. Vitamin D can increase the production and secretion of IGF-1 (insulin-like growth factor-1) and IGFBR-3 (insulin like growth factor binding protein 3) in the liver. On the other hand, both STH and IGF-1 increase renal production of vitamin D by increasing the kidney activity

of 1α-hydroxylase [8]. During the vitamin D therapy the effect of recombinant GH(rGH) on the bone formation in GH treatment [9].

The main function of the vitamin D substances is, first of all, to regulate the bone metabolism. Only 10-15 % of calcium and about 60 % of phosphorus are absorbed without the vitamin D participation. 1.25(OH)2D – a hormone-active form of vitamin D, interacting with the VDR, increases the absorption of intestinal calcium and phosphorus by 30-40 % and 80 %, respectively. At the same time, the process of calcium mobilization from the bone tissue is controlling by vitamin D, which is also necessary to create optimal conditions for its growth. Calcium and phosphate metabolism are regulated not only by vitamin D but also by the level of ionized calcium, PTH and calcitonin [10].

The vitamin D receptor belongs to a family of transactive transcription regulatory factors and has a similarity to the steroid and thyroid hormone receptors, confirming the functioning of vitamin D as a hormone [11,12].

Vitamin D receptors are known to be encoded by the eponymous VDR gene (also known as NR1I1) localized on chromosome 12q12-q14.

This gene is characterized by polymorphism, that is, the existence of its different allelic variants in the population. The most significant are polymorphisms of the VDR gene involved in disease development: BsmI, FokI, TaqI Fokl, ApaI [12]. To date, 1518 single-nucleotide polymorphisms (SNPs) of the human VDR gene have been described.

Among them is BsmI, localized in the eighth intron. The nature of the BsmI polymorphism lies in the fact that guanine is substituted for adenine at position 58980. By themselves, polymorphisms in introns are not functionally significant, since they do not change the sequence of the nitrogenous bases in the content part of the gene, however, being linked to the regulatory regions of the gene, may serve as markers of functional relationships of other polymorphisms with the development of pathological processes and diseases. The association of BsmI polymorphism with different pathological processes and diseases has been studied in many trials of different populations. In some of them, this SNP was associated with osteoporosis [13], pathological fractures [14], type 2 diabetes [15], prostate cancer [16], breast cancer [17], Parkinson's disease [18].

THE AIM

The aim was to investigate the polymorphism of the VDR BsmI gene in children with growth hormone deficiency and the level of their vitamin D supply.

MATERIALS AND METHODS

The serum 25-hydroxycalciferol (25(OH)D) level was determined by immunochemiluminescent method. The GH and IGF-1 levels were studied using radioimmunoassay and enzyme immunoassay methods. The control group consisted of 250 healthy children and adolescents aged 9 to 18 (mean age 8.24 ± 3.83 years) [19]. Sixteen children were examined with diagnosis of GH deficiency who were treated at the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of NAMS of Ukraine». The patient's sex and age, anthropometric data, vitamin D level in the blood (excluded summer months of patient recruitment), bone age, GH level after stimulation tests (clonidine, insulin), IGF-1 levels, blood levels of total and ionized calcium were taken into account. The mean age of children (11 boys, 5 girls) who were included in the study was 10 ± 3.0 years. The average growth delay was minus 2.25 (± 0.85) SDS. At the time of the examination, all the patients were in a euthyroid state. The study included children who had not received the calcium and vitamin D drugs for 6 months. Children with growth hormone deficiency had a significant decrease in IGF-1 levels (from 27.83 to 94.89 ng / ml). Statistical processing of the study results was performed using Microsoft Excel statistical programs.

In order to verify the diagnosis of vitamin D insufficiency and deficiency, the classification (2011) was adopted by the International Institute of Medicine and Endocrine Medicine, *Committee practical guidelines*. According to this classification, the vitamin D deficiency in children and adults is considered to be a clinical syndrome due to low serum 25(OH)D level (below 20 ng/ml or 50 nmol/l). The serum 25(OH)D level from 21 ng / ml to 29 ng/ml (from 50.1 to 74.9 nmol/l) should be considered as the vitamin D deficiency. The normal level of vitamin D is equal to the serum 25(OH)D concentration above 30 ng / ml.

The determination of VDR BsmI gene (rs1544410) polymorphism was performed using the polymerase chain reaction (PCR) method, followed by analysis of the length of the restriction fragments upon their detection by agarose gel electrophoresis.

For genotyping, the venous blood was collected under sterile conditions in 2.7 ml monovets with potassium salt of ethylenediaminetetraacetic acid ("Sarstedt", Germany), which served as an anticoagulant. First, DNA was eliminated from the peripheral blood using a commercial Quick-DNA™ Miniprep Plus Kit (manufactured by Zymo Research, USA).

The genes studied were amplified using specific primers (Metabion, Germany) and commercial Dream Taq Green PCR Master Mix (Thermo Scientific, USA). The tubes with the final amplification mixture were transferred to the Flex Cycler BU amplifier (Analytic Jena, Germany) to provide the appropriate temperature regime.

The amplification products of DNA fragments (amplicons) of the VDR gene were subjected to hydrolytic cleavage by restriction endonuclease BsmI (Thermo Scientific, USA), respectively. Separate mixtures were prepared for restriction analysis and transferred to pre-labeled tubes, and then the amplicons were added.

The fragment limiting reaction for the BsmI G / A (rs1544410) of the VDR gene was performed according to the manufacturer's recommendations in a solid-state micro thermostat at 37 ° C for 16 hours.

The process was stopped by increasing the temperature to 65°C for 20 minutes. The state of the restriction fragments

of the VDR gene was analyzed by a 3% agarose gel (agarose firm Cleaver Scientific, UK), with the addition of ethidium bromide, a marker of molecular weight "GeneRuler 50 bp DNA Ladder" (Thermo Scientific, USA) and subsequent visualization using a transilluminator stained with ethidium bromide by computer program "Vitran".

Amplifiers of the VDR BsmI G / A gene (rs1544410) were hydrolytically cleaved in the presence of a 5'-GAATGCN ↓ -3 'restriction site, resulting in the restriction formations with molecular mass a 644 bp and 179 bp – the GG genotype. The restriction site disappeared with nucleotide replacement from G to A, if the size of the amplified DNA fragments remained unchanged after interaction with the restriction nuclease (823 bp), then the AA genotype was recorded. Accordingly, all three types of fragments: 823, 644 and 179 pp in the heterozygous genotype (GA) were simultaneously observed.

The data obtained were statistically analyzed using Statistica 6.1 and SPSS17.0 software package (SPSS, Inc., Chicago, IL, USA). General statistical analysis included median (Me) and interquartile interval' (UQ-LQ) calculations. Laboratory parameters were presented in the form of arithmetic data (mean (M ± m), standard error of mean) (SEM). For nominal variables, the ratios were calculated using the Pearson test ($\chi 2$) and the Fisher test (two-sided); these differences were considered statistically significant for which the P value was <0.05.

The study was conducted in accordance with the basic principles of bioethics of the Council of Europe Convention on Human Rights and Biomedicine (the 4th of April 1997), the World Health Association Helsinki Declaration on Ethical Principles for Conducting Medical Research with the Participation of People (1964-2013). Commission on Biomedical Ethics of the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine" did not find violations of moral norms during the study. Informed consent was obtained from the participants and their parents.

RESULTS

Acting through its receptor, the hormone-active form of vitamin D – $1.25(OH)_2D$ can cause many effects that affect various biological processes in the body.

In the target tissues, the vitamin D receptors are functioning in both the cell nuclei (gene regulation level) and plasma membranes (non-gene regulation level). At the gene level, active metabolites of vitamin D bind to specific receptors, forming the hormone receptor complex D3-VDR, which has its own specific DNA-binding domain (a specific DNA sequence), thereby controlling the transcription of the corresponding genes. This process, in one's turn, leads to the biosynthesis of new mRNA molecules and the translation of the corresponding proteins involved in the physiological responses) [11,12].

Analysis of the distribution of allele and genotype frequencies of the polymorphic locus BsmI (rs1544410) gene in the group of patients with GH deficiency and in the control sample [19] is statistically significant (Table 1).

The allele frequency of the VDR BsmI polymorphism was 62.5% for the G allele (n = 20) and 37.5 % for the allele A (n=12). The study found that the *G allele carriers* of polymorphic locus BsmI (rs1544410) of the vitamin D receptor gene (rs11568820) is associated with an increased risk of growth hormone deficiency OR = 1,31 (95 % CI 0.62-2.75; p = 0.47).

It was also shown that the risk of growth hormone deficiency is increased in the presence of the G/A genotype, OR = 1.096 (95% CI 0.39-3.02; p=0.86); also in G/G variant, the risk of growth hormone deficiency was OR = 1.27 (95 % CI 0.44-3.63; p = 0.65); in the A/A genotype variant, the risk of growth hormone deficiency was minimal OR = 0.56 (95 % CI 0.12-2.58; p=0.46).

For BsmI, mean values of height, weight, height SDS, serum 25(OH)D, in the studied population (16 children) were 123.49 ± 19.62 cm, 26.96 ± 11.11 kg, -2.25 ± 0.85, 48,86 ± 16,71 nmol/l, respectively, level of total calcium – 2,40 ± 0,12 mmol/l, serum phosphorus -1,43 ± 0,11 mmol/l (Table 2).

The vitamin D deficiency is occurred in all the children with growth hormone deficiency regardless of the polymorphic locus rs1544410 BsmI of the vitamin D receptor gene. Vitamin D was significantly lower (32.05 ± 11.67 nmol/l) in children with polymorphic variant G/C than that in children with other VDR BsmI polymorphisms, but not significantly.

The GH level after the clonidine stimulation test was significantly lower in group with the VDR BsmI polymorphic variant A / A (0.65 ± 0.05 ng/ml), and significantly higher in the VDR BsmI polymopphic G/G variant (5.59 ± 0.42 ng/ml).

Growth SDS (Standard Deviation Score) was significantly lower in the group of children with polymorphic variant A / A (-3.09 ± 0.12) compared to variants of the polymorphic locus BsmI (rs1544410) of the VDR gene G/A (-2.02 ± 0, 42) and G / G (-2.51 ± 1.35).

IGF-1 in all surveyed was low with polymorphic variant G / G VDR BsmI (94.89 ± 44.34 ng/ml), with polymorphic variants G / A and A / A as well, but not significantly (27.83 ± 12.61 ng/ml and 37.75 ± 18.03 ng/ml, respectively). Normal levels of total and ionized calcium in serum were found in all the children examined.

DISCUSSION

The vitamin D and IGF-1 levels affect each other: on the one hand, the increase in vitamin D increases the level of IGF-1 [20], and on the other hand, IGF-1 stimulates the activity of the enzyme 1α-hydroxylase, which, in one's turn, regulates the renal production of vitamin D : $1,25(OH)_2D$ or calcitriol [21].

In addition, GH itself has a direct stimulating effect on the production of $1.25(OH)_2D$ [22]. Besides, both GH and IGF-1 seem to increase the activity of CYP27A1, a multifunctional cytochrome P450 enzyme that catalyzes 25-hydroxylation of vitamin D in hepatoblastoma cells [23].

Table 1. Distribution of allele and genotype frequencies of the polymorphic locus of vitamin D receptor gene BsmI-rs1544410 in the group of patients with growth hormone deficiency and in the control sample

Groups and number individuals (n)	Allele' frequencies, %		χ 2; df = 1	Genotype frequencies, %			χ 2; df = 2
	G	A		G/A	G/G	A/A	
Population sample (218)*	244 (56)	192 (44)	0,52 p=0,47	104 (47,7)	70 (32.1)	44 (20.2)	0.59 p=0.74
Patients with GH deficiency	20 (62.5)	12 (37.5)		8 (50)	6 (37.5)	2 (12.5)	

Note: Hardy-Weinberg equilibrium for BsmI (P = 0.79)
* – data from source19.

Table 2. Effect of VDR polymorphism on growth values and some serum biochemical parameters in children with growth hormone deficiency

Values	Genotype		
	BsmI (rs1544410), n=16		
	GA	GG	AA
Growth SDS	-2.02±0.42	-2.51±1.35	-3.09±0.12*
GH level after stimulation testwith clonidine, ng / ml	2.12±0.12	5.59±0.42*	0.65±0.05**
25(OH)D, nmol/l	47.63±16.91	32.05±11.67	41.52±4.41
IGF-1, ng / ml	27.83±12.61	94.89±44.34	37.75±18.03
The total calcium, mmol/l	2.44±0.09	2.43±0.11	2.20±0.13
Calcium ionized, mmol/l	1.19±0.06	1.22±0.07	1.11±0.01

Note: * - the significance level between values of the BsmI (rs1544410) GA and GG genotypes (p<0.05);
** - significance level between the values of BsmI (rs1544410) GA and AA genotypes (p<0.05).

Another target, which is rich in VDR, is represented by the pituitary gland. It is likely that $1.25(OH)_2D$ acts on the human pituitary VDR, stimulating GH secretion and modulating the expression of some genes [24].

G. Saggese et al. [25] studied the status of vitamin D in 26 children with GH deficiency and found normal concentrations of 25OHD but low levels of $1.25(OH)_2D$ before growth hormone (treatment and a significant increase in $1.25(OH)_2D$ levels after 12 months of rGH treatment. Data of 80 Sicilian children with growth hormone deficiency were analyzed by A. Ciresi et al.[8] These authors reported of the higher 25OHD values in children with growth hormone deficiency in the solar seasons (31.1 ± 11.1 ng/ml in June – September) than in the cold season (17.3 ± 5.3 ng / ml in November – February), 35% of children have vitamin D insufficiency and 40% – vitamin D deficiency . E. Witkowska-Sedek et al. studied 84 children and adolescents with GH deficiency and found the low concentrations of 25OHD (22.3 ± 6.9 ng / ml) [26], and M.C. Savanelli et al. analyzed 41 adult patients with growth hormone deficiency and found an average 25OHD concentration of 21.3 ± 12.3 ng/ml, the vitamin D deficiency was found in 51% of patients compared with 14.6% of the control group[27]. In addition, P. Ameri et al. found 69 adult patients with GH deficiency and only 6 patients (8.7%) had a normal serum concentration of 25OHD more than 30 ng/ml. They also reported a positive correlation between the vitamin D status and IGF-1, and found a tendency in treatment to increase doses of growth hormone in patients with the vitamin D deficiency (25OHD <15 ng/ml), suggesting that the better vitamin D level may facilitate the achievement of normal IGF-1 level in patients with growth hormone deficiency. In the literature review, these authors suggested that the assessment of the vitamin D levels may be an appropriate method for determining the doses of recombinant GH for the treatment of adult patients with GH deficiency [6].

Taking into account that GH increases $1.25(OH)_2D$ level [20,25,28-29], although very likely indirectly by IGF-1, it can be considered that patients with growth hormone deficiency due to detachment of the pituitary leg have a low concentration of serum $1.25(OH)_2D$. In our study, all the children with GH deficiency had the 25(OH)D deficiency.

The analysis of important VDR polymorphisms in the pathogenesis of various diseases is difficult. Discovering the genetic variants associated with susceptibility to diseases may be the key to their preventing. Thus, VDR regulates the expression of a number of genes in the bone cells, many of which are encoded bone remodeling, have catabolic or anabolic actions, as well as stimulate the secretion of hormones that affect the vitamin D metabolism and mineral metabolism.

The role of VDR gene polymorphisms in the formation of skeletal pathology was actively studied. Thus, a study of the association of BsmI polymorphism with osteoporosis in different parts of the skeleton in postmenopausal women revealed a positive association of the disease with the genotype G/G (p = 0.009) and the G allele (p = 0.016) polymorphism [30]. L. Bao et al. in a meta-analysis showed that genetic BsmI polymorphism correlates with the level of bone mineral density in children, in particular the b(G) allele and the b/b (G/G) genotype are more likely to occur in children with the higher bone mineral density [3].

CONCLUSIONS

1. The allele frequency of the VDR BsmI polymorphism was 62.5% for the G allele (n = 20) and 37.5% for the A allele (n = 12). *G allele carriers* of the BsmI (rs1544410) polymorphic locus of the VDR gene (rs11568820) was

associated with an increased risk of growth hormone deficiency OR = 1.31 (95% CI 0.62-2.75; p = 0.47).
2. It was also shown that in the presence of the G / G genotype, the risk of growth hormone deficiency was OR = 1.27 (95% CI 0.44-3.63; p = 0.65).
3. The vitamin D deficiency occurred in all children with growth hormone deficiency regardless of the BsmI rs1544410 polymorphic locus of the vitamin D receptor gene. In children with the BsmI polymorphic variant of G / G VDR the level of vitamin D was significantly lower (32.05 ± 11.67 nmol / l) than in children with other VDR BsmI polymorphisms, but not significantly.

REFERENCES

1. Baldock P.A., Thomas G.P., Hodge J.M. et al. Vitamin D action and regulation of bone remodeling: suppression of osteoclastogenesis by the mature osteoblast . J Bone Min Res. 2006; 21(10): 1618-1626 . doi: 10 .1359/jbmr .060714.
1. Pike J.W., Zella L.A., Meyer M.B. et al. Molecular actions of 1,25-Dihydroxy vitamin D 3 on genes involved in calcium homeostasis. J Bone Min Res. 2007; 22(1): 16-19. doi: 10 .1016/0026-0495(75)90055-4 .
2. Bao L., Chen M., Lei Y. et al. Association between vitamin D receptor BsmI polymorphism and bone mineral density in pediatric patients: A meta-analysis and sys tematic review of observational studies. Medicine (Baltimore). 2017; 96(17): e6718. doi: 10.1097/MD.0000000000006718.
3. Ciresi A., Giordano C. Vitamin D across growth hormone (GH) disorders: From GH deficiency to GH excess. Growth Hormone & IGF Research. 2017; 33: 35-42. doi: 10.1016/j.ghir.2017.02.002.
4. Hamza R.T., Hamed A.I., Sallam M.T. Vitamin D status in prepubertal children with isolated idiopathic growth hormone deficiency: effect of growth hormone therapy Journal of Investigative Medicine. 2018; 66(5): 1-8. doi: 10.1136/jim-2017-000618.
5. Ameri P., Giusti A., Boschetti M. et al. Vitamin D increases circulating IGF1 in adults: potential implication for the treatment of GH deficiency. European Journal of Endocrinology. 2013; 169(6): 767-72. doi: 10.1530/EJE-13-0510.
6. Bogazzi F., Rossi G., Lombardi M. et al. Vitamin D status may contribute to serum insulin-like growth factor I concentrations in healthy subjects. Journal of Endocrinological Investigation. 2011; 34(8): e200–3. doi: 10.3275/7228.
7. Ciresi A., Cicciò F., Giordano C. High prevalence of hypovitaminosis D in Sicilian children affected by growth hormone deficiency and its improvement after 12 months of replacement treatment. Journal of Endocrinological Investigation. 2014; 37(7): 631-8. doi: 10.1007/s40618-014-0084-7.
8. Witkowska-Sędek E., Stelmaszczyk-Emmel A., Majcher A. et al. The relationship between alkaline phosphatase and bone alkaline phosphatase activity and the growth hormone/insulin-like growth factor-1 axis and vitamin D status in children with growth hormone deficiency. Acta Biochimica Polonica. 2018; 65(2): 269-275. doi: 10.18388/abp.2017_2541.
9. Shih E.V., Milotova N.M. Rol polimorfizma gena VDR, kodiruyuschego retseptor vitamina D, v patogeneze arterialnoy gipertonii [Role of the VDR gene encoding the vitamin D receptor in the pathogenesis of hypertension]. Biomedttsina. 2009; 1: 55-67 (in Russian).
10. Holick M.F., Binkley N.C., Bischoff-Ferrari H.A. et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011; 96(7): 1911–30. doi: 10.1210/jc.2011–0385.
11. Uitterlinden A.G., Fang Y., Van Meurs J.B. et al. Genetics and biology of vitamin D receptor polymorphisms: Review. Gene. 2004; 338: 143–156. doi: 10.1016/j.gene.2004.05.014.
12. Tanriover M.D., Tatar G.B., Uluturk T.D. et al. Evaluation of the effects of vitamin D receptor and estrogen receptor 1 gene polymorphisms on bone mineral density in postmenopausal women. Clin Rheumatol. 2010; 29(11): 1285-93. doi: 10.1007/s10067-010-1548-6.
13. Chatzipapas C., Boikos S., Drosos G.I. et al. Polymorphisms of the Vitamin D Receptor Gene and Stress Fractures. Horm Metab Res. 2009; 41(8): 635-40. doi: 10.1055/s-0029-1216375.
14. Ortlepp J.R., Lauscher J., Hoffmann R. et al. The VDR gene variant is associated with the prevalence of Type 2 diabetes mellitus and coronary artery disease. Diabet Med. 2001; 18(10): 842-5. doi: 10.1046/j.1464-5491.2001.00585.x.
15. Habuchi T., Suzuki T., Sasaki R. et al. Association of vitamin D receptor gene polymorphism with prostate cancer and benign prostatic hyperplasia in a Japanese population. Cancer Research. 2000; 60(2): 305-8.
16. Sinotte M., Rousseau F., Ayotte P. et al. Vitamin D receptor polymorphisms (FokI, BsmI) and breast cancer risk: association replication in two case-control studies within French Canadian population. Endocrine-Related Cancer. 2008. 15(4): 975-83. doi: 10.1677/ERC-08-0056.
17. Kim J.S., Kim Y.I., Song C. et al. Association of Vitamin D Receptor Gene Polymorphism and Parkinson's Disease in Koreans. J Korean Med Sci. 2005; 20(3): 495-8. doi:10.3346/jkms.2005.20.3.495.
18. Montazeri-Najafabady N., Dabbaghmanesh M.H., Mohammadian A.R. et al. Association of Vitamin D Receptor BsmI Gene Polymorphism with BMD Z-Score in Iranian Children and Adolescents (9-18 Years Old). Int J Endocrinol Metab, 2019; 17(2): e82677. Published online 2019 Apr 23. doi: 10.5812/ijem.82677.
19. Wei S., Tanaka H., Seino Y. Local action of exogenous growth hormone and insulin-like growth factor-I on dihydroxyvitamin D production in LLC-PK1 cells. Eur J Endocrinol. 1998; 139(4): 454-60. doi: 10.1530/eje.0.1390454.
20. Henry H.L. Regulation of vitamin D metabolism. Best Pract Res Clin Endocrinol Metab. 2011; 25(4): 531–41. doi: 10.1016/j.beem.2011.05.003.
21. Marcus R., Butterfield G., Holloway L. et al. Effects of short term administration of recombinant human growth hormone to elderly people. J Clin Endocrinol Metab. 1990; 70(2): 519-27. doi:10.1210/jcem-70-2-519.
22. Araya Z., Tang W., Wikvall K. Hormonal regulation of the human sterol 27-hydroxylase gene CYP27A1. Biochem J. 2003; 372(2): 529–34. doi: 10.1042/bj200 21651.
23. Pérez-Fernandez R., Alonso M., Segura C. et al. Vitamin D receptor gene expression in human pituitary gland. Life Sci. 1997; 60(1): 35–42.
24. Saggese G., Baroncelli G.I., Bertelloni S. et al. Effects of longterm treatment with growth hormone on bone and mineral metabolism in children with growth hormone deficiency. J Pediatr. 1993; 122(1): 37-45. doi: 10.1016/s0022-3476(05)83484-5.
25. Witkowska-Sedek E., Kucharska A., Ruminska M. et al. Relationship between 25(OH)D and IGF-I in children and adolescents with growth hormone deficiency. Adv Exp Med Biol. 2016; 912: 43-9. doi: 10.1007/5584_2016_212.
26. Savanelli M.C., Scarano E., Muscogiuri G. et al. Cardiovascular risk in adult hypopituitaric patients with growth hormone deficiency: is there a role for vitamin D? Endocrine. 2016; 52(1): 111-9. doi: 10.1007/s12020-015-0779-3.

27. Wei S., Tanaka H., Kubo T. et al. Growth hormone increases serum 1,25-dihydroxyvitamin D levels and decreases 24,25-dihydroxyvitamin D levels in children with growth hormone deficiency. Eur J Endocrinol Eur Fed Endocr Soc. 1997; 136(1): 45-51. doi: 10.1530/eje.0.1360045.
28. Condamine L., Menaa C., Vrtovsnik F. et al. Local action of phosphate depletion and insulin-like growth factor 1 on in vitro production of 1,25-dihydroxyvitamin D by cultured mammalian kidney cells. J Clin Invest. 1994; 94(4): 1673-9. doi: 10.1172/JCI117512.
29. Maylyan E.A. Vliyanie polimorfizma gena retseptora vitamina D 283 A> G (BSMI) u zhenschin s osteoporozom v postmenopauze. [The influence of vitamin D receptor gene 283 A>G (BSMI) polymorphism on osteoporosis in postmenopausal women]. Medicinskij vestnik Juga Rossii. 2016; 4: 32–38. (in Russian).

"Investigation of Vitamin D3 (VDR3) receptor Bsm1 gene polymorphism, VDR gene TaqI (rs731236) polymorphism, and VDR Gene ApaI polymorphism (rs7975232) and establishing an association of identified disorders with clinical manifestations of short stature and patient phenotype".
"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study".

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ORIGINAL ARTICLE

RESULTS OF QUESTIONING PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE IN THE CONDITIONS OF DENTAL PRACTICE

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ABSTRACT

The aim: Is to develop and test questionnaires allowing systematization of the main dental complaints and knowledge on hygienic oral care in patients with non-alcoholic fatty liver disease (NAFLD) given their individual characteristics.

Materials and methods: The developed questionnaires and database have been tested on 36 patients with NAFLD and 10 patients of the control group.

Results: As a result of the study, it appears that the main dental complaints in patients with NAFLD are dry mouth and the associated need for frequent mouth rinsing with water, bleeding gums, mainly during teeth brushing, distortion of taste and bad breath. It has been noted that only 33.3% of patients regularly brush their teeth every morning and evening, while 25.0% of respondents brush their teeth only once a day. It is also characteristic that more than 25.0% of patients with NAFLD use mainly carbohydrate foods in their diet, and the smoking rate among this group is at least 58.0%.

Conclusions: The use of this questionnaire makes it necessary to take into account all the "bottlenecks" in selection of measures for dental disease prevention in NAFLD, which currently is of high relevance and necessity.

KEY WORDS: non-alcoholic fatty liver disease, periodontitis, dryness questionnaire

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INTRODUCTION

The relevance of studying combined somatic and dental diseases is beyond doubt, since the internal organs pathology in patients can cause development of structural and functional changes in the oral cavity and have a significant impact on their course and prognosis [1-3]. According to various scientific studies, there is a close relationship between changes in the oral cavity and the digestive system pathology, which is due to the anatomical and morphofunctional unity. One of the most globally widespread somatic diseases is non-alcoholic fatty liver disease (NAFLD), which currently takes the form of a pandemia [4-5].

Today NAFLD is considered as a chronic disease with morphological changes in the form of steatosis, non-alcoholic steatohepatitis and fibrosis (cirrhosis) [6-7]. Despite the extensive study of this disease, there is no data on any single etiological factor in NAFLD development, but several risk factors inherent in the metabolic syndrome are distinguished, namely: obesity, hypodynamia, insulin resistance, hypertriglyceridemia, hypertension, age-related aspects [8-9].

There are a number of works containing data that prove the relationship between periodontal disease and NAFLD. Thus, in 2017, W. Alazawi with a group of colleagues studying the relationship between chronic periodontitis, NAFLD and diabetes showed that these conditions had common risk factors [10]. The authors noted that patients with steatosis were significantly more likely to suffer from chronic periodontitis, with more severe stages of NAFLD having a high correlation with the severity of periodontitis. A similar study was conducted by a group of scientists who studied periodontal disease in the inhabitants of Pomerania (Aderonke A. Akinkugbe, 2017). Researchers noted that the prevalence of periodontal disease was related to the incidence of NAFLD [11].

Inflammatory periodontal diseases are known to have a multifactorial nature and arise when several risk factors are combined, such as poor oral hygiene, smoking, nutritional faults, genetic predisposition, and somatic diseases. However, the aggressive effect of Porphyromonas gingivalis in case of oral hygiene violation is recognized as the main etiological factor, while NAFLD can serve as a background and significantly aggravate the course of dental pathology [12]. The influence of P. gingivalis on NAFLD pathogenesis was noted by Japanese scientists in their study (Masato Yoneda and et., 2012). The results of the study showed that the frequency of P. gingivalis occurrence in patients with NAFLD was statistically higher than in somatically healthy patients [13]. It was found that P. gingivalis could cause significant changes in the composition of the entire oral cavity microflora, stimulate the proteinase activity of neutrophils and increase development of periodontal tissue destruction, as well as exert a systemic effect associated with their toxic and immunopathogenetic effects. It is characteristic that P. gingivalis in the same way affects the intestinal microflora, since the intestinal composition of the microbiota reacts very actively and shifts in response to changes in a person's lifestyle (diseases, eating pattern, mood, etc.).

Table 1. Example of the questionnaire for patients involved into the study

Dear patient, please fill out this questionnaire (choose the answer to the question), carry out self-assessment for the presence and severity of dental complaints, which largely reflect the state of your dental health. This will help us in diagnostics and make the treatment process more effective!				
Seq. No.	**Complaints and questions**	**Never**	**Often**	**Always**
1	Do you ever have a dry mouth sensation?			
2	Do you feel dryness in your mouth, regardless of food intake?			
3	Do you wake up at night to drink water due to a dryness sensation?			
4	Do you have a need to rinse your mouth with water throughout the day?			
5	Do you feel taste distortion?			
6	Do you have bad breath?			
7	Do you feel pain and itching in your gums?			
8	Do you have gum bleeding? - when brushing teeth - when eating - spontaneous bleeding			
9	Do you have communication difficulties due to the above complaints?			
10	Are you undergoing periodontal treatment?			
11	Do you visit your dentist regularly for professional oral hygiene?			
12	Do you use dental hygiene products recommended by your dentist?			
13	Do you brush your teeth 2 times a day?			
14	Do you rinse your mouth after eating?			
15	How often do carbohydrate foods prevail in your diet?			
16	How often do you drink sugary carbonated beverages?			
18	Do you often eat foods containing probiotics?			
19	Do you smoke and how often?			

In this regard, development of questionnaires to identify the prevalence of major dental complaints and perform daily hygienic care of the oral cavity will allow the formation of a program aimed at prevention of possible dental disorders in this category of patients.

THE AIM

The aim of this work is to develop and test questionnaires allowing systematization of the main dental complaints and knowledge on hygienic oral care in patients with NAFLD given their individual characteristics.

MATERIALS AND METHODS

Detection of dental complaints, data on the nature of a diet and methods of individual oral hygiene, bad habits has been carried out though interviewing and questioning patients with self-completion (Table I). When compiling the list of questions, the necessary requirements for the questionnaire have been taken into account: simplicity and unambiguity of questions; possibility of self-completion by the patient; brevity; registration of modern domestic and international dental questionnaires.

The questions in the questionnaire are represented in certain blocks, namely:

Block 1. Basic dental complaints (dry mouth, the need for mouth rinsing with water due to dryness, taste distortion, bad breath, pain and discomfort in the gums, bleeding gums, difficulties in communicating with people due to the above complaints);

Block 2. This block includes questions regarding visits to the dentist and individual oral hygiene (regularity of visits to the dentist for professional oral hygiene, selection of means and items of oral hygiene, frequency of teeth brushing);

Block 3. This block concerns nutrition issues (nature of food, drinking sugary carbonated beverages, eating probiotic products, bad habits).

To process complaints and questionnaire data, an electronic database has been developed based on SPSS 21 software, which allows entering and storing data collected during the survey. The questionnaires have been tested on 36 respondents with verified diagnosis of NAFLD and 10 somatically healthy patients (control group). At the time of the survey, none of the patients in the control group had any complaints or anamnesis data of respiratory and cardiovascular system diseases, endocrine diseases, acute inflammatory processes in the oral cavity, and also none of them was taking any medications at the time of the study. All the results of somatically healthy patients collected during the study completely coincided with those that can be found in modern literature, which made it possible to use these data as reference control values.

RESULTS

When questioning and testing questionnaires, the following results have been obtained: for example, the most common complaint is dry mouth, which is suffered by 91.6% of patients, and 64.9% of them complain of constant dry mouth. Patients note that the dryness sensation appears gradually and increases over time. A large half of the interviewed patients with this complaint notes that dryness cannot be eliminated, since all means turn out to be ineffective. Almost 42.0% of respondents note that night dryness causes sleep interruption, there is a need for water drinking and mouth rinsing with water, even though everyone agrees on low effectiveness of such procedures, since they bring immediate relief, but its duration is no longer than several hours. Dryness causes discomfort when chewing and swallowing food. The respondents note that the dryness sensation is accompanied by a change in the saliva properties, which becomes viscous, foamy, and unable to moisten the oral cavity.

Besides, 83.33% of patients with NAFLD complain of bad breath (halitosis), and 21 patients (58.33%) continuously have this complaint, while only 6 patients say that they never have such sensations. Halitosis cause inconvenience in communication with others, bring discomfort, reduce the quality of patients' lives. Most patients associate the halitosis phenomenon with a feeling of lack of saliva and its inability to excrete food debris.

Most patients with NAFLD also suffer from taste distortion when eating (25 patients), pain and itching in the gums (25 patients), and 88.8% of the respondents note bleeding gums, mainly when brushing teeth. It should be noted that none of the patients surveyed notes spontaneous bleeding of the gums.

When processing data in the questionnaires of somatically healthy patients, it has been noted that only 2 patients (20%) complain of periodic dry mouth and inconveniences associated with it, and 5 respondents suffer from halitosis, 1 of them claims constant halitosis. Also, a frequent complaint among somatically healthy patients is bleeding gums, 40% – when brushing teeth, and 1 patient – when eating solid foods (hard vegetables, fruits).

It has been found that 77.7% of patients with NAFLD have been undergoing periodontal treatment over the past 5 years, but only 27.7% of all regularly visit the dentist for professional oral hygiene and follow the recommendations, which is probably due to low motivation regarding own dental health. Besides, it should be noted that only 33.3% regularly brush their teeth 2 times a day (every morning and evening), while most patients brush their teeth once a day or once every two days (5 respondents).

When asking more specific questions about the nature of food, it has been revealed that carbohydrate foods predominate in the diet of most patients with NAFLD, and half of the respondents drink sugary carbonated beverages, but only 19.4% of surveyed report their regular consumption.

We also received disturbing data regarding cigarette smoking. Thus, in the group of patients with NAFLD, more than 80.0% of patients smoke, and 58.33% of the respondents have a very high degree of nicotine addiction.

DISCUSSION

Our research confirms once again that all pathology of internal organs is directly displayed in the oral cavity [14,15]. The developed questionnaire allows evaluating the main complaints of patients, identifying deficiencies in hygienic oral care, and assessing the nature of a diet to achieve effective control of dental status.

It was noted that the most frequent complaints for the patients with NAFLD were xerostomia, halitosis and distortion of taste sensitivity, which is probably associated with a violation of the qualitative and quantitative parameters of the oral fluid. It is known that the oral fluid performs many functions, the main of which are protective (moisturizing the tissues of the oral cavity, utilizing food and epithelial debris, forming a barrier of antibodies and other active substances), trophic (maintaining constant hydration and physiological regeneration of the mucosa), etc. Since NAFLD is often accompanied by overweight, symptoms of carbohydrate metabolism disorders and arterial hypertension, xerostomia can be caused not only by the action of pathological processes in the body, but also by taking medications prescribed for this. [16]. As a result, during xerostomia, the oral mucosa is affected, burning sensations appear, taste changes and bad breath appears, and the risk of periodontal disease increases [17]. The pH of the oral fluid is decreased, that contributes to the accumulation of plaque.

The survey shows that the questions of the questionnaire are understandable for respondents and provide basic subjective information for compiling individual preventive programs [18]. The data collected in the survey show significant differences in the estimated parameters in the groups of patients, which confirm the need to create individual programs to improve effectiveness of the dental disease prevention. Therefore, development of a universal form of an outpatient questionnaire for patients with NAFLD seems extremely relevant. The developed questionnaire is an important tool for quality improvement, since it is intended for use in real work in an outpatient dental reception.

CONCLUSIONS

So, owing to scientific researches, consideration of the etiopathogenesis of periodontal tissue diseases separately from somatic pathology is impossible. The pathogenesis of periodontal disease in patients with NAFLD is complex and multifactorial. Development and implementation of the questionnaire allow evaluating data on the frequency of occurrence and characteristics of dental complaints, the nature of a diet and oral hygiene in patients with NAFLD.

Future prospectus: The developed methodology has proven its effectiveness and is recommended for implementation in medical institutions in order to reduce the risk factors for dental morbidity in the specified category of patients.

REFERENCES

1. Jepsen S., Caton J.G., Albandar J.M. et al. Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. 2018; 45(20):219–S229. doi.org/10.1111/jcpe.12951.

2. Atezhanov D., Supiev T., Bakiev B. Relationship of Somatic Pathology and Dental Diseases in Children, Prevention Measures and Treatment. Bulletin of Science and Practice. 2019;(8):56-65. doi.org/10.33619/2414-2948/45/06. (in Russian).
3. Singh S., Gupta K., Garg K.N. et al. Dental Management of the Cardiovascular Compromised Patient: A clinical approach. J Young Pharm. 2017; 9(4):453-456.
4. Pandyarajan V., Gish R.G., Alkhouri N., Noureddin M. Screening for Nonalcoholic Fatty Liver Disease in the Primary Care Clinic. Gastroenterol Hepatol (N Y). 2019;15(7):357–365.
5. Spengler E.K., Loomba R. Recommendations for diagnosis, referral for liver biopsy, and treatment of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Mayo Clinic Proceedings. 2015; 90(9):1233–1246.
6. Azzam H., Malnick S. Non-alcoholic fatty liver disease—the heart of the matter. World Journal of Hepatology. 2015; 7(10):1369–1376.
7. Ma X., Liu S., Zhang J. et al. Proportion of NAFLD patients with normal ALT value in overall NAFLD patients:a systematic review and meta-analysis. BMC Gastroenterol. 2020. http://doi.org/10.1186/s12876-020-1165-z .
8. Younossi Z., Anstee Q.M., Marietti M. et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018; 15:11–20.
9. Sheng X., Che H., Ji Q. et al. The Relationship Between Liver Enzymes and Insulin Resistance in Type 2 Diabetes Patients with Nonalcoholic Fatty Liver Disease. Horm Metab Res. 2018; 50:397–402.
10. Alazawi W., Bernabe E., Tai D. et al. Periodontitis is associated with significant fibrosis in patients with Nonalcoholic Fatty Liver Disease PLoS One. 2017; 12. doi: 10.1371/journal.pone.0185902, PMC5722374.
11. Akinkugbe A., Slade G., Barritt A.S. et al. Periodontitis and Non-alcoholic Fatty Liver Disease, a population based cohort investigation in the Study of Health in Pomerania. J Clin Periodontol. 2017; 44(11):1077–1087. doi: 10.1111/jcpe.12800 .
12. Nakahara T., Hyogo H., Ono A. et al. Involvement of Porphyromonas gingivalis in the progression of non-alcoholic fatty liver disease. J Gastroenterol. 2018; 53: 269–280.
13. Yoneda M., Naka S., Nakano K. et al. Involvement of a periodontal pathogen, Porphyromonas gingivalis on the pathogenesis of non-alcoholic fatty liver disease. BMC Gastroenterol. 2012; 12: 16. doi: 10.1186/1471- 230X-12-16.
14. Emelyanova N. Relapses of desquamative glossitis in patients with gastroesophageal reflux disease. Biomedical Research and Therapy. 2020; 7(10):4041-4044. doi.org/10.15419/bmrat.v7i10.638.
15. Golla K., Epstein J.B., Cabay R.J. Liver disease: current perspectives on medical and dental management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 98:516–521. doi.org/10.1016/s1079210404006432.
16. Fadieienko G., Nikiforova Y. Eating disorders and visceral adipose tissue – two interconnected informative markers of prognosis of disorders of nutritional status and risk of development of comorbidity chronic non-communicable diseases. Georgian Med News. 2017; 262:58-64.
17. Emelyanova N., Komir I. Effect of drug-induced pathomorphosis on oral cavity organs and tissues in chronic obstructive pulmonary disease combined with coronary heart disease: A clinical case. Biomedical Research and Therapy. 2021; 8(1):4197-4202. doi.org/10.15419/bmrat.v8i1.657.
18. Isayeva G., Rieznik L., Buriakovska O. et al. The impact of group and individual training on hemodynamics, lipid metabolism, physical activity and quality of life in patients with high and very high cardiovascular risk. Wiad Lek. 2019;72(12):2315-2323.

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ORIGINAL ARTICLE

APPLICATION OF ANTIBIOTICS AND PROBIOTICS FOR PREVENTION OF ANTIBIOTIC-ASSOCIATED DISBIOSIS IN PATIENTS WITH GENERALIZED PERITONITIS AND ENTERAL DYSFUNCTION SUPPORTS STAFF AWARENESS

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ABSTRACT

The aim: To clarify the efficacy of probiotics use as a preventive measure for post-antibiotic treatment in acute peritonitis and increase staff awareness related to antibiotic and probiotic use.

Materials and methods: The study design included determination of the proper antibiotic and probiotic strain combination and clinical application of probiotic strains. The control group consisted of 63 (48.46%) patients who underwent traditional multimodal treatment of peritonitis and the study group of 67 (51.54%) individuals, with inclusion of different antibiotic/probiotic combinations.

Results: Prior to antimicrobial therapy 67.7% patients of both groups' patients had severe dysbiosis, proving dysbiosis as a sign of peritonitis. *S. boulardii* showed widest resistance spectrum and was used for probiotic therapy in study group. Intestinal dysbiosis grades distribution in control group significantly worsened, while in study group ratio of severe dysbiosis significantly dropped from 58.2% to 38.8% with significant growth of grade II dysbiosis to 61.2%. No visible differences in disease course and clinical picture, duration or complications rate between study and control groups were observed.

Conclusions: Most of probiotic strains lack antibacterial resistance that makes meaningless their use during systemic antibiotic therapy of acute peritonitis. It is characterized by harsh changes of intestinal microbiota (severe intestinal dysbiosis). While probiotic strains showed antibiotic tolerance, their use presented no significant clinical efficacy, though high level of positive influence on intestinal dysbiosis was observed.

KEY WORDS: peritonitis, treatment, antibiotics, probiotics, physiology, microbiota

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INTRODUCTION

Acute peritonitis (AP) is a comparatively common, but deadly condition, generally described as an acute inflammation of the peritoneum resulting mainly from the bacterial infection as well as other causes such as chemicals, irradiation, and foreign-body injury [1, 2]. It is a major contributor to non-trauma deaths despite improvements in diagnosis and surgical and intensive care management. Influence on the peritoneal lining by any of these agents can lead to an inflammatory response, known as AP [3]. The structure of the etiological causes leading to AP consists of sources in the colon (32% of patients), appendix (31%), stomach/duodenum (18%), small bowel (13%), or biliary tract (6%). About 78% patients with AP present with generalized peritonitis and 26% with severe peritonitis. The overall mortality rate significantly decreased (15-25%) over last decades but remains high [4-7].

Multiorgan failure, or multiorgan dysfunction syndrome (MODS) is essential for peritonitis and abdominal sepsis (AS) and is observed in the vast majority of such patients [8]. The role of endotoxemia in the pathogenesis of multiorgan failure is confirmed by data on the homogeneity of systemic changes in different pathologies, which leads to AP and AS, as well as the lack of direct correlation between the type of pathogen and the nature of the disease [9-11]. Moreover, the understanding of the role of bacteremia in the pathogenesis of AS has changed radically. At the present stage, bacteremia is no longer considered as the only decisive component in the diagnosis of abdominal sepsis. It is the high concentration of endotoxins and exotoxins of bacterial cells in the blood that becomes the main factor in the activation of mediator systems, in particular cytokines [12, 13]. Among them, TNF, interleukins, system of complement, and interferons play an important role. Damage to the epithelium in the target organs under the influence of mediators leads to significant functional disorders and forms MODS, which is similar to many inflammatory and immune dependent conditions and considered to have strong genetic background [14-16].

Development of intestinal insufficiency as a part of MODS is often underevaluated, whilst playing an extremely important

role in the pathogenesis of peritonitis and abdominal sepsis. Under the influence of inflammatory mediators, hypercatabolism, impaired coagulation, systemic and visceral blood flow disorders, damage of enterocytes develops rapidly, almost all functions of the digestive tract are disrupted – barrier, metabolic, immunoreactive, endocrine, etc. Intestinal insufficiency is a key moment in the development of the "vicious" circle in AP and AS, because the translocation of microorganisms and their toxins supports the general inflammatory reaction, aggravating metabolic disorders [17-19].

Intestinal dysbiosis is known to be either a background or aggravating factor for multiple conditions including chronic and acute inflammatory processes, inflammatory bowel disease, non-alcoholic fatty liver disease, several types of tumors, etc. Possible mechanisms of microbiota involvement in their pathogenesis are complex and not clearly understood, though immune, metabolic and even genetic factors associated with intestinal microbiota are important in many diseases [20, 21].

Relationship between virulence factors of pathogens and host resistance explains diverse clinical picture of AP and formation of AS in many patients. Furthermore, existing multimodal treatment approach including massive antimicrobial therapy seems to partially add to both developments of intestinal insufficiency and MODS. However, linkage of antibacterial therapies and intestinal insufficiency, including intestinal microbiota changes and translocation is not clearly understood. Moreover, there is related issue of insufficient staff awareness related to antimicrobial therapy application.

THE AIM

The aim of the study was to clarify the efficacy of possible use of different probiotic compositions as a preventive measure for post-antibiotic treatment in acute peritonitis and increase staff awareness related to antibiotic and probiotic use.

MATERIALS AND METHODS

Design of the study included two consecutive components, determination of the proper antibiotic and probiotic strain combination and clinical application of probiotic strains aimed on finding the probiotic activity in patients undergoing treatment. According to the aim of the study, the examination was conducted of 130 patients with acute surgical diseases of the abdominal cavity the course of which was complicated by the development of different forms of peritonitis. The study was conducted in accordance with the principles of the Council of Europe Convention on Human Rights and Biomedicine, Declaration of Helsinki on the ethical principles for medical research involving human subjects, and other valid international and national legislations in bioethics (including GCP, EU directives, etc. The study protocol was approved by the institutional ethics committee. All patients signed an informed consent prior to participating in the study.

Among study individuals, there were 73 men (56.15%) and 57 women (43.85%), indicating frequency of AP in both men and women. The age-related division of AP occurrence: in age categories from 40 to 60 years (41.5%), from 61 to 80 years (28.46%), from 20 to 40 years (23.85%). The number of patients with concomitant somatic pathology (71 patients, 54.62%) significantly exceeded the number of patients without comorbidities (45.38%). Only surviving patients were included into the study.

All patients are divided into two groups. The control group consisted of 63 (48.46%) patients who underwent traditional comprehensive multimodal treatment of peritonitis [4, 17, 22] and patients of the study group consisting of 67 (51.54%) individuals, who underwent multimodal treatment with inclusion of different antibiotic/probiotic combinations. Distribution of patients with peritonitis is presented in Table I.

The peculiarities of the probiotics' and antibiotics' action on the microflora were studied according to the standard microbiological methods: a culture of a probiotic strain was previously grown from a registered biological product and a suspension of 10^9 CFU/ml was made from it using an optical standardization approach. In this study we tested following strains of probiotic microorganisms: *Esherichia coli strain M-17* (Bifikol, Biofarma, Ukraine); *Bacillus subtilis 3, Bacillus licheniformis 31* (Biosporin, Biofarma, Ukraine); Bacillus clausii (Enterogermina, Sanofi, France); *Lactobacillus fermentum 90 TC-4* (Lactobacterin, Biofarma, Ukraine); *Lactobacillus acidophilus, Lactobacillus bifidus, Lactobacillus bulgaricus, Streptococcus thermophilus* (Canadian yoghurt, Astrapharm, Ukraine), *Lactobacillus rhamnosus R0011, Lactobacillus rhamnosus R0049, Streptococcus thermophilus, Lactobacillus debrueckii spp. bulgaricus* (Yoghurt Rosell, Astrapharm, Ukraine) *Saccharomyces boulardii* (Enterol 250, Biocodex, France), *Enterococcus faecium* (Bifiform, Ferrosan AS, Denmark), *Lactobacillus GG* (Bifiform Baby, Ferrosan AS, Denmark), and *Aerococcus viridans* (A-bacterin, Biolik, Ukraine). Sensitivity of probiotic strains to chemotherapeutic agents was studied by disc-diffusion (growth retention area size in mm) method on dense medium using commercially available standard disc sets according to their manuals. Dysbiosis severity was determined in colonic content by standard microbiologic methods, and calculated as a 1/3 deviation steps (grades I-III) from normal microbiota values, where 1/3 deviation was grade I, 2/3 deviation was grade II and more than that was grade III. Microsoft Excel 365 and Statsoft Statistica 7.0 (USA) software packages were used for data collection and statistical analysis.

RESULTS

Probiotic strains of L. fermentum 90TS-4 and S. boulardii have been shown to be antagonistic to *Enterobacteria, Pseudomonas, Staphylococci, Proteus* and *Bacilli*, while Enterol 250 is resistant to most antibiotics. Obtained study results are presented in Tables II-IV, while summary of compatible antibiotic and probiotic combinations is shown in Table V.

E. coli M-17, A. viridans, B. subtilis 3, B. licheniformis 31, B. Clausii, and *L. fermentum 90 TC-4* showed insufficient resistance to antibiotics applying choice limits on their use and alleviating possibilities for successful systemic antimicrobial therapy of peritonitis. Probiotic strains

included in Bifiform (*E. faecium*) and lactose-containing drugs (*Lactobacterin, Bifiform Baby, Canadian yoghurt* and *Roselle yoghurt*) are sufficiently resistant to cephalosporins, so they can be used in combination with these drugs. As shown at Table V, *S. boulardii* seems to be a better choice for use in patients undergoing systemic antibiotic treatment.

Prior to antimicrobial therapy, 75 (67.7%) patients of both groups' patients had severe dysbiosis (grade III). Thirty-six (48.0%) of them were in control group, the rest 39 (52.0%) belonged to the study group. Grade II had 46 (35.4%) and grade I – 9 (6.9%) patients. Distribution of grades I-II between both groups was similar (grade II – 25 and 21 patients in study and control groups, respectively), grade I – 3 in study group and 6 in control. No significant correlation of dysbiosis and peritonitis severity before treatment was observed in both groups.

As *S. boulardii* showed widest possible resistance for most of tested antibiotics, it was used for probiotic therapy in study group patients in the form of Enterol-250, 500 mg, twice a day for seven days as a part of early enteral feeding approach.

After the treatment, intestinal dysbiosis grades distribution in control group significantly worsened. No patients had grade I dysbiosis, and 13 (20.6%) had grade II, twice as low compared to the period before the antimicrobial therapy. The majority of control group patients (50 or 79.4%) had grade III dysbiosis after antibiotics use. Study group patients after combined use of antibiotics and selected probiotic demonstrated absence of grade I dysbiosis, too. However, ratio of severe dysbiosis (grade III) significantly dropped from 58.2% to 38.8% (26 patients) with significant growth of grade II dysbiosis to 61.2% (41 patients). No visible differences in disease course and clinical picture, as well as duration of treatment or complications rate between study and control groups were observed in this study.

DISCUSSION

Acute peritonitis and its generalized forms often lead to systemic changes defined as abdominal sepsis (AS) and multiple organ dysfunction syndrome (MODS). While antibiotic use is an essential part of peritonitis managements it may under these circumstances have negative influence, too. This is exactly one of the reasons why the vast majority of hospitals employ different programs for increasing staff awareness towards antibiotic use as an addition to more common antibiotic resistance awareness. However, very few of these programs including globally spread initiatives or organizations like APUA (Alliance for the Prudent Use of Antibiotics) pay attention for the alleviation of the antibiotic use side effects, focusing mainly on preventing unnecessary use only [23]. Comparatively few papers published under the auspices of APUA deal with inter-bacterial relationships as a part of understanding antibiotic resistance [24].

Multiple genetic studies of both intestinal microbiome and proteome, show existence of complex cross-related links between human genomic single nucleotide polymorphisms (SNP) and occurrence of different conditions serving as a background for AS and MODS. Moreover, there are ties connecting intestinal microbiome and human genome. Interestingly, connections of several SNPs, metabolic and endothelial changes are well associated with changes in gut microbiota and vice versa [25]. Such complex interrelations make situation even more complex. While influence of antibiotics on intestinal microflora seems to be well established, the use of probiotics to alleviate negative influence of antibacterial therapy remains generally confusing. There are both supporting and challenging reports on this issue, especially concerning acute conditions like AP and AS [26].

In this study we attempted to add clarification on the problem while finding out the appropriate combination of antibiotic and probiotic to avoid preliminary elimination of probiotic strain by the drug used. In addition, both clinical and microbiologic efficacy of such approach were evaluated. Our findings support the idea, that insufficient efficacy of the probiotic use in AP may be associated with inappropriate selection of probiotic strain and its elimination or neutralization by the applied antibiotic itself. But even when appropriate antibiotic and probiotic composition is used, whilst there is a good impact on microbiota's balance as shown by better dysbiosis grades proportions compared to control, no reliable support in terms of clinical picture or complication rates was observed. It rises multiple questions concerning the possible mechanisms of microflora involvements into the acute inflammatory process, metabolic and immune changes and AS/MODS formation during acute peritonitis. Further study of these mechanisms may produce sufficient data to understand why clinical efficacy of probiotic and antibiotic use is confusing, while being effective in correction of dysbiosis itself. Furthermore, this study supports awareness of medical personnel towards the proper use of antibiotics.

CONCLUSIONS

Most of probiotic strains lack antibacterial resistance that makes meaningless their use during systemic antibiotic therapy of acute peritonitis. Acute peritonitis is characterized by harsh changes of intestinal microbiota as proved by severe intestinal dysbiosis registered in this study. While selected probiotic strains of S. boulardii showed good antibiotic tolerance when combined with common antimicrobial drugs, their use presented no significant clinical efficacy, though high level of positive influence on intestinal dysbiosis was observed.

REFERENCES

1. Tochie J.N., Agbor N.V., Frank Leonel T.T. et al. Global epidemiology of acute generalised peritonitis: a protocol for a systematic review and meta-analysis. BMJ Open. 2020;10(1):e034326.
2. Riché F.C., Dray X., Laisné M.J. et al. Factors associated with septic shock and mortality in generalized peritonitis: comparison between community-acquired and postoperative peritonitis. Crit Care. 2009;13(3):R99.
3. Sartelli M., Abu-Zidan F.M., Labricciosa F.M. et al. Physiological parameters for Prognosis in Abdominal Sepsis (PIPAS) Study: a WSES observational study. World J Emerg Surg. 2019;14(34)..
4. Gauzit R., Péan Y., Barth X. et al. Mistretta F, Lalaude O; Top Study Team. Epidemiology, management, and prognosis of secondary non-postoperative peritonitis: a French prospective observational multicenter study. Surg Infect (Larchmt). 2009;10(2):119-27.

5. Ivashchuk S.I., Sydorchuk L.P. The parameters of liver functional state as a risk factor of edematous pancreatitis development providing of genetic determination of IL-4 production. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2019;72(4):639-644.
6. De Simone B., Ansaloni L., Sartelli M. et al. The Operative management in Bariatric Acute abdomen (OBA) Survey: long-term complications of bariatric surgery and the emergency surgeon's point of view. World J Emerg Surg. 2020;15(2).
7. Chromik A.M., Meiser A., Hölling J. et al. Identification of patients at risk for development of tertiary peritonitis on a surgical intensive care unit. J Gastrointest Surg. 2009;13(7):1358-67.
8. Abaziou T., Vardon-Bounes F., Conil J.M. et al. Outcome of community- versus hospital-acquired intra-abdominal infections in intensive care unit: a retrospective study. BMC Anesthesiol. 2020;20(1):295.
9. Sydorchuk L., Dzhuryak V., Sydorchuk A. et al. The cytochrome 11B2 aldosterone synthase gene rs1799998 single nucleotide polymorphism determines elevated aldosterone, higher blood pressure, and reduced glomerular filtration, especially in diabetic female patients. Endocrine Regulations. 2020;54(3):217-226.
10. Sydorchuk L.P., Dzhuryak V.S., Sydorchuk A.R. et al. Association of lipids' metabolism disorders with aldosterone synthase CYP11B2 (-344C/T) gene polymorphism in hypertensive patients depending on glomerular filtration rate. PharmacologyOnLine. 2020;2:230-242.
11. Hameed T., Kumar A., Sahni S. Et al. Emerging Spectrum of Perforation Peritonitis in Developing World. Front Surg. 2020;7:50.
12. Sydorchuk L.P., Syrota B.S., Sydorchuk A.R. et al. Clinical markers of immune disorders in the pathogenesis of Escherichia coli enteritis. Arch Balk Med Union. 2019;54(1):89-96.
13. Yoon Y.K., Kim J., Moon C. Et al. Antimicrobial Susceptibility of Microorganisms Isolated from Patients with Intraabdominal Infection in Korea: a Multicenter Study. J Korean Med Sci. 2019;34(47):e309.
14. Tochie J.N., Agbor N.V., Frank Leonel T.T. et al. Global epidemiology of acute generalised peritonitis: a protocol for a systematic review and meta-analysis. BMJ Open. 2020;10:e034326.
15. Sheremet M.I., Sydorchuk L.P., Shidlovskyi V.O. et al. Association of the blood serum cytokines' rate and lymphocytes' apoptosis with polymorphic variants of the BCL-2 (Rs17759659), CTLA-4 (rs231775) and APO-1/FAS (rs2234767) genes in patients with nodular goiters in autoimmune thyroiditis and thyroid adenoma. Romanian Journal of Morphology and Embryology. 2017;58(3):931-939.
16. Sydorchuk L.P., Serdulets Y.I., Sydorchuk A.R. et al. The polymorphism of matrilin-3 (rs77245812) and interleukin-10 (rs1800872) genes in osteoarthritis patients with arterial hypertension, obesity and type 2 diabetes mellitus. Arch Balk Med Union. 2017;52:422-429.
17. Membrilla-Fernández E., Sancho-Insenser J.J., Girvent-Montllor M. et al. Secondary Peritonitis Spanish Study Group. Effect of initial empiric antibiotic therapy combined with control of the infection focus on the prognosis of patients with secondary peritonitis. Surg Infect (Larchmt). 2014;15(6):806-14.
18. Sydorchuk L.I., Hrushko O.I., Sydorchuk I.Y. et al. Early changes of luminal colonic microbiome in experimental abdominal sepsis. Infection. 2019;47(Suppl. 1):5.
19. Maseda E., Ramírez S., Picatto P. et al. HELP Investigators and the Perioperative Infection Research Group. Critically ill patients with community-onset intraabdominal infections: Influence of healthcare exposure on resistance rates and mortality. PLoS One. 2019;14(9):e0223092.
20. Lobo L.A., Benjamim C.F., Oliveira A.C. The interplay between microbiota and inflammation: lessons from peritonitis and sepsis. Clin Transl Immunology. 2016;5(7):e90.
21. Fay K.T., Klingensmith N.J., Chen C.W., et al. The gut microbiome alters immunophenotype and survival from sepsis. FASEB J. 2019;33(10):11258-11269.
22. Doklestić S.K., Bajec D.D., Djukić R.V. et al. Secondary peritonitis – evaluation of 204 cases and literature review. J Med Life. 2014 Jun 15;7(2):132-8.
23. O'Brien T., Stelling J. Integrated multilevel surveillance of the world's infecting microbes and their resistance to antimicrobial agents. Clin. Microbiol. Rev. 2011;24(2):281-295.
24. Garbeva P., Silby M.W., Raaijmakers J.M. et al. Transcriptional and antagonistic responses of Pseudomonas fluorescens Pf0-1 to phylogenetically different bacterial competitors. ISME J. 2011;5:973-985.
25. Sydorchuk L.P., Sokolenko A.A., Sydorchuk A.R. et al. Insulin resistance in patients with arterial hypertension and abdominal obesity depending on ACE and PPAR-γ2 genes polymorphism: A new opinion concerning an old problem. New Armenian Medical Journal 2015; 9:43-51.
26. Barnett R.E., Conklin D.J., Ryan L. et al. Anti-inflammatory effects of miR-21 in the macrophage response to peritonitis. J Leukoc Biol. 2016;99(2):361-371.

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ORIGINAL ARTICLE

ASSESSMENT OF THE DEMYELINATING PROCESS ACTIVITY IN PATIENTS WITH HERPESVIRAL MENINGITIS AND MENINGOENCEPHALITIS BASED ON THE LEVEL OF MYELIN BASIC PROTEIN (MBP) IN THE CEREBROSPINAL FLUID

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ABSTRACT

The aim: To study the peculiarities of demyelination by detection of changes in the levels of myelin basic protein (MBP) in CSF of patients with acute herpesviral meningitis (M) and meningoencephalitis (ME).

Materials and methods: A total of 136 CSF samples from 68 patients with herpesviral M and ME were collected. The control group consisted of patients with acute respiratory infection and meningismus. MBP level in CSF was identified at the admission and after 10-12 days of treatment. Analysis of MBP concentrations in CSF was performed using an enzyme immunoassay.

Results: Examination of patients on the first day of hospitalization showed the presence of a significant increase of MBP in the CSF in all patients with viral M/ME compared with the indicators of the comparison group ($p<0.01$). In all groups of patients with ME, the level of MBP in CSF was significantly higher than the indicators of comparison group and M groups of the suitable etiology of the disease ($p<0.01$). In patients with lethal outcome, the MBP level was significantly higher ($p<0.01$) than in all meningitis groups, but we did not find a significant difference with the patients with ME ($p>0.05$).

Conclusions: The increase of MBP level identified in patients with acute M/ME confirms the presence of the demyelinating process that occurs in all patients, but it is more pronounced in patients with ME.

KEY WORDS: herpesvirus, meningitis, meningoencephalitis, CSF, MBP, demyelination

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INTRODUCTION

CNS infections make up a unique problem for physicians due to the rapid course of the disease, the high mortality rate and the complications they cause, as well as the inherent difficulties associated with diagnostics and treatment. These infections include meningitis (M) and meningoencephalitis (ME). They are usually characterized by high morbidity and mortality [1]. Acute M and ME rare occur, compared to other infectious diseases – an average of 4-30 cases/100,000 population and 3-7 cases/100,000 population accordingly in developed countries and Europe each year, but mortality and economic losses are significant [2, 3]. At present, in the developed countries and Europe, acute M/ME in immunocompetent adults are more often caused by enteroviruses, herpesviruses, arboviruses (70-90% of all cases of CNS infectious lesions) [4, 5]. The highest mortality and severe course of disease are observed in herpesviral lesions of the nervous system [6].

The pathogenesis of brain damage during herpesvirus neuroinfection has not been sufficiently studied. The isolation of molecules, which localized solely in the cells of the nervous system – neuronal markers, was a significant impetus in the study of the pathogenesis of various neurological diseases. Determination of the content of these markers in the CSF or blood of the patient allows us to evaluate both the degree of damage of certain brain cells and the severity of the demyelinating process [7, 8]. Markers of CNS pathology are becoming increasingly important and are being investigated in acute and chronic diseases [7, 9].

Myelin basic protein (MBP) is considered the best marker for determining the activity of the demyelinating process [10]. MBP forms 30% of the myelin protein content and is the second most common protein of myelin sheath in the CNS. MBP is regarded as the "most important" myelin molecule due to its essential role in the formation of CNS myelin [11]. It was demonstrated that myelin degeneration

Table I. Demographic and clinical characterization of the cohort

Etiology of neuroinfection	HSV-1,2 (n=20)	EBV (n=19)	VZV (n=15)	HHV-6 (n=14)
Age in years (Mean±SD)	35.47±14.71	36.43±16.09	38.27±18.24	31.69±13.03
Sex, Men (n/%)	4/20.00	7/36.84	9/60.00	8/57.14
Sex, Women (n/%)	16/80.00	12/63.16	6/40.00	6/42.86
Meningitis (n/%)	15/75.00	10/52.63	11/73.33	9/64.29
Meningoencephalitis (n/%)	5/25.00	9/47.37	4/26.67	5/35.71
Non survivors (n/%)	1/5.00	2/10.53	1/6.67	1/7.14

occurs after CNS lesions such as chemical intoxication, brain injury and demyelinating diseases [12].

Determination of the neuronal markers level promotes to early diagnostics because significant changes in their concentration often occur earlier than those damages that can be detected by instrumental examination. In addition, the ability of assess of the severity of CNS lesions and prognosis of the disease course, monitoring of the treatment of patients with various nervous system diseases, such as Alzheimer's disease, Huntington's disease, brain injury, hypoxic conditions in newborns, strokes was proved [13-16].

Infection of the CNS with influenza virus induces long-term upregulation of MBP expression while decreasing the thickness of myelin sheaths surrounding axons in the cerebellum and hippocampus of influenza-recovered mice. Influenza infection may induce MBP expression by increasing proinflammatory cytokines without affecting oligodendrocyte viability [17]. The demyelinating process in patients with herpes virus neuroinfections remains uncertain.

THE AIM

To study the peculiarities of demyelination by detection of changes in the levels of myelin basic protein in CSF of patients with acute herpesviral meningitis and meningoencephalitis.

MATERIALS AND METHODS

Potential study participants were admitted in Kharkiv Regional Clinical Infectious Diseases Hospital (Kharkiv, Ukraine). The inclusion of patients in the research program conducted with the selection criteria. Inclusion criteria: clinical symptoms typical for acute M or ME, etiological confirmation of herpesviral etiology of disease by CSF PCR, age of patients – 18 to 65 years, voluntary consent of the patient to participate in the study. Patients were excluded in the following cases: the presence of comorbidities, which can influence the level of neurospecific proteins – HIV, Alzheimer's disease, multiple sclerosis, hematological diseases, malignant neoplasms.

68 patients with herpesvirus M or ME were involved to the research according to the selection criteria. Among them: 20 patients with herpes simplex virus 1,2 (HSV-1,2) M/ME, 19 with Epstein-Barr viral M/ME, 15 with Varicella-Zoster M, 14 with Human herpesvirus 6 (HHV-6) M/ME. The control group consisted of 15 patients with acute respiratory infection and meningismus.

At hospital admission, demographic data, clinical indicators were obtained from patients. Anamnesis of the disease, complains and neurological status were recorded briefly.

CSF was aspirated by lumbar puncture. Performing of lumbar puncture was in a line with the usual protocols of diagnostics and treatment of patients with signs of meningitis. Patients involved in the study were not subjected to additional invasive procedures. Collecting CSF in patients with ARI and meningismus performed only at the beginning of treatment to exclude neuroinfection. The CSF samples were immediately frozen at – 70°C until subsequent analysis. CSF MBP level were identified on admission and after 10-12 days of treatment. Analysis of MBP CSF concentrations was performed using an enzyme immunoassay based on the sandwich technique («AnshLabs», ELISA, USA) in Central scientific research laboratory of Kharkiv National Medical University. Work conducted in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of Kharkiv National Medical University, Kharkiv, Ukraine. All data were analyzed using «BiostatPro», AnalystSoft Inc program. We used the Mann Whitney U test for continuous variables. P value of <0.05 was used for significance.

RESULTS

The highest age was observed in patients with VZV meningitis – 38.27±18.24 years, the youngest – in patients with HHV-6 infection 31.69±13.03 (P<0.05) (table 1).

The quantity of women and men was the same almost in all groups, however, among patients with HSV-1,2 neuroinfection, women significantly predominated – 16 (80%) of 20 cases. The most severe was EBV and HHV-6 neuroinfection, a severe course of the disease was observed in 47.37% and 35.71% of patients (table 1).

Severity of the condition of all patients with ME was caused by development of focal neurological symptoms and brain edema symptoms. In patients with EBV and HHV- 6 infection, symptoms of focal lesions of the brain in the form of paresis, paralysis and cognitive impairment were more common. Meningitis was observed in 15 (75%) patients with

Table II. MBP level in CSF of patients with acute herpesvirus meningitis and meningoencephalitis on the first day of hospitalization

Etiology of neuroinfection	MBP, ng/ml		p
	Meningitis	Meningoencephalitis	
HSV-1,2	4.41±0.51 1	9.40±0.53 [1]	p=0.0019
VZV	4.35±0.71 1		
EBV	4.11±0.34 1	11.05±1.32 [1]	p=0.0001
HHV-6	4.57±0.28 1	13.30±0.99 [1]	p=0.0001
Control group	2.13±0.15		

Notes: p – the level of significance of the difference between the indicators of patients with meningitis and meningoencephalitis of the same etiology, obtained using the Mann– Whitney test; [1] – significant difference with comparison group metrics (p<0.05).

Table III. MBP level in CSF in patients with acute bacterial and viral M /ME on the 10-12 day of hospitalization

Etiology of the disease	MBP, ng/ml		p
	M	ME	
HSV 1,2	6.37±0.34 [1,2]	8.29±0.27 [1]	p=0.0023
VZV	4.52±0.21 [1]		
EBV	4.95±0.35 [1]	8.56±0.70 [1,2]	p=0.0067
HHV-6	5.22±0.38 [1]	9.11±0.77 [1,2]	p=0.0036
Comparison group	2.13±0.15		

Notes: p – the level of significance of the difference between the indicators of patients with M and ME of the same etiology, obtained using the Mann– Whitney test; [1] – significant difference with comparison group metrics (p<0,05); [2] – significant difference with the level of the first day of hospitalization (p<0,05).

HSV-1,2 infection, in 10 (52.63%) with EBV, in 11 (73.33%) with VZV, in 9 (64.29%) with HHV-6 etiology of the disease. The highest mortality was observed in patients with EBV (10.53%) and HHV-6 (7.14%) neuroinfection (table I).

On the first day of hospitalization the MBP content in patients with meningitis was equal to: at HSV 1,2 meningitis – 4.41±0.51 ng/ml, at VZV meningitis – 4.35±0.71 ng/ml, with EBV meningitis – 4.11±0.34 ng/ml, with HHV-6 meningitis – 4.57±0.28 ng/ml, in the comparison group – 2.13±0.15 ng/ml (table 2). In patients with ME, the MBP content on the first day of hospitalization was: at HSV 1,2 ME – 9.40±0.53 ng/ml, EBV ME – 11.05±1.32 ng/ml, HHV-6 ME – 13.30±0.99 ng/ml (Table II).

Thus, the MBP level in the CSF of all patients with herpes virus neuroinfection was significantly higher than the indicators of the comparison group (p<0.01). In all patients with ME, the MBP level in the CSF was higher than the indicators of comparison group and M groups of the corresponding etiology (p<0.01) (table 2).

According to the received data, the MBP content on the 10-12 day of hospitalization in the CSF of patients with M was: at HSV 1,2 M – 6.37±0.34 ng/ml, at VZV M – 4.52±0.21 ng/ml, at EBV M – 4.95±0.35 ng/ml, at HHV-6 M – 5.22±0.38 ng/ml (table 3).

In patients with ME, the MBP content in the CSF on the 10-12 day of hospitalization was: for meningococcal infection – 10,48±0,92 ng/ml, pneumococcal – 11.63±0.74 ng/ml, HSV 1,2 ME – 8.29±0.27 ng/ml, EBV ME – 8.56±1.40 ng/ml, HHV-6 ME – 9.11±0.77 ng/ml.

Thus, the direct dependence of the MBP level from the severity of CNS lesions remained on the 10-12 day of hospitalization. The highest levels of MBP were determined in patients with ME, but in all groups of patients with meningitis, the level of MBP remained significantly higher than the indicators of the comparison group (table III).

The level of MBP in the period of early convalescence was significantly reduced only in patients with HSV 1,2 M, and EBV, and HHV-6 ME compared with the indicators which were obtained on the first day of hospitalization.

DISCUSSION

In the mammalian CNS, oligodendrocytes constituting glial cells with microglia and astrocytes are myelin–forming cells [18]. It has been found that oligodendrocyte damage, which ultimately undergoes to apoptosis, to be responsible for myelin destruction and subsequent demyelination [19]. The detected changes of MBP levels indicate the presence of oligodendrocyte damage in patients with acute herpes virus M /ME. Such lesions are most pronounced in patients with ME, but they occur even in patients who do not have neurological symptoms.

The present results showed that demyelination commonly occurs in the brain of patients with herpesvirus CNS infection. Demyelination is a common feature in the brain that is infected by encephalitis viruses as seen in patients with HIV infection [20]. It is well known that free MBP causes a number of reactions: it changes the platelet shape, destroys cell membranes and acidic lipid vesicles, stimulates proliferation of astrocytes and Schwann cells and depolarizes the neural membrane [12]. The specific toxic effect of MBP on neurons at a concentration of 30 μg / ml

and above has been proven [12]. MBP toxicity is due to the fact that this protein is an intrinsically nonstructural protein with a very positive charge [11], and upon release from the myelin cell membrane begins to interact with various molecules, including negatively charged lipids, sialic acids, polyanionic proteins, and neuron plasmas [12], which in turn causes inflammation and death of nerve cells [23]. Virus-mediated autoimmunity seen in multiple sclerosis and Theiler's virus infection was reported to cause T cell-mediated autoimmune disease related to demyelination [22]. The development of a myelin-specific autoimmune response may be a relatively important cause of demyelination in patients with Japanese encephalitis and other viral infections [23].

The limitations of our study do not answer the question of whether the development of demyelination in patients with herpesvirus neuroinfection is the result of direct cytopathic action of the virus or is it the result of immune responses. However, the dependence of the development of neurological symptoms from the degree of increase in the concentration of MBP in CSF in patients revealed us indicates about significant diagnostic and prognostic potential of this marker.

In addition, the MBP content in the CSF in patients with M/ME in the early convalescence period is higher than the content in comparison group in all patients with herpes virus M/ME (table 3). Such dynamics of the MBP level testify about presence of long-term disorders of myelin cells in the pathogenesis of herpesvirus M/ME, which are determined longer than the clinical manifestations of the disease.

CONCLUSIONS

The increase of MBP level determined in patients with acute M/ME confirms the presence of the demyelinating process that occurs in all patients, but it is more pronounced in patients with ME. MBP release is one of the factors affecting of CNS tissues during acute herpesviral M/ME. Demyelination may continue longer than the clinical manifestation of the disease.

REFERENCES

1. Parikh V., Tucci V., Galwankar S. Infections of the nervous system [published correction appears in Int J Crit Illn Inj Sci. 2013 Jan–Mar;3(1):97]. Int J Crit Illn Inj Sci. 2012;2(2):82–97. doi:10.4103/2229–5151.97273.
2. George B.P, Schneider E.B., Venkatesan A. Encephalitis hospitalization rates and inpatient mortality in the United States, 2000–2010. PLoS One. 2014;9(9):e104169. doi:10.1371/journal.pone.0104169.
3. Oordt–Speets A.M., Bolijn R., van Hoorn R.C., et al. Global etiology of bacterial meningitis: A systematic review and meta–analysis. PLoS One. 2018;13(6):e0198772. doi:10.1371/journal.pone.0198772.
4. McIntyre P.B., O'Brien K.L., Greenwood B., Van de Beek D. Effect of vaccines on bacterial meningitis worldwide. Lancet. 2012;380(9854):1703–1711. doi: 10.1016/S0140–6736(12)61187–8.
5. Swanson P.A., McGavern D.B. Viral diseases of the central nervous system. Curr Opin Virol. 2015;11:44–54. doi:10.1016/j.coviro.2014.12.009.
6. Bloch K.C., Glaser C.A. Encephalitis Surveillance through the Emerging Infections Program, 1997–2010. Emerg Infect Dis. 2015;21(9):1562–1567. doi:10.3201/eid2109.150295.
7. Papa L., Robertson C.S., Wang K.K. et al. Biomarkers improve clinical outcome predictors of mortality following non–penetrating severe traumatic brain injury. Neurocrit Care. 2015;22(1):52–64. doi: 10.1007/s12028–014–0028–2.
8. Mayer C.A., Brunkhorst R., Niessner M. et al. Blood levels of glial fibrillary acidic protein (GFAP) in patients with neurological diseases. PloS one. 2013;8(4):e62101. doi:10.1371/journal.pone.0062101.
9. Rees C.L., White C.M., Ascoli G.A. Neurochemical Markers in the Mammalian Brain: Structure, Roles in Synaptic Communication, and Pharmacological Relevance. Current Medicinal Chemistry. 2017;24(28), 3077–3103.
10. Brunkhorst R., Pfeilschifter W., Foerch C. et al. Astroglial Proteins as Diagnostic Markers of Acute Intracerebral Hemorrhage–Pathophysiological Background and Clinical Findings. Translational Stroke Research. 2010;1(4):246–51. doi: 10.1007/s12975–010–0040–6.
11. Harauz G., Ladizhansky V., Boggs J.M. Structural polymorphism and multifunctionality of myelin basic protein. Biochemistry. 2009;48:8094–8104.
12. Zhang J., Sun X., Zheng S. et al. Myelin Basic Protein Induces Neuron–Specific Toxicity by Directly Damaging the Neuronal Plasma Membrane. PLoS ONE. 2014;9(9):e108646. http://doi.org/10.1371/journal.pone.0108646.
13. Rohlwink U.K., Figaji A.A. Biomarkers of Brain Injury in Cerebral Infections. Clin Chem. 2014;60(6):823–34. doi: 10.1373/clinchem.2013.212472.
14. Yokobori S., Hosein K., Burks S. et al. Biomarkers for the clinical differential diagnosis in traumatic brain injury––a systematic review. CNS Neurosci Ther. 2013;19(8):556–565. doi:10.1111/cns.12127.
15. Vinther–Jensen T., Börnsen L., Budtz–Jørgensen E. et al. Selected CSF biomarkers indicate no evidence of early neuroinflammation in Huntington disease. Neurol Neuroimmunol Neuroinflamm. 2016;3(6):e287. doi:10.1212/NXI.0000000000000287.
16. Takano R., Misu T., Takahashi T. et al. Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. Neurology. 2010;75(3):208–16. doi: 10.1212/WNL.0b013e3181e2414b.
17. Jin H. K., Ji E.Yu., Byung–Joon Ch., Sang–Soep N. Neonatal influenza virus infection affects myelination in influenza–recovered mouse brain. J Vet Sci. 2018; 19(6): 750–758. doi: 10.4142/jvs.2018.19.6.750.
18. Baumann N., Pham–Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev. 2001;81:871–927.
19. Zivadinov R., Zorzon M., Weinstock–Guttman B. et al. Epstein–Barr virus is associated with grey matter atrophy in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2009;80:620–625. doi: 10.1136/jnnp.2008.154906.
20. Stohlman S.A., Hinton D.R. Viral induced demyelination. Brain Pathol. 2001;11:92–106. doi: 10.1111/j.1750–3639.2001.tb00384.x.
21. Sun X, Wang X., Chen T. et al. Myelin activates FAK/Akt/NF–kappaB pathways and provokes CR3–dependent inflammatory response in murine system. PLoS One. 2010;5:e9380.
22. Grigoriadis N., Hadjigeorgiou G.M. Virus–mediated autoimmunity in multiple sclerosis. J Autoimmun Dis. 2006;3:1. doi: 10.1186/1740–2557–3–1.
23. Fazakerley J.K., Walker R. Virus demyelination. J Neurovirol. 2003;9:148–164.

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ORIGINAL ARTICLE

STUDY OF RELATIONSHIP OF PSYCHOSOCIAL FACTORS WITH SMOKING IN NORTHERN POPULATION

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ABSTRACT

The aim: Of our study was to identify the relationship between the main risk factors for heart disease and social factors among northern population.

Materials and methods: We polled 3092 native and non-native habitants of the Sakha Republic (Yakutia). The poll consists of several sections. It includes questions related to social, demographic and life record data, heredity, physical activity and unhealthy habits.

Results: Among the people diagnosed with hypertension (HT), there are more smokers than among the people without this medical condition. On the contrary, in the group of people diagnosed with HT at the examination for taking blood pressure, there were fewer smokers than in the group, in which HT was not registered. Patients with CHD, Myocardial infarction (MI) in past medical history, cerebrovascular accident (CVA) and type 2 diabetes (T2D), are smokers to the same degree as the other group. This indicates that this category of the examined are exposed to the risk of cardiovascular aggravations. We determined a high spread of psychosocial risk factors for the examined respondents – the relationship between smoking, overweight, obesity, abdominal obesity and HT, and the level of education, marital status and labor specificity. The increase in the level of education is associated with fewer amounts of smokers among both non-native and native habitants. Overweight is more frequently observed for the people who are not single, have low level of education and are engaged in manual labor. In particular, it is applicable to native habitants.

Conclusions: Abdominal obesity did not have any relationship with psychosocial factors for native habitants. However, in relation to non-native habitants, overweight, obesity, abdominal obesity and HT are associated with marital status (married) and with manual labor.

KEY WORDS: smoking, risk factors, psychosocial risk

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INTRODUCTION

Smoking is commonly recognized as a reason for development of various diseases. It is a cause of deaths for 50% of smokers (half of whom die from cardiovascular diseases), which could have been avoided. Such aspects of lifestyle as smoking and psychosocial factors lead to cardiovascular diseases [1]. Many countries consider that this problem results in premature mortality and increased costs of healthcare system, which damages social and economic spheres.

Regardless of decrease observed in smoking tendency in Europe, it is still a common phenomenon among people with low level of education. Level of education determines the difference in the amount of people who give up smoking in many European countries [2].

Smoking-related risk primarily depends on the number of cigarettes smoked per day, taking into consideration an obvious dose-addiction effect. At the same time, there is no lower limit with the absence of negative effect [3].

The accumulated data indicates the increase of coronary heart disease (CHD) risk for passive smokers to a greater extent than it was expected [4, 5]. Cardio vascular disease (CVD) risk increases by about 30% for a non-smoker person living with a smoker partner [6, 7], or for an employee who inhales cigarette smoke at the workplace [8, 9].

THE AIM

The aim of our study was to identify the relationship between the main risk factors for heart disease and social factors among northern population.

MATERIALS AND METHODS

We polled 3092 native and non-native habitants of the Sakha Republic (Yakutia). The poll consists of several sections. It includes questions related to social, demographic and life record data, heredity, physical activity and unhealthy habits. A regular smoker shall be considered a person who has been smoking at least one cigarette per day for the last 12 months.

Social status was estimated using standard questionnaire. We estimated such parameters as education level and marital status.

In accordance with level of education we divided the examined into four groups:
Group 1: people with primary (or lower) level of education.
Group 2: people with vocational education.
Group 3: people with secondary education.
Group 4: people with a degree.
In accordance with marital status we divided the examined into 4 groups:

Table I. Smoking rate for native population of the Sakha Republic (Yakutia).

Age groups	All	Men	Women	P
All	37.4	52.6	31.9	<0.001
Age 18–59 (1)	42.3	57.8	36.6	<0.001
Age 60+ (2)	20.9	35.4	16.2	<0.001
P_{1-2}	<0.001	<0.001	<0.001	

Table II. Smoking rate among native population of the Sakha Republic (Yakutia) districts.

Age groups	All	Men	Women	P
Tattinsky District				
All	37.7	55.6	31.3	<0.001
Age 18–59 (1)	43.9	64.0	36.7	<0.001
Age 60+ (2)	23.8	36.4	19.4	0.108
P_{1-2}	<0.01	<0.05	<0.01	
Verkhnevilyuisky District				
All	31.4	41.1	29.0	<0.05
Age 18–59 (1)	36.5	44.1	34.8	0.150
Age 60+ (2)	16.5	33.3	12.0	<0.01
P_{1-2}	<0.001	0.335	<0.001	
Verkhoyansky District				
All	39.7	54.1	34.0	<0.001
Age 18–59 (1)	44.3	60.9	37.5	<0.001
Age 60+ (2)	20.9	24.0	19.4	0.654
P_{1-2}	<0.001	<0.001	<0.01	
Eveno-Bytantaysky District				
All	43.5	58.7	35.1	<0.001
Age 18–59 (1)	46.9	61.3	38.8	<0.01
Age 60+ (2)	27.7	47.1	17.2	<0.05
P_{1-2}	<0.05	0.280	<0.05	

Table III. Smoking rate among native population of Yakutia having different chronic NCDs.

	Pathology	No pathology	P
HT (according to BP)	29.4	39.1	<0.001
HT (diagnosed by a specialist)	38.3	29.1	<0.01
Type 2 diabetes	33.3	37.3	0.460
Stroke in past medical history	46.4	36.9	0.149
CHD	33.1	38.2	0.136
MI in past medical history	35.8	37.4	0.823

Group 1: married or having common-law relationship.
Group 2: single.
Group 3: widow (widower).
Group 4: divorced or living apart.
Statistical data was processed by SPSS v19.0 software package. Statistic estimation includes descriptive analysis of indicators numerical characteristics (averaged values, standard deviations) and their division. We used such methods as selections comparison (Mann–Whitney U test, Student's t-test) and contingency table analysis (Fisher's exact test, Pearson's chi-squared test). Normality of distribution was confirmed by the Kolmogorov–Smirnov test. The results were considered significant if $p < 0.05$.

RESULTS

According to our data, the occurrence of smoking among adult population of the Sakha Republic (Yakutia) amounts to 37.4%. There are more male smokers (52.6%) than female (31.9%). We noticed that there are two times less smokers among older people. This tendency is traced in both gender groups (Table I).

Table IV. Smoking rate among non-native population of the Sakha Republic (Yakutia).

Age groups	All	Men	Women	P
All	41.8	62.8	31.4	<0.001
Age 18–59 (1)	43.5	63.3	33.5	<0.001
Age 60+ (2)	22.5	55.6	11.3	<0.001
P_{1-2}	0.001	0.513	0.001	

Table V. Smoking rate related to psychosocial factors.

Factors	All	men	women	P
Education level				
Secondary level; secondary vocational level; lower than secondary level (1)	41.3	54.0	35.9	<0.001
Higher (2)	25.8	43.5	21.8	<0.001
P_{1-2}	<0.001	0.111	<0.001	
Marital status				
Married	37.6	52.0	31.1	<0.001
Single	37.0	54.4	33.2	<0.001
P_{1-2}	0.831	0.684	0.474	
Labor specificity				
Sedentary work	27.3	43.8	23.9	<0.001
Standing work	35.0	63	29.4	<0.001
Manual work	47.8	58.3	40.6	<0.001
Nervous work	39.5	50.0	35.8	0.098
Domestic work	32.6	40.4	30.4	0.175
P	<0.001	0.083	<0.001	
Depression				
Presence of depression (1)	37.1	46.7	34.0	0.378
Absence of depression (2)	37.0	51.4	31.7	<0.001
P_{1-2}	0.987	0.718	0.735	

Analysis of the smoking rate for native habitants from different districts of the republic has proven that the spread of smoking does not vary to a large extent; it is almost the same for all the districts and does not exceed 40%, except for Evenko-Bytantaysky District, where the smoking rate is a bit higher (43.5%). The higher smoking rate among men is consistent in all the districts. However, we can observe more explicit age-related gradation of the women's smoking rate (Table II).

We can observe age-related decrease in the men's smoking rate in Tattinsky and Verkhoyansky Districts. In Verkhnevilyuisky and Eveno-Bytantaysky Districts the number of young and mature men smokers is approximately equal. It is important to note that almost 60% of working-age men from the examined districts are smokers, except for men from Verkhnevilyuisky District (44.1%). This negative situation can be observed for women as well. More than 30% of women aged 18-59 smoke (Table II).

In our study, we analyzed the smoking rate among patients with CVD and T2D (Table III). Among the people diagnosed with hypertension (HT), there are more smokers than among the people without this medical condition. On the contrary, in the group of people diagnosed with HT at the examination for taking blood pressure, there were fewer smokers than in the group, in which HT was not registered. Patients with CHD, Myocardial infarction (MI) in past medical history, cerebrovascular accident (CVA) and type 2 diabetes (T2D), are smokers to the same degree as the other group. This indicates that this category of the examined are exposed to the risk of cardiovascular aggravations.

The analysis of smoking rate among non-native population has shown that this risk factor is frequent for general group of the examined non-native habitants. We observed age-related decrease of this parameter (Table IV). At the same time, we noticed that the smoking rate is 2 times higher among non-native men compared to non-native women: 62.8% and 31.4% respectively. The smoking rate is high for men regardless of the age. As for non-native women, the smoking rate is 3 times higher for working-age representatives of this group, compared to the senior ones.

We did not find any differences in the smoking rate for native and non-native habitants. However, we discovered that the smoking rate for non-native men is higher than for native men. Such difference is more explicit for senior men.

Table VI. Smoking rate related to psychosocial factors in Tattinsky District.

Age groups	All	men	women	P
Secondary level; secondary vocational level; lower than secondary level (1)	42.0	58.6	34.1	<0.001
Higher (2)	30.3	50.0	25.9	0.101
P_{1-2}	0.095	0.583	0.279	
Marital status				
Married	36.1	56.9	27.9	<0.001
Single	45.7	57.9	41.2	0.212
P_{1-2}	0.162	0.938	0.085	
Labor specificity				
Sedentary work	26.5	44.4	22.5	0.178
Standing work	36.7	80.0	28.0	0.028
Manual work	52.4	69.2	40.5	0.025
Nervous work	55.2	85.7	45.5	0.062
Domestic work	23.4	27.3	22.2	0.729
P	<0.001	<0.05	0.164	
Depression				
Presence of depression (1)	38.3	40.0	33.3	0.850
Absence of depression (2)	37.5	58.0	31.8	<0.001
P_{1-2}	0.963	0.439	0.955	

Table VII. Smoking rate related to psychosocial factors in Verkhnevilyuisky District.

Age groups	All	men	women	P
Secondary level; secondary vocational level; lower than secondary level (1)	33.8	41.1	31.8	0.037
Higher (2)	23.9	30.8	22.8	0.532
P_{1-2}	0.070	0.483	0.127	
Marital status				
Married	31.3	40.4	28.4	0.189
Single	33.3	33.3	33.3	0.352
P_{1-2}	0.678	0.599	0.357	
Labor specificity				
Sedentary work	24.4	25.0	24.3	0.948
Standing work	22.4	50.0	17.1	0.041
Manual work	40.0	40.0	40.0	1.000
Nervous work	32.3	33.3	31.9	0.919
Domestic work	28.4	38.5	25.9	0.368
P	0.068	0.738	<0.05	
Depression				
Presence of depression (1)	37.5	60.0	42.1	0.475
Absence of depression (2)	38.3	35.7	29.8	0.337
P_{1-2}	0.131	0.278	0.259	

In this study we analyzed the relationship between psychosocial factors and smoking, which is a key risk factor for CVDs. The smoking rate is higher for people with secondary and higher level of education among the examined native habitants. However, the rate in both groups is high for men, while it is lower for women with higher education (Table V). The relationship between smoking and labor specificity is also observed for women. Smoking rate is

Table VIII. Smoking rate related to psychosocial factors in Verkhoyansky District.

Age groups	All	men	women	P
Secondary level; secondary vocational level; lower than secondary level (1)	42.9	52.0	38.3	<0.05
Higher (2)	23.1	75.0	13.6	<0.01
P_{1-2}	0.50	0.367	<0.05	
Marital status				
Married	39.7	52.6	35.2	0.181
Single	38.8	53.1	34.6	0.059
P_{1-2}	0.901	0.973	0.939	
Labor specificity				
Sedentary work	20.3	53.8	13.1	<0.001
Standing work	37.5	57.1	33.3	0.237
Manual work	49.5	60.4	38.3	<0.05
Nervous work	36.0	33.3	36.8	0.876
Domestic work	41.0	38.1	42.1	0.749
P	<0.001	0.419	<0.01	
Depression				
Presence of depression (1)	23.1	0	25.0	0.569
Absence of depression (2)	39.9	52.5	33.8	<0.01
P_{1-2}	0.223	0.296	0.529	

Table IX. Smoking rate related to psychosocial factors in Eveno-Bytantaysky District.

Age groups	All	men	women	P
Eveno-Bytantaysky District				
Secondary level; secondary vocational level; lower than secondary level (1)	50.4	62.7	43.2	<0.05
Higher (2)	29.7	42.9	26.0	0.222
P_{1-2}	<0.01	0.181	<0.05	
Marital status				
Married	48.7	58.2	43.2	0.108
Single	31.4	66.7	23.8	<0.05
P_{1-2}	<0.05	0.631	<0.05	
Labor specificity				
Sedentary work	38.0	58.3	33.9	0.112
Standing work	43.5	60.0	38.9	0.400
Manual work	56.1	54.2	58.8	0.767
Nervous work	46.9	75.0	30.0	<0.05
Domestic work	12.5	50.0	0	0.064
P	0.147	0.816	0.102	
Depression				
Presence of depression (1)	38.5	50.0	33.3	0.569
Absence of depression (2)	42.8	59.6	34.0	<0.01
P_{1-2}	0.763	0.706	0.968	

higher for the representatives of professions related to manual labor. Regardless of marital status and depression, we observe high smoking rate both for men and women.

Following the detailed analysis, we can observe that the relationship between psychosocial factors and CVD risk factors varies between districts. In Tattinsky District we do not trace the relation

of the difference factors with education level, marital status and depression. However, we can see explicit relationship between labor specificity and smoking, especially among men (Table VI).

For Verkhnevilyuisky District, the same as for Tattinsky District, we observe a clear relationship between labor specificity and smoking, especially among women (Table VII). We did not discover any differences as regards to the rest of the factors.

In Verkhoyansky District, the smoking rate is higher for women with secondary or secondary vocational level of education. However, this relationship is smoothed out in the total reference set as there is a large number of smoking men with higher education. We can see explicit relationship between labor specificity and smoking, especially among women (Table VIII). We did not discover any differences as regards to the rest of the factors.

For Eveno-Bytantaysky District, we can observe a small number of married women and women with higher education. We did not discover any relationship of smoking with the rest of psychosocial factors (Table IX).

DISCUSSION

Smoking is a recognized cause of many diseases and the cause of 50% of avoidable deaths of smokers, half of them are due to cardiovascular diseases [4].

In our study, it was found that patients with coronary artery disease, myocardial infarction (MI), acute cerebrovascular accident (ACVA), and type 2 diabetes, compared with those without these pathologies, smoke equally, which indicates a high risk of cardiovascular complications in this category of patients. The results of clinical studies and laboratory experiments conducted by Capewell S. et al. also show that a decrease in mortality from coronary heart disease can occur quickly after individual or population measures to change the diet or quit smoking [3].

Higher education levels are associated with lower smoking prevalence among indigenous and newcomers. Despite a decline in smoking prevalence in Europe, it remains widespread among people with low educational attainment; differences in the frequency of smoking cessation depending on the level of education in recent years have been observed in many European countries [6]. In previous studies, the risk associated with smoking in women is significantly higher than in men [8], which is confirmed by our study. This may be due to differences in the metabolism of nicotine, and is also confirmed by the data that women are more passive smokers, which does not reduce their risk of developing diseases of the cardiovascular system [7].

Studies carried out by many scientists [7, 8], as well as our data have shown that low socioeconomic status, lack of social support, stress at work and in the family, depression, anxiety, hostility, in association with smoking, increase the risk of developing cardiovascular diseases, contribute to the deterioration of the clinical course and prognosis of cardiovascular diseases.

CONCLUSIONS

We determined a high spread of psychosocial risk factors for the examined respondents – the relationship between smoking, overweight, obesity, abdominal obesity and HT, and the level of education, marital status and labor specificity. Taking into consideration high CHD occurrence and wide spread of smoking, we can assume that decrease in the spread of passive smoking will significantly improve the situation.

REFERENCES

1. Wipfli H. L., Samet J. M. Second-hand smoke's worldwide disease toll. Lancet. 2011; 377: 101–102.
2. NcNeely E., Mordukhovich I., Staffa S. et al. Legacy health effects among never smokers exposed to occupational secondhand smoke. PLos One. 2019;14(4): e0215445. DOI: 10.1371/journal.pone.0215445. 3. Capewell S., O'Flaherty M. Rapid mortality falls after risk-factor changes in populations/Lancet. 2011;378:752–753.
4. McAlinden K.D., Sohal S.S., Sharma P. There can be smoke without fire: warranted caution in promoting electronic cigarettes and heat not burn devices as a safer alternative to cigarette smoking. ERJ Open Research. 2019;5:00114-2019. doi:10.1183/23120541.00114-2019.
5. Oakes J.M., Fuchs R.M., Gardner J.D. et al. Nicotine and the reninangiotensin system. Am J Physiol Regul Integr Comp Physiol. 2018;315(5):R895-R906.5. doi:10.1152/ajpregu.00099.2018.
6. Hackshaw A., Morris J.K., Boniface S. et al. Low cigarette consumption and risk of coronary heart disease and stroke: meta-analysis of 141 cohort studies in 55 study reports. BMJ. 2018;360:5855. 7. Lawrence H., Hunter A., Murray R. et al. Cigarette smoking and the occurrence of influenza–Systematic review. J Infect. 2019;79(5):401-6. doi:10.1016/j.jinf.2019.08.014.
8. Girard D., Delgado-Eckert E., Schaffner E. et al. Longterm smoking cessation and heart rate dynamics in an aging healthy cohort: Is it possible to fully recover? Environ Res. 2015; 143 (A): 39–48.
9. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388(10053):1659-1724.

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ORIGINAL ARTICLE

RATIO OF MAIN PHYLOTYPES OF GUT MICROBIOTA IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE DEPENDING ON THE BODY MASS INDEX

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ABSTRACT

The aim: To analyze the status of Gut microbiota (GM) at the level of the main phylotypes in patients with NAFLD, depending on the body mass index (BMI) and gender in comparison with a group of practically healthy individuals.

Materials and methods: The study involved 120 patients with NAFLD, who were divided into two groups depending on BMI and the control group containing 20 practically healthy individuals.

Results: In patients with NAFLD with comorbid obesity, a statistically significant increase in the relative amount of Firmicutes (52.12 [42.38; 67.39]%) and Firmicutes/Bacteroidetes ratio (3.75 [1.7; 9.5]) against the background of a significant decrease in the amount of Bacteroidetes (13.41 [7.45; 26.07]%); in NAFLD patients with overweight, the relative amount of Firmicutes was 49.39 [37.47; 62.73]%, Firmicutes / Bacteroidetes ratio was 1.98 [1.15; 5.92], and the relative amount of Bacteroidetes was 23.69 [12.11; 36.16]%. In the control group, the distribution of the basic GM phylotypes was significantly different; the relative amount of Bacteroidetes was almost the same as of Firmicutes — 34.65 [24.58; 43.53]% and 29.97 [22.52; 41.75]% respectively, and the Firmicutes/Bacteroidetes ratio was 0.64 [0.52; 1.47].

Conclusions: The most statistically significant changes in the composition of IM occur due to the increase in the relative amount of Firmicutes and the ratio of Firmicutes/Bacteroidetes against the background of a decrease in the relative amount of Bacteroidetes. These changes were directly proportional to the increase in BMI, but had no gender features.

KEY WORDS: non-alcoholic fatty liver disease, gut microbiota, body mass index

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a wide range of liver lesions, which ranges from simple steatosis to steatohepatitis that can progress to liver fibrosis and cirrhosis and transform into hepatocellular carcinoma [1,2].

During the last decades, the incidence of NAFLD has increased worldwide, making it the most common pathology: 25.2% of the world population is suffering from NAFLD, which is a significant burden for health care [3,1].

In addition, NAFLD is considered to be a polysystemic disease that occurs on the background of concomitant lesions of other organs and systems, including obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome, chronic kidney diseases and cardiovascular disease [4,5].

It is now convincingly proven that gut microbiota (GM) is an important factor of regulation of gastrointestinal homeostasis; its qualitative and quantitative changes can contribute to immune system and metabolism impairments [6].

Increasing evidence suggests that GM is involved in the development of obesity and metabolic syndrome, considering the potential role in the pathogenesis of NAFLD.

However, the data of the conducted studies are ambiguous, which is explained by the influence of numerous genetic and environmental factors.

For instance, when comparing the composition of GM in patients with obesity and in individuals with normal weight or underweight, Ley et al. (2006) [7,8] found relatively low amount of Bacteroidetes and impaired GM diversity in patients with obesity. However, the findings are contrary to many other studies. Duncan et al., (2008) [9], as well as Jumpertz et al., (2011) [10] excluded differences between normal and overweight individuals regarding the relative amount of Bacteroidetes, Firmicutes, or Actinobacteria. Schwiertz et al (2010) concluded in their study that the relative amount of Bacteroidetes increases only in patients with obesity [11].

Despite considerable progress in the study of the status and features of the impact of GM on formation and course of NAFLD, some issues remain insufficiently considered.

THE AIM

To analyze the status of GM at the level of the main phylotypes in patients with NAFLD, depending on the body mass index and gender in comparison with a group of practically healthy individuals.

MATERIALS AND METHODS

The study involved 120 patients with NAFLD. The first group included 89 patients with diagnosed comorbid obesity, whose body mass index (BMI) was 34.50 [31.50; 39.40] kg/m2 (47 men and 42 women aged from 42.00 to 61.00 years with a median of 49.00 years). The second group included 31 overweight patients with the BMI of 28.00 [27.20; 29.80] kg/m2 (17 men and 12 women aged from 36.00 to 56.00 years with a median of 44.00 years).

The control group was formed from 20 practically healthy volunteers, including 8 men and 12 women aged from 37.50 to 55.50 years (median — 50.50 years) with the BMI of 23.50 [21.30; 25.85] kg/m2). The groups were comparable in age and gender ratio.

In all the examined patients, NAFLD was established by standard procedures. An additional mandatory criterion for inclusion in the study was the presence of excess body weight or obesity. This concomitant pathology was diagnosed using a standard technique – measurement of BMI.

All the patients were interviewed to determine the etiologic factors for the development of secondary liver steatosis and other conditions that affect the intestinal microbiota composition. The energy value and quality of the daily diet and eating habits were estimated using a diet diary that participants completed within 3 –7 days.

All the patients underwent general clinical examination (complaint analysis, medical history, life history, objective status) and anthropometry with BMI calculation, assessment of the functional state of liver, carbohydrate and fat metabolism, ultrasound examination of liver.

Blood serum insulin concentration was determined by enzyme-linked immunosorbent assay using DRG reagent kits (Germany) and the NOMA-IR index by the standard formula.

The state of lipid metabolism was analyzed by the level of total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein cholesterol. Ultrasound examination of liver, assessment of existence and severity of liver fibrosis was performed using elastometry on Siemens ACUSON S2000 according to conventional methods.

Diagnosis of the bacterial overgrowth syndrome was performed by measuring the changes of concentration of hydrogen in the exhaled air after consumption of 50 g of glucose (Gastro + Gastrolyzer gas analyzer, UK).

For determination of the basic GM phylotypes, the stool samples collected in sterile containers were aliquoted, rapidly frozen and stored until extraction at -20 °C. DNA was extracted from 400 mg of stool using the Ribo-prep nucleic acid extraction kit (AmpliSens, RF) according to the manufacturer's instructions.

DNA concentration in the extracts was measured using a Qubit 3 fluorometer with the Qubit dsDNA HS Assay Kits reagents (Thermo Scientific, USA) and equilibrated to 1010 ng/μL. Determination of the composition of GM at the level of basic phylotypes was performed by identification of total bacterial DNA and DNA of Bacteroidetes, Firmicutes, and Actinobacteria by real-time quantitative polymerase chain reaction (PCR) using universal primers for 16S rRNA gene and taxon-specific primers [12].

PCR was performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA).

Amplification program:

- the initial stage of denaturation for 5 min at 95 °C — 40 cycles: 15 s at 95 °C, 15 s at 61.5 °C, and 30 s at 72 °C with the reading of fluorescence signal;
- the final stage of elongation — 5 min at 72 °C.

Statistical processing was performed using Statistica 13.1 package. According to Kolmogorov-Smirnov test, the distribution of all the studied indicators did not meet the normal (Gaussian) one, so the data processing was performed using nonparametric statistics methods. Data are given as Me [LQ; UQ], where Me is the median, and LQ and UQ are the lower and upper quartiles, respectively.

The Bioethics Committee of the State Institution "L.T. Mala National Institute of Therapy of the National Academy of Medical Sciences of Ukraine" approved this study. All the patients were provided with written information regarding the purpose and nature of the study before being included in the study, and they were informed of the possibility of withdrawal from the study at any time without further explanation of their decision. All the examined patients signed a form of informed consent for participation in this study.

RESULTS

According to the data obtained (tab.1) in both groups of patients with NAFLD, statistically significant changes in anthropometric parameters were noted in comparison with the control group: for instance, an increase of BMI by 46.8% ($p < 0.05$), an increase of waist circumference by 54.2% ($p < 0.05$), and an increase of hips circumference by 15.1% ($p < 0.05$) was recorded in patients with comorbid obesity. This trend was also observed in the overweight group, though it was less pronounced: BMI was increased by 19.1% ($p < 0.05$), waist circumference was increased by 33.3% ($p < 0.05$), and hips circumference was increased by 7.9% ($p < 0.05$). It should be noted that these indicators were also statistically significant between the first and the second group. The obtained data confirm the abdominal type of obesity typical for patients with NAFLD.

NAFLD is a hepatic manifestation of metabolic syndrome. Its prevalence increases with increasing rates of obesity and insulin resistance, and this statement was also confirmed with our study.

During the study, patients in both groups showed signs of formation of insulin resistance syndrome (for the first group, glycated hemoglobin content was 22.34% ($p < 0.05$)

Table 1. General characteristics of the examined patients

Parameter	Main group (n=120)		Control group (n=20)
	NAFLD with comorbid obesity (n=89)	NAFLD with overweight (n=31)	
BMI, kg/m^2	34.50 [31.50; 39.40]	28.00 [27.20; 29.80]	23.50 [21.30; 25.85]
	p<0.05	p<0.05	p<0.05
Waist circumference, cm	118.00 [108.00; 128.00]	102.00 [94.00; 111.00]	76.50 [72.00; 80.50]
	p<0.05	p<0.05	p<0.05
Hips circumference, cm	110.00 [105.00; 122.00]	103.00 [97.00; 111.00]	95.50 [88.5; 101.00]
	p<0.05	p<0.05	p<0.05
Glycated hemoglobin, %	6.57 [5.99; 7.36]	6.04 [5.43; 6.47]	5.37 [5.01; 5.45]
	p<0.05	p<0.05	p<0.05
HOMA, units	5.29 [4.06; 6.84]	3.80 [3.08; 4.761]	2.42 [1.73; 2.97]
	p<0.05	p<0.05	p<0.05
LDL, mmol/L	3.24 [2.52; 3.87]	2.57 [2.05; 3.21]	1.26 [1.06; 1.46]
	p<0.05	p<0.05	p<0.05
TG, mmol/L	1.73 [1.17; 2.37]	1.40 [0.99; 2.30]	0.87 [0.7; 1.12]
	$p<0.05^{c1c2}$	$p<0.05^{c1c2}$	$p<0.05^{c1c2}$
TC, mmol/L	5.43 [4.98; 6.20]	5.03 [4.57; 5.38]	4.59 [4.05; 5.15]
	$p<0.05^{12c1}$		$p<0.05^{12c1}$
VLDL, mmol/L	0.81 [0.58; 1.13]	0.78 [0.45;1.67]	0.65 [0.39; 0.77]
	$p<0.05^{c1}$		$p<0.05^{c1}$
ALT, U/L	29.00 [23.00; 42.00]	22.00 [18.00; 32.00]	17.5 [16.00; 20.00]
	p<0.05	p<0.05	p<0.05
GGT, U/L	22.30 [13.90; 41.60]	20.00 [13.10; 29.00]	17.70 [16.20; 19.85]
	$p<0.05^{c1}$		

Note. p<0,05 — statistically significant changes between all the studied groups;
$p<0,05^{c1}$ — statistically significant changes in the first group compared with the control group;
$p<0,05^{c1c2}$ — statistically significant changes in the first and the second group compared with the control group;
$p<0,05^{12c1}$ — statistically significant changes in the first group compared with the control group and statistically significant changes between the first and the second group.

higher, and the NOMA index was 2.19 times higher (p <0.05) than in the control group; for the second group, it was 12.48% (p <0.05) higher and 1.6 times (p <0.05) higher, respectively).

Lipid metabolism was also analyzed.

Patients in both groups showed a statistically significant increase in low-density lipoprotein (LDL) content compared with the control group — by 2.57 times (p <0.05) in the first group and by 2.03 times (p <0.05) in the second group, and by 1.26 times when comparing the first and the second group (p <0.05). Triglycerides (TG) concentrations in the NAFLD groups also significantly exceeded those of the control group: 1.99 times (p <0.05) for the first group and 1.6 times (p <0.05) for the second group; however, it was a trend when the first and the second group were compared.

Total cholesterol (TC) concentrations were statistically different in patients with comorbid obesity by 18.30% (p <0.05) and by 7.95% (p <0.05) compared with the overweight group. Changes in TC values in the second group

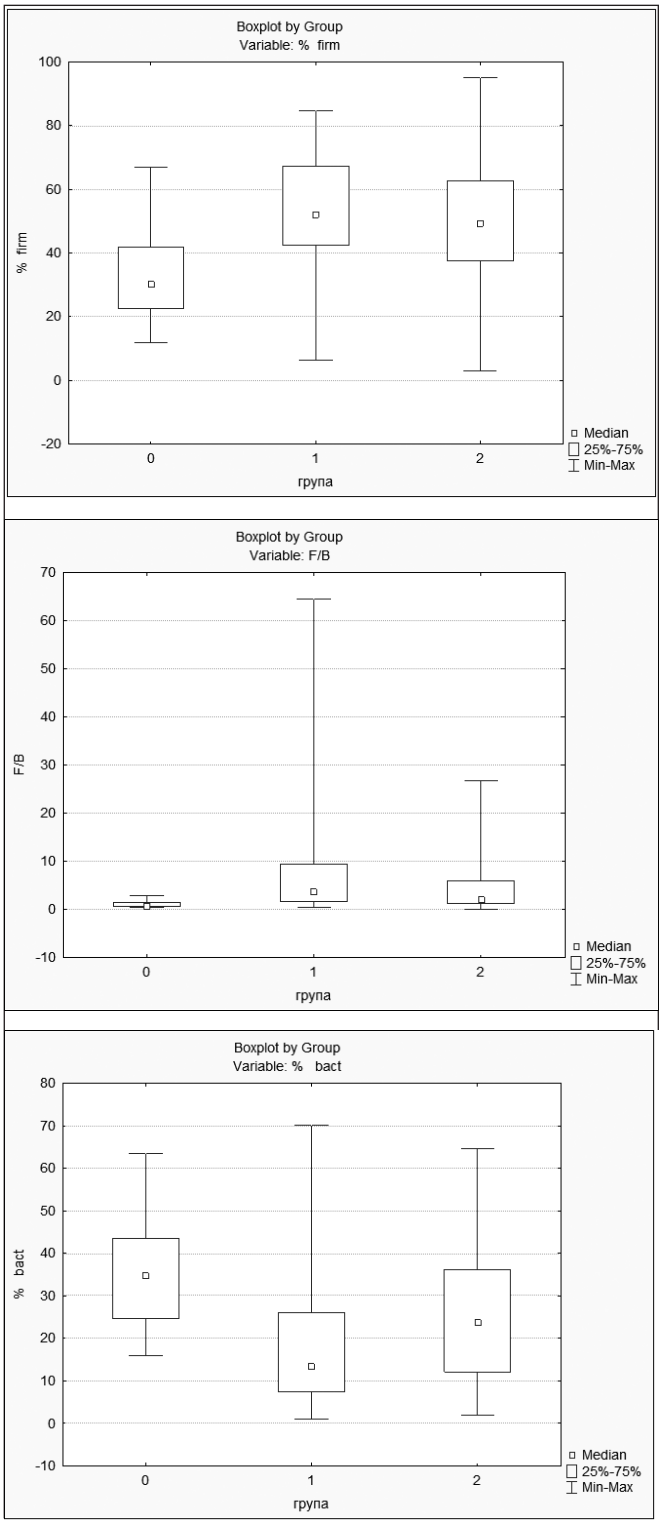

Fig 1. Relative composition main phylotypes of GM in examined patients

when compared to the control group were typical. Very low-density lipoprotein (VLDL) was statistically different only for patients in the first group compared with the control group.

Changes were also noted in parameters showing the functional state of liver: a statistically significant increase by 65.71% ($p < 0.05$) in the level of alanine aminotransferase (ALT) and gamaglutamyltranspeptidase (GGT) — by 25.99% ($p < 0.05$) in patients with comorbid obesity; this pattern was observed in overweight patients, but less pronounced for ALT concentrations: by 25.71%, and for GGT it was typical.

A comparative analysis of GM of patients with NAFLD depending on BMI and patients from the control group with normal body weight was performed (fig.1).

The study of relative quantitative composition of GM in the studied groups revealed a number of group dependencies (Kruskall-Wallis test) — namely, in patients with NAFLD with comorbid obesity, a statistically significant increase in the relative amount of Firmicutes (52.12 [42.38; 67.39]%) and Firmicutes/Bacteroidetes ratio (3.75 [1.7; 9.5]) against the background of a significant decrease in the amount of Bacteroidetes (13.41 [7.45; 26.07]%), while in NAFLD patients with overweight this tendency was observed only for the relative amount of Firmicutes (49.39 [37.47; 62.73]%), and Firmicutes/Bacteroidetes ratio was slightly lower (1.98 [1.15; 5.92]) due to increase of the amount of Bacteroidetes (23.69 [12.11; 36.16]%). However, changes in the relative composition of the intestinal microbiota in both the first and the second group were significantly different from the control group by all parameters: Firmicutes — 29.97 [22.52; 41.75]%, Bacteroidetes — 34.65 [24.58; 43.53]%, and the ratio Firmicutes/Bacteroidetes amounted to 0.64 [0.52; 1.47]. It should be noted that the relative amount of Actinobacteria, was similar in all the groups: 5 [2.87; 9.14]%, 4.28 [1.53; 7.56]% and 5.47 [1.64; 12.79]% according to (pict.2).; the recorded general trend corresponds to our previous results in a smaller study group (n=82) [13].

DISCUSSION

Importance of GM in energy homeostasis and nonspecific inflammation, which probably contributes to the development of obesity and metabolic diseases, has been currently proven [14,15].

Recent studies suggest a possible correlation between human GM and obesity. It has been suggested that the *Firmicutes/Bacteroidetes* ratio may play a role in the development of obesity [16].

Our study findings indicate that GM composition in patients with NAFLD on the background of obesity or overweight (considering the age and gender) may depend on BMI.

The study indicates that the ratio of *Firmicutes/Bacteroidetes*, which is of great importance in the development of obesity, may vary with BMI. On the one hand, this confirms the data obtained in previous studies, and on the other hand, it refutes them: some authors claim that intestinal dysbiosis develops with NAFLD regardless of the presence of concomitant obesity and/or insulin resistance [17].

These findings were based on the results of a prospective cross-sectional study involving patients with histologically confirmed NAFLD (n = 39), including 15 patients with steatosis, 24 participants with NASH, and 28 healthy individuals. The authors have shown that against the background of NAFLD, the amount of *Lactobacillus* and *Lac-*

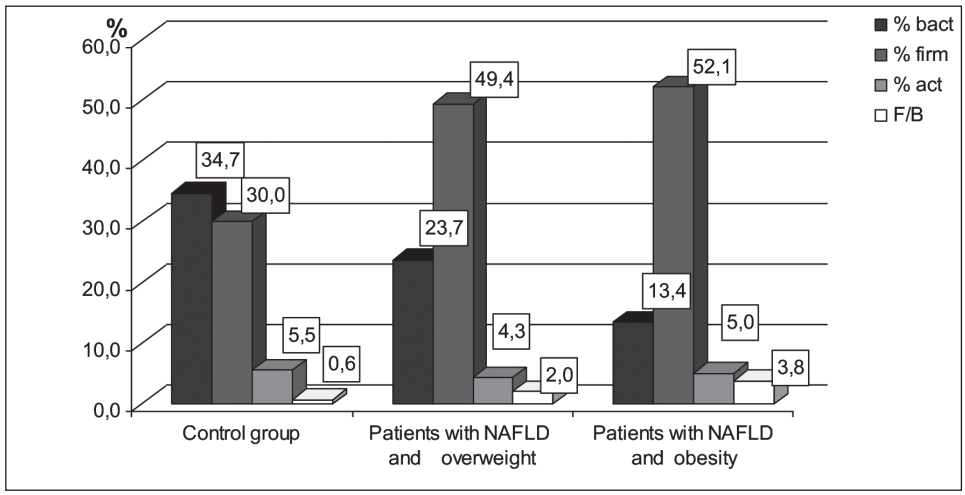

Fig 2. Relative composition main phylotypes of GM in control group (0), patients with comorbid course NAFLD and obesity (1), and patients with NAFLD and overweight (2): firm. – Firmicutes, %; bact. – Bacteroidetes, %;, Firmicutes/ Bacteroidetes- F/B

tobacillaceae increased compared to controls. Regardless of the stage of NAFLD, body weight, presence / absence of insulin resistance, scientists noted a decrease in the population of *Ruminococcus, Faecalibacterium prausnitzii,* and *Coprococcus* [17].

Researchers from California also refute the correlation between the *Firmicutes/Bacteroidetes ratio [18]* and agree with the [19] study findings, claiming that the differences in the *Firmicutes/Bacteroidetes ratio are much greater among the studies than between individuals with normal weight and overweight in any study*.

Moreover, the researchers had initially analyzed the quantitative association of BMI and *Bacteroidetes/Firmicutes* ratio, determining the relative abundance of the five major bacterial types in each sample, and had made a composite profile at the type level for each individual. No communication was found as a result of the performed work. They then had considered the existence of an association of BMI with a more accurate taxonomic composition, without identifying significant associations, confirming the study performed by Smith MI et al. [20].

The scientists had also analyzed the hypothesis that BMI depends not on the amount, but on the diversity of taxons; however, they refuted this hypothesis, unlike their colleagues Turnbaugh et al. [8] and Le Chatelier et al. [21], who confirmed it.

No gender or age peculiarities were revealed in the ratio of the main GM phylotypes in the patients examined by us.

However, some studies reported differences in the composition of GM in terms of gender. For instance, in the study performed by Haro et al. [22], in patients with a BMI of 23.44 to 41.88 kg/m^2, it was found that women had a lower relative amount of *Bacteroidetes* compared to men. It should be noted that this study also observed an increase in the *Firmicutes/Bacteroidetes* ratio, which varied with increasing BMI. However, in a similar study conducted earlier, no differences in GM depending on BMI were found; this may have been due to the fact that only patients with normal and excess weight, and no patients with obesity, were included in the study. In addition, a lower amount of *Bacteroidetes* was observed in women [23].

Our results have shown that the composition of GM at the level of the main phylotypes differs in individuals with and without obesity, which suggests that these changes are related to body mass and are typical for the studied Ukrainian population.

However, it is difficult to draw any conclusions about the importance of different groups of bacteria in obesity, since not all influential parameters, such as diet, genetic background, race, and living conditions, have been taken into account.

CONCLUSIONS

The data obtained by PCR method in qRT-PCR mode using universal primers for the 16S rRNA gene and taxon-specific primers from 140 Ukrainians show the most statistically significant changes in the composition of GM by means of increase of the relative amount of *Firmicutes* and the *Firmicutes/Bacteroidetes* ratio on the background of decrease of the relative amount of *Bacteroidetes.* These changes were directly proportional to the increase in BMI, but had no gender features.

In patients with NAFLD, regardless of BMI, a significant violation of enzymatic, lipid, carbohydrate metabolism and changes in the state of insulin resistance were determined, which testifies to the pathogenetic role of metabolic disorders in the development of NAFLD.

Both parallels and differences in the composition of GM associated with both NAFLD and BMI were identified between our study and other studies.

The obtained data confirm the essential role of GM in the pathogenesis of NAFLD. Therefore, it is important to continue the study of the composition of GM at a grand-scale level, which in future can serve as a good prognostic marker in the diagnosis and treatment of NAFL in patients with obesity or excess weight.

REFERENCES

1. Kim D., Siddique O., Perumpail B.J., Ahmed A. Clinical epidemiology of NAFLD. In Clinical Epidemiology of Chronic Liver Diseases. Springer International Publishing: Cham, Switzerland. 2019; 211–227.

2. Younossi Z.M., Koenig A.B., Abdelatif D. et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73–84.
3. Kim D., Chung G.E., Kwak M.S. et al. Body fat distribution and risk of incident and regressed nonalcoholic fatty liver disease. Clin. Gastroenterol. Hepatol. 2016;14:132–138.e4.
4. Bae J.C., Han J.M., Cho J.H. et al. The persistence of fatty liver has a differential impact on the development of diabetes: The Kangbuk Samsung Health Study. Diabetes Res. Clin. Pract. 2018;135:1–6.
5. Sinn D.H., Cho S.J., Gu S. et al. Persistent nonalcoholic fatty liver disease increases risk for carotid atherosclerosis. Gastroenterology. 2016;151: 481–488.
6. Sunkara T., Rawla P., Ofosu A., Gaduputi V. Fecal microbiota transplant – A new frontier in inflammatory bowel disease. J. Inflamm. Res. 2018;11: 321–328.
7. Ley R.E., Turnbaugh P.J., Klein S., Gordon J.I. Human gut microbes associated with obesity. Nature. 2006; 444: 1022–1023.
8. Turnbaugh P.J., Ley R.E., Mahowald M.A. et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444 :1027–1031. doi: 10.1038/nature05414.
9. Duncan S.H., Lobley G.E., Holtrop G. et al. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond). 2008;32(11):1720-4. doi: 10.1038/ijo.2008.155.
10. Jumpertz R., Le D.S., Turnbaugh P.J. et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr. 2011;94(1):58-65. doi: 10.3945/ajcn.110.010132.
11. Schwiertz A. Microbiota and SCFA in lean and overweight healthy subjects. 2010;18:190-195. doi: 10.1038/oby.2009.167.
12. Turnbaugh P. J. A core gut microbiome in obese and lean twins Nature. 2009; 457:480-484. doi: 10.1038/nature07540.
13. Chereliuk N.I. Ratio of the main phylotypes of gut microbiota in patients with non-alcoholic fatty liver disease and obesity. Modern Gastroenterology. 2019;5(109): 26-33.
14. Backhed F., Ding H., Wang T. et al. The body as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. 2004.
15. Turnbaugh P. J., Hamady M., Yatsunenko T. et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457: 480–484.
16. Bajzer M., Seeley R.J. Physiology: obesity and gut flora. Nature. 2006;444:1009–1010.
17. Finucane M.M., Sharpton T.J., Laurent T. J., Pollard K.S. Taxonomic Signature of Obesity in the Microbiome? Getting to the Guts of the Matter PLoS One. 2014;9(1):e84689. doi: 10.1371/journal.pone.0084689.
18. Arumugam M., Raes J., Pelletier E. et al. Enterotypes of the human gut microbiome. Nature 473: 174–180. Ta The Human Microbiome Project Consortium Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486: 207–214.
19. Smith M.I. et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science. 2013;339(6119):548-54. doi: 10.1126/science.1229000.
20. Le Chatelier E., Nielsen T., Qin J. et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500: 541–546.
21. Haro A. et al Intestinal Microbiota Is Inuenced by Gender and Body Mass Index Carmen. 2016. https://doi.org/10.1371/journal.pone.0154090.
22. Dominianni C., Sinha R., Goedert J.J. et al. Sex, body mass index, and dietary fiber intake influence the human gut microbiome. PloS one. 2015;10:e0124599.

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ORIGINAL ARTICLE

THE PRINCIPLE OF COMBINED PREOPERATIVE DIAGNOSIS OF THYROID TUMORS

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ABSTRACT

The aim of the research is to increase the effectiveness of preoperative diagnosis of patients with thyroid tumors and to assess the use of cancer-embryonic antigen and immunocytochemical research.

Materials and methods: Patients were interviewed about their complaints and lifestyle; performed ultrasound with fine-needle aspiration, determination of the level of cancer-embryonic antigen (CEA), cytological and immunocytochemical researches.

Results: The Benign process in the thyroid gland is low serum REA (less than 0.95 ng / ml), poor expression of thyroglobulin (77.8%), negative reaction with TTF-1 (100%) and cytokeratin-19 (55.6%). Differential-prognostic markers of thyroid neoplasms with risk of malignancy include increased serum REA (0.95 ng / ml and above), the presence of a moderate reaction with antibodies to thyroglobulin (80.0%), a positive reaction — to TTF-1 (100.0%) and E-cadherin (90.0%), with moderate or strong expression of cytokeratin-19 (90.0%). Statistically significant markers of malignant thyroid disease were determined: the presence of harmful factors at work (45.5%), smoking (27.3%), elevated serum REA (0.95 ng / ml and above), the presence of strong cytoplasmic expression of thyroglobulin (63.6%), moderate or strong expression of TTF-1 (90.9%) and cytokeratin-19 (81.8%).

Conclusions: The most appropriate and practically significant for preoperative diagnosis of thyroid tumors is a set of several diagnostic methods, which are carried out in one hospital – ultrasound with fine-needle aspiration, cytomorphological, and immunocytochemical and REA levels in a primary screening.

KEY WORDS: thyroid gland, nodular diseases of the thyroid gland, carcinoma, diagnosis, fine-needle aspiration puncture biopsy

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INTRODUCTION

Today thyroid disease is taking one of the leading places among endocrine pathology in terms of overall morbidity and prevalence in the world. Due to the environmental situation in Ukraine, this problem is attracting more and more attention every year. This indicator varies significantly in different regions of the country and depends on the influence of environmental factors like stress, iodine deficiency, lifestyle, malnutrition, micronutrient deficiencies, and comorbidity. In general, diseases of the endocrine glands and, in particular, pathology of the thyroid gland cause great socioeconomic damage which is determined by the cost of medical care and social security (due to disability, premature death) [1, 2, 3].

Mass surveys of the population after the accident revealed a high prevalence of thyroid enlargement (in 20-40% of those who were surveyed) [4, 5]. O.B. Kaminsky and his co-authors (2016) conducted a retrospective analysis of data and found that the incidence of thyroid pathology among all victims of the Chernobyl accident was 40.3%, among the participants in the aftermath of the accident — 35.4%, among those who evacuated from the exclusion zone — 27.2%, residents of radiation-contaminated areas — 28.6%, which is much more ($p<0.0001$) than the general population (3.9%) of Ukraine [6]. According to the National Cancer Registry of Ukraine, 137,266 new cases of malignant neoplasms (MN) were registered in 2017; the total incidence of ZN was 381.4 per 100 thousand of the population, including 392.5 for men and 371.7 for women. Thyroid cancer is more often diagnosed among malignant tumors of the endocrine organs and in the overall structure of cancer is 1-3%. In Ukraine, the prevalence of thyroid cancer was 8.2 per 100 thousand of the population in 2018 and 8.3 per 100 thousand of the population in 2019. The incidence rate per 100 thousand of the population in Dnipropetrovsk region was 6.7, of which 3.1 — among men and 9.8 — among women [7]. The share of thyroid cancer is 0.5-1.5% in the structure of all malignant neoplasms depending on age, gender, radiation exposure, hereditary history and other factors. According to world data, the incidence of thyroid cancer is 20-80 cases per 100 thousand of the population per year, mortality — 5 per 1 million of the population per year [8, 9, 10]. However, there are no unified clinical protocols in endocrinology for the provision of medical care to adults with thyroid pathology in Ukraine. Therefore, it is important to supplement modern approaches to the diagnosis of such diseases, analysis of scientific and practical aspects

of treatment and monitoring of thyroid nodules based on current international guidelines of the American Thyroid Association for adult patients with thyroid nodules and differentiated thyroid cancer, year 2015; joint consensus of the American Association of Clinical Endocrinologists, the Italian Association of Clinical Endocrinologists, the European Thyroid Association, year 2010, with taking into the account of evidence-based medicine [11].

THE AIM

The aim of the research is to increase the effectiveness of preoperative diagnosis of patients with thyroid tumors and to assess the use of cancer-embryonic antigen and immunocytochemical research.

MATERIALS AND METHODS

During the the research work the results of diagnostics of 60 patients with thyroid neoplasms aged 21 to 83 years (mean age 54.6 ± 1.9 years) who underwent fine-needle aspiration puncture biopsy were obtained in the polyclinic Communal Institution "Dnipropetrovsk Regional Clinical Hospital named after I.I. Mechnykov".

Patients were divided into three groups according to the Bethesda System (BSRTC) classification category. The 1st group included 18 patients of category II BSRTC (Benign, benign lesions), of which 88.9% were women and 11.1% were men and the mean age was 46.6 ± 3.7 years. Group 2 included 20 patients with category V BSRTC (Suspicious for Malignancy – SFM, suspected malignancy), female (100%) and mean age 58.7 ± 3.1 years. In addition, other 22 patients with category VI BSRTC (malignant tumor) formed the 3rd group, of which 90.9% were women and 9.1% were men, the mean age was 57.4 ± 2.4 years. The research included patients with the first established TR-4 category of thyroid nodule on the TI-RADS scale (4-6 points, suspected of malignant thyroid changes).

Patients were interviewed about their complaints, history, lifestyle and negative health factors; quality of life assessment MOS SF-36 (Medical Outcomes Study Short Form Health Status Survey), ultrasound on the device Toshiba SSA-580A with electronic linear sensors with a frequency of 9-12 MHz and a scanning surface length of 6 cm, fine-needle aspiration 23G (0.6x25 mm), determination of the level of cancer-embryonic antigen (CEA) on the analyzer Cobas e-411 (Roche Diagnostics GmbH), cytological and immunocytochemical researches.

Informative were considered drugs from at least 6 groups of thyroid cells, at least ten follicular cells each (preferably in one drug). The puncture material on the slide was air-dried and fixed with methanol for 5 minutes, staining for 30 minutes by the Romanovsky method. The results of the cytological examination were evaluated by the Bethesda System (BSRTC) with a standard cytological protocol. Immunocytochemical researches were conducted at points fixed with methanol and stained according to Romanovsky method by restoring the activity of antigenic determinants. An indirect immunoperoxidase method using mouse monoclonal antibodies was used (Dako Cytomation, Denmark). Antibodies against mouse g-globulins labeled with horseradish peroxidase (Dako Cytomation, Denmark) were used as secondary. Control tests were used to assess the adequacy of the methodological procedure. Evaluation of the specificity of the interaction of antibodies with tissue antigens was performed using positive and negative controls. The results were analyzed by determining the number of positive (+) cells using an Axiostar plus microscope (Carl Zeiss, Germany) at a magnification of 320 times, and evaluated using the classical H-Score method. Statistical processing of the results was performed using the methods of parametric and nonparametric statistics, implemented in the application packages MDCalc statistical software and Microsoft Excel 2019 (Microsoft). The assessment of the conformity of the distribution of quantitative values of the data to the normal law was performed using the Shapiro-Wilk test. Under the conditions of the normal distribution law, we used the arithmetic mean and it's standard error (M ± m), one-way analysis of variance ANOVA (F) with a posteriori comparison of groups with each other by Tukey (HSD); in other cases – median (Me), interquartile range (25-75%), nonparametric analysis of Kruskal-Wallis ANOVA (H) followed by Multiple Comparisons (MS). Comparison of relative indicators were conducted according to the Pearson Chi-square test (c2) and the two-sided Fisher's Exact Test (FET). The correlation between the factors was determined by criterion c2 and Spearman's rank correlation coefficients (r). To assess the discriminant significance of the REA indicator for predicting the malignancy of the process, an ROC analysis was conducted with the calculation of the area under the ROC curve (AUC) and operational characteristics (sensitivity, specificity) with a 95% confidence interval (95% CI). The level of $p < 0.05$ (5%) was considered statistically significant.

RESULTS

A comparative analysis of the studied indicators (Table I) showed that the selected groups probably did not differ in the gender of patients ($\chi 2 = 2.21$, $p > 0.05$), body mass index ($F = 0.25$, $p > 0.05$), the size and area of the tumor according to ultrasound ($H = 2.30$, $p > 0.05$ and $H = 1.92$, $p > 0.05$, respectively). At the same time, patients of the 1st group were younger in age than (when) compared with patients of other groups ($p < 0.05$ according to the HSD criterion). It should be noted that more than half of patients in all research groups were overweight or had grade I-II of obesity (WHO, 1997) – 55.6%; 50.0%; 72.7%, respectively, in the 1st, 2nd and 3rd groups.

According to the results of the questionnaire (Table II), hereditary burden of thyroid disease was noted by 10 patients (16.7%) from the 1st (n = 4 – 22.2%) and 2nd (n = 6 – 30.0) %) group, while in the 3rd group there were no such kind of cases (p <0, 05). One in five patients in the research groups was in areas of high radiation exposure, with no significant differences between the groups ($\chi 2 = 0.10$, $p > 0.05$). However, almost half of the patients of the

Table I. General characteristics of patients and thyroid tumors

Indicator	Research groups			General difference between the groups
	Group 1 (n=18)	Group 2 (n=20)	Group 3 (n=22)	
Gender, % ffemale/ male	88,9/ 11,1	100/ 0	90,9/ 9,1	χ^2=2,21, p=0,331
Age, years, M±m	46,6±3,7	58,7±3,1*	57,4±2,4*	F=4,51, p=0,015
BMI, kg/m^2, M±m	28,7±1,3	27,5±1,4	27,8±0,8	F=0,25, p=0,777
Max diameter of formation, mm, Me (25-75%)	15,0 (13,0-17,0)	11,0 (10,0-20,0)	12,0 (10,0-27,0)	H=2,30, p=0,316
Area of formation, mm^2, Me (25-75%)	135,0 (112,0-225,0)	106,0 (60,0-312,0)	120,0 (70,0-513,0)	H=1,92, p=0,383

Note. * - p <0.05 compared to group 1 (by HSD criterion)

Table II. Frequency of detection of risk factors and clinical signs of thyroid tumors of patients of research groups (abs. / %)

Indicator	Research groups			General difference between the groups
	Group 1 (n=18)	Group 2 (n=20)	Group 3 (n=22)	
Hereditary (genetic) factor	4/ 22,2*	6/ 30,0*	0/ 0	χ^2=7,36, p=0,025
Harmful factors at work	4/ 22,2	2/ 10,0*	10/ 45,5	χ^2=6,99, p=0,030
Smoking	0/ 0*	0/ 0*	6/ 27,3	χ^2=11,52, p=0,003
Staying in areas of high radiation exposure	4/ 22,2	4/ 20,0	4/ 18,2	χ^2=0,10, p=0,951
The pressure in the neck	8/ 44,4	6/ 30,0	6/ 27,3	χ^2=1,46, p=0,481
Hoarseness	2/ 11,1	6/ 30,0	4/ 18,2	χ^2=2,18, p=0,335
Impaired swallowing	2/ 11,1	6/ 30,0	2/ 9,1	χ^2=3,87, p=0,145
Delayed passage of food	6/ 33,3	2/ 10,0	4/ 18,2	χ^2=3,30, p=0,192

Note. * - p <0.05 compared to group 3 (by FET criterion)

Table III. Level of cancer-embryonic antigen (CEA, ng / ml) of patients of the research groups

Research group	Statistical characteristics			Reference values
	Min – max	Me (25-75%)	General difference between the groups	
Group 1 (n=18)	0,20 - 3,31	0,90 (0,53-1,92)	H=9,01, p=0,011	1. All subjects: 20 – 69 years to 4,7 40-69 to 5,2 2. Do not smoke or have smoked in the past: 20-69 years to 3,8 40-69 years to 5,0 3. Smoke now: 20-69 years to 5,5 40-69 years to 6,5
Group 2 (n=20)	0,84 - 3,70	2,01 * (1,36-2,23)		
Group 3 (n=22)	0,50 - 85,83	2,05 * (1,17-3,99)		

Note. * - p <0.05 compared to the 1st group (according to the MC criterion)

3rd group (45.5%) had harmful factors at work against 10.0% of the patients in the 2nd (p <0.05) and 22.2% in the 1st group (p> 0, 05). Smoking was observed only among patients of group 3 (27.3%) at p <0.05 compared with other groups. Thus, the analysis of anamnestic data showed that the most characteristic risk factors for the development of malignant processes in the thyroid gland are the presence of harmful factors at work and smoking.

Analysis of patient complaints showed the absence of pathognomonic clinical signs characteristic of thyroid

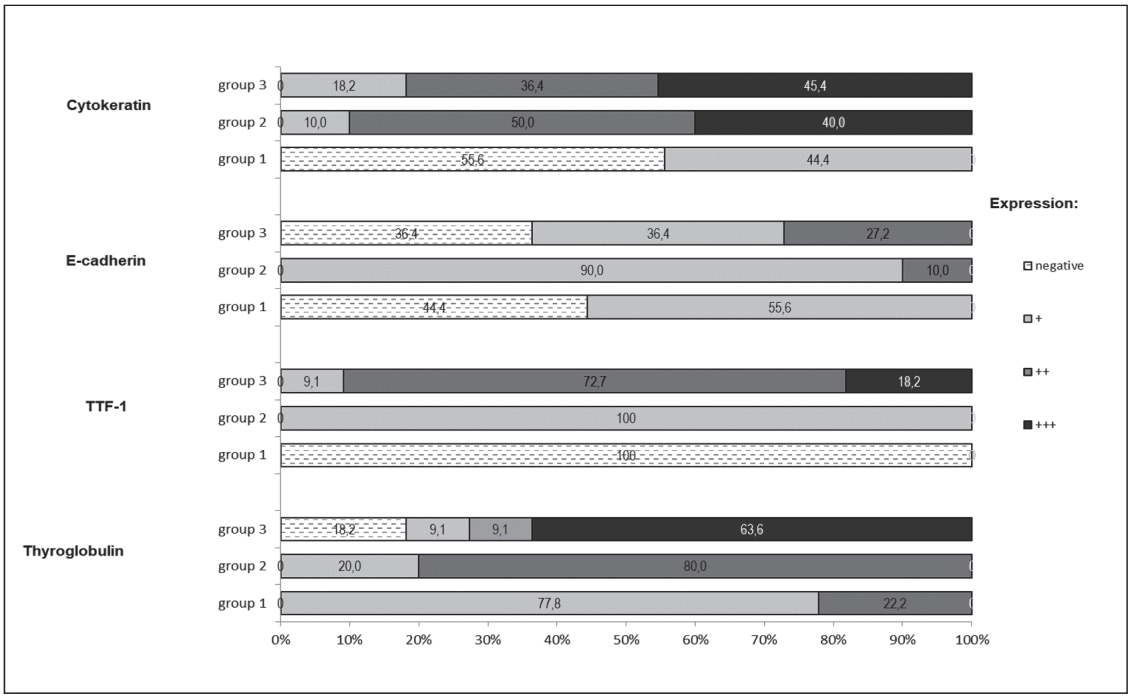

Fig. 1. The frequency of expression of thyroglobulin, TTF-1, E-cadherin and cytokeratin-19 of different intensity in the cells of thyroid tumors with different classification category according to BSRTC

tumors with different classification category according to BSRTC (Table II). Pressure in the neck was felt by about a third of patients – 20 (33.3%), hoarseness was noted by 12 (20.0%), swallowing disorders – 10 (16.7%), delayed passage of food – 12 (20.0). %) patients, without probable differences between groups (p> 0.05). At the same time, according to the correlation analysis, a direct correlation was established between the delay in the passage of food and the size of the tumor – r = 0.34; p <0.01.

The research of the level of cancer-embryonic antigen (CEA) in the serum of thematic patients showed the presence of a direct correlation between the indicators depending on the degree of malignancy of the process in the thyroid gland – r = 0.33; p <0.01. In benign thyroid disease (group 1), the level of CEA of all patients did not exceed the reference range, ranged from 0.20 to 3.31 ng / ml and averaged 0.90 (0.53-1.92) ng / ml (Table III). Patients with category B BSRTC (group 2), the rate ranged from 0.84 to 3.70 ng / ml, also without exceeding the reference values. However, the median index was 2.2 times higher than in the 1st group (p <0.05). In 18.2% of patients in group 3 (n = 4), who were confirmed in the postoperative stage of metastatic lymph node involvement, the level of PEA was above normal. The mean for this cohort of patients was 2.05 (1.17-3.99) ng / ml, probably exceeded the corresponding value of patients with a benign process by 2.3 times (p <0.05), but did not differ statistically from indicator of the 2nd group (p> 0.05). Therefore, the serum level of REA can serve as a differential diagnostic criterion and indicate a potentially malignant origin of the tumor. According to ROC-analysis, it is established that a high probability of malignant transformation of the thyroid lymph node is predicted by increasing serum REA to 0.95 ng / ml and above – the area under the ROC curve AUC 0.746 (95% CI 0.617-0.850) at p < 0.001, which provides the sensitivity of the criterion 85.7 (95% CI 75.1-96.3)%, specificity – 66.7 (95% CI 44.9-88.4)%.

Comparative analysis of immunocytochemical data of patients with thyroid tumors revealed the correlation between the expression of thyroglobulin, thyroid transcription factor-1 (TTF-1), E-cadherin and cytokeratin-19 with the potential for tumor malignancy for pragmatic aspect of preoperative diagnosis.

It was found that patients with BSRTC-2 (group 1) had a weak expression of thyroglobulin (thyroid hormone) in 77.8% (n = 14) cases and moderate in 22.2% (n = 4). In the 2nd group of patients with V category BSRTC there was an increase in the intensity of the reaction with antibodies to thyroglobulin: only in 20.0% of cases (n = 4) the expression was weak, and in 80.0% (n = 16) – moderate (p <0.001 compared with group 1). With metastatic thyroid disease (group 3), high thyroglobulin expression was observed in 63.6% (n = 14) of patients, moderate – in 9.1% (n = 2), weak – in 9.1% (n = 2), a negative result was obtained in 18.2% (n = 4) cases (p <0.001 against the data in the 1st and 2nd groups) (Fig. 1). Correlation analysis confirms a direct correlation between the frequency of increased immunogenic expression of thyroglobulin and the potential for malignancy of the thyroid gland – $\chi 2 = 62.53$; r = 0.49; p <0,001, which emphasizes the prognostic value of this marker for the identification of malignant processes in the preoperative stage.

The research of the proliferative activity of thyroid tissue based on the expression of TTF-1 protein showed it's high differential prognostic significance for assessing the risk of

malignant process in the thyroid gland – $\chi 2 = 109.6$; $r = 0.96$; $p < 0.001$. Thus, in the benign process in all cases (100%) the negative status of TTF-1 expression was established, in the case of suspected tumor malignancy – a weak reaction (+) in 100% of patients ($p < 0.001$), in metastatic lesions – in 18.2% cases of strong expression (+++), in 72.7% – moderate (++), in 9.1% – weak ($p < 0.001$ compared to previous groups).

The research of the reaction of E-cadherin, which functions as an antitumor suppressor (anti-oncogene), in the studied samples of thyroid tumors showed mixed results (Fig. 1). In the benign nature of the thyroid tumor (group 1), suppression of E-cadherin expression was determined: in 44.4% of cases, the expression was absent, in 55.6% – a weak reaction was noted. The presence of a positive reaction of E-cadherin was noted in all biopsies (100%) with suspected malignancy (group 2); including weak expression was observed in 90.0% of cases, moderate reaction – in 10.0% ($p < 0.001$ compared with benign lesions). At the same time, there is no clear link between E-cadherin expression and neoplasia. Negative expression occurred in 36.4% of cases of metastatic thyroid disease, weak reaction – in 36.4%, moderate reaction – in 27.2% ($p = 0.053$ and $p = 0.001$ compared with the 1st and 2nd groups in accordance). This partially satisfies the requirements of timely preoperative diagnosis.

In contrast to the previous marker, the expression of cytokeratin-19 with varying degrees of intensity of cytoplasmic staining of tumor cells was determined in all studied neoplasms of V and VI category on the BSRTC scale. Strong expression of cytokeratin-19 was observed in 40.0% of cases with BSRTC-5 and 45.4% with BSRTC-6, moderate – in 50.0% and 36.4% of cases, respectively, weak – in 10.0% and 18.2%, without significant differences between groups ($p > 0.05$). At the same time, in the benign process in 55.6% of cases there was no reaction of cytokeratin-19, and in 44.4% – weak expression ($p < 0.001$ in comparison with the 2nd and 3rd groups). Therefore, on the puncture material with the thyroid gland cytokeratin-19 is a promising marker for the identification of malignant processes.

DISCUSSION

The relevance of this study is because the pathology of the thyroid gland is one of the main places among endocrine pathology in terms of overall morbidity [1]. It is obvious that predicting an increase in the number of cases of thyroid cancer for the next 30 years reflects the problem of diagnosis [4, 5]. In modern international protocols of endocrinology, there are no data on the use of the above set of markers in the preoperative stage of diagnosis [11]. Cytological examination of thyroid tumors is considered a minimally invasive procedure, but to obtain adequate material is not always possible and depends on many factors. Based on this, the introduction of immunocytochemical and immunochemiluminescent analysis was proposed.

The reliable results of these methods obtained by us testify to the expediency of including them in the basic set of diagnostic procedures used for dynamic monitoring of patients. Given the fact that after the analysis of pathomorphological study it was concluded that the sensitivity of the level of CEA is high only in the serum of patients with poorly differentiated carcinoma. Use as an auxiliary marker for predicting the course of the tumor process is appropriate. Only one-time application of these diagnostic stages can bring closer the earlier appointment of adequate treatment and reduce the cost of expensive procedures.

CONCLUSIONS

Our comprehensive researches of patients with thyroid tumors suggest that immunocytochemical diagnosis plays a key role in assessing the malignant potential of the tumor in the preoperative stage.

Benign thyroid disease (category II BSRTC) is likely to be associated with younger patients (46.6 ± 3.7 years), low serum REA (less than 0.95 ng / ml), poor thyroglobulin expression (77.8 %), negative reaction with TTF-1 (100%) and cytokeratin-19 (55.6%).

Differential-prognostic markers of thyroid cancer with risk of malignancy (V category BSRTC) include elevated serum REA (0.95 ng / ml and above), the presence of a moderate reaction with antibodies to thyroglobulin (80.0%), a positive reaction – to TTF-1 (100.0%) and E-cadherin (90.0%), with moderate or strong expression of cytokeratin-19 (90.0%).

Statistically significant markers of malignant thyroid disease (category VI BSRTC) are: the presence of harmful factors at work (45.5%), smoking (27.3%), elevated serum REA (0.95 ng / ml and above), the presence of severe cytoplasmic expression of thyroglobulin (63.6%), moderate or severe expression of TTF-1 (90.9%) and cytokeratin-19 (81.8%).

Thus, the most appropriate and practically significant for preoperative diagnosis of thyroid tumors is a set of several diagnostic methods, which are carried out in one hospital – ultrasound with fine-needle aspiration, cytomorphological, and immunocytochemical and REA levels in a primary screening.

REFERENCES

1. Kravchenko V. Iodine deficiency as a cause of high prevalence of thyroid pathology in the population of regions affected by the Chernobyl accident. Journal of the National Academy of Medical Sciences of Ukraine. 2016; 22:.222-229.
2. Tronko M., Brenner A., Bogdanova T. Thyroid neoplasia risk is increased nearly 30 years after the Chernobyl accident. Int J Cancer. 2017;141: 1585–1588.
3. Palamar B.I., Gruzieva T.S. The estimation of economic effectiveness of preventive measures of non-infectious diseases. Wiad. Lek. 2019; 72 (8): 1532-1541.
4. Shibata Y., Fuzik M., Prysyazhnyuk A. Age and gender patterns of thyroid cancer incidence in Ukraine depending on thyroid radiation doses from radioactive iodine exposure after the Chernobyl NPP accident. Problem Radiac Med Radiobiology. 2013; 18: 144-55.
5. Taylor P.N., Albrecht D., Scholz A., et al. Okosieme OE. Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol. 2018 May; 14(5): 301-316. doi: 10.1038/nrendo.2018.18. Epub 2018 Mar 23. PMID: 29569622.

6. Kaminskyi, O., Kopylova, O., Afanasyev, D. et al. Non-cancer thyroid and other endocrine disease in children and adults exposed to ionizing radiation after the Chernobyl NPP accident. Problems of radiation medicine and radiobiology. 2015; 20: 341-55.
7. Fedorenko Z., Michailovich Y. Goulak L. Mortality from malignant neoplasms. State of oncological care to the population of Ukraine in 2017-2018. Bulletin of National Cancer Registry of Ukraine. 2019; 20:6.
8. Park Y.J., Kim J.A., Son E.J., et al. Thyroid nodules with macrocalcification: sonographic findings predictive of malignancy. Yonsei Med J. 2014 Mar; 55(2):339-44. doi: 10.3349/ymj.2014.55.2.339. PMID: 24532501; PMCID: PMC3936639.
9. Perros P., Boelaert K., Colley S. et al. Guidelines for the management of thyroid cancer. Clinical endocrinology. 2014; 81 (1): 1–122. https://doi.org/10.1111/cen.12515--
10. Tronko M., Bogdanova T., Saenko V. Thyroid cancer in Ukraine after Chernobyl. [Nagasaki]: Nagasaki Association for Hibakusha's Medical Care. 2014.
11. Haugen B. R., Alexander E. K., Bible K. C., et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid: official journal of the American Thyroid Association. 2016; 26(1): 1–133. https://doi.org/10.1089/thy.2015.0020.

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ORIGINAL ARTICLE

CHANGES IN BIOCHEMICAL BLOOD VALUES DURING COMPLEX TREATMENT OF RHINOSINUSITIS POLYPOSA PATIENTS

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ABSTRACT

The aim: To increase the effectiveness of treatment of sinusitis polyposa patients, develop and introduce a new non-traditional complex method of influencing several links of the pathogenesis of this disease, including laser therapy and laser puncture, used in the postoperative period.

Materials and methods: In 60 rhinosinusitis polyposa patients the indicators of lipid peroxidation and the state of antioxidant protection were studied. Depending on the type of treatment, the patients were divided into two groups: the first included 30 people who underwent traditional surgical intervention, and the second – 30 people who used complex therapy, including in the postoperative period endonasal laser exposure and laser puncture.

Methods: clinical data, the functional state of the nasal mucosa, indicators of lipid peroxidation (hydroperoxide, malondialdehyde) and antioxidant activity (glutathione peroxidase, glutathione reductase, reduced glutathione, superoxide cismutase, catalase) of erythrocyte membranes and blood serum were studied.

Results: The proposed complex method for treating lipids of erythrocyte membranes and serum, which includes endonasal surgical intervention, endonasal laser therapy and laser puncture leads to the normalization of physiological functions of the nose, activates antioxidant protection and reduces the peroxide activity of lipids in the membranes of erythrocytes and blood serum.

Conclusions: When examining patients in the long-term (after 1 year) period, a significant improvement in 85.7% of cases, an improvement in 10.7%, and absence of effect in 3.6% was achieved. Thus, the proposed method of therapy can be recommended for widespread use in medical institutions.

KEY WORDS: Treatment method, rhinosinusitis polyposa

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INTRODUCTION

Among the urgent problems of modern practical otorhinolaryngology, the issues of increasing the efficiency of diagnosis and treatment of inflammatory diseases of the nose and paranasal sinuses occupy one of the leading positions [1].

In recent years, there has been a significant increase in the number of diseases of the nose and paranasal sinuses both in absolute figures and in percentage of the total number of ENT diseases [2, 3]. The increasing number of patients with inflammatory diseases of the paranasal sinuses causes significant expenditures in the public health system associated with modern technologies of treatment, as well as using a large number of expensive drugs [2, 4].

Chronic rhino-sinusitis polyposa accounts for 13% of all diseases of the nasal cavity and paranasal sinuses, and this indicator tends to further increase [5; 6]. The actuality of this issue is due to the increase of incidence of chronic polypous rhino-sinusitis and frequent recurrence of the process, in addition, the lack of clarity of many elements of pathogenesis. Polypoid rhino-sinusitis contributes to severe lower respiratory tract pathology such as pre-asthma and bronchial asthma, and can lead to life-threatening intraocular and intracranial complication.

When nasal breathing is impaired, the physiological functions of the nose change, leading to a weakening of ventilation capacity, gas exchange in the lungs, the development of hypoxia and hypercapnia [7].

Sinusitis polyposa is characterized by a prolonged course, frequent relapses. In this case, both local changes in the mucous membrane of the nasal cavity and paranasal sinuses are observed, as well as metabolic disorders, immunological reactivity, impairment of function of the endocrine and non-vascular system [8 – 11].

A large percentage of relapses of chronic sinusitis polyposa after surgery cause the search for and development of new treatment methods.

Medical methods of therapy for chronic sinusitis polyposa, prescribed after surgical treatment, are in most cases not effective and often intensify the manifestations of allergies, which worsens the course of the disease and contributes to an increase in relapses.

THE AIM

To increase the effectiveness of treatment of sinusitis polyposa patients to develop and introduce a new nonconventional complex method of influencing several links of the pathogenesis of this disease, including laser therapy and laser puncture used in the postoperative period.

MATERIALS AND METHODS

Under the supervision there were 60 sinusitis polyposa patients, treated in the ENT clinic on the basis of the regional hospital named after I.I. Mechnikov. The control group included 34 clinically healthy individuals. Among the subjects, there were 36 men and 24 women aged 14 – 70 years. Methods: clinical data, the functional state of the nasal mucosa, indicators of lipid peroxidation (hydroperoxide, malondialdehyde) and antioxidant activity (glutathione peroxidase, glutathione reductase, reduced glutathione, superoxide cismutase, catalase) of erythrocyte membranes and blood serum were studied.

RESULTS

The effectiveness of therapy over time (before and 1,12 months after its completion), patients' clinical data, the functional state of the nasal mucosa, indicators of lipid peroxidation (hydroperoxide, malondialdehyde) and antioxidant activity (glutathione peroxidase, glutathione reductase, reduced glutathione, superoxide cismutase, catalase) of erythrocyte membranes and blood serum were studied.

The state of lipid peroxidation (LPO) processes was assessed by the level of hydroperoxides using a color reaction with ammonium thiocinate, by the accumulation of LPO secondary products, represented mainly by malonic dialdehyde and determined during the reaction with 2-thiobarbaturic acid, as well as by resistance of erythrocytes to peroxide effects based on the content of free hemoglobin, formed after hemolysis of red blood cells.

We studied the antioxidant defense system using the determination of SOD by the non-enzymatic method, as well as catalase – by the decrease in H2O2, the activity of GLP, GLP and reduced glutathione in erythrocytes using standardized methods.

The diagnosis of sinusitis polyposa was established on the basis of patients' complaints, anamnesis, objective data, and the results of additional research methods: determination of the pathogenic microflora located on the nasal mucosa and radiography of the paranasal sinuses. Computed tomography was performed according to indications.

The leading complaint in 89% of the patients examined was difficulty or absence of nasal breathing and in 76% – disturbed olfaction in the form of hypo- or anosmia. Periodically attacks of rhinorrhea, paraxysmal sneezing occurred. General symptoms included headache, general weakness, decreased performance and disturbed sleep.

In anterior and posterior rhinoscopy, an edematous cyanotic mucous membrane, single or multiple polyps filling the nasal cavity were observed. In all clinical groups, significant impairments of the respiratory function, dynamic activity of the ciliated epithelium and the pH of the nasal secretion were revealed.

In sinusitis polyposa patients before treatment, activation of lipid peroxidation processes of erythrocyte membranes was found (an increase in the concentration of thiobarbituric acid hydroperoxide and malonic dialdehyde in erythrocytes and serum), as well as a decrease in antioxidant activity (AOA) in the initial stages of the disease due to glutathione enzyme system. Antioxidants still effectively inhibit the intensity of LPO processes, but the activity of the glutathione system is already decreasing. The activity of SOD remains unchanged or even increases due to pronounced compensatory and adaptive capabilities aimed at detoxifying LPO products and interrupting the processes of radical formation. With a disease duration of 10 years or more, LPO processes are significantly activated, which leads to a weakening of all links of antioxidant protection.

Depending on the type of treatment, the patients were divided into two groups: the first included 30 people who underwent the traditional surgical intervention and the second included 30 people who used complex therapy, including endonasal laser exposure and laser puncture in the postoperative period.

After traditional surgical treatment 1 month after in all the subjects of the first group nasal breathing, the dynamic activity of the ciliated epithelium and the pH of the nasal secretion were restored, the indicators of the patency of the nasal passages improved. Headache, disturbed sleep and other complaints disappeared in 16 (53.3%) of them, anosmia persisted in 12 (40%).

In rhinoscopy in 18 (60%) individuals, the mucous membrane remained cyanotic or moderately edematous, scanty mucus discharge in the nasal cavity was noted and in 8 (26.7%) individuals crusts were found.

In the study of LPO and AOA in the 1st group, there was a slight decrease in the level of hydroperoxide – by 1.1% compared to its indicators before treatment and the MDA content in the blood serum – by 3.7%, and in erythrocytes – by 8.4%; the antioxidant protection increased slightly: the concentration of reduced glutathione increased by 2.1%, catalase – by 4.2%, glutathione peroxidase – by 0.3%, glutathione reductase – by 3%, SOD – by 1.9% (Table I, II).

The effectiveness of therapy was assessed according to the criteria – "significant improvement", "improvement" and "no effect".

DISCUSSION

In the long-term period (1 year after), 28 individuals from the 1st group were examined. Significant improvement was noted in 14 (50%) of them, improvement in 7 (25%), no effect was observed in 7 (25%).

Consequently, traditional surgical treatment was effective only in 53.6% of patients. Indicators of physiological functions of the nose, LPO, AOA, objective data after the operation did not change significantly.

In patients of the 2nd group, on the next day after surgery, a session of local endonasal laser therapy was first performed in each half of the nasal cavity then a session of laser puncture performed daily. The course of treatment consisted of 8-10 sessions. The laser beam was supplied to the acupuncture points of the skin using a flexible light guide with special nozzles for focusing.

Biologically active points and points of general action (depending on the state of the body) segmentary connect-

Table I. Indicators of lipid peroxidation of erythrocyte membranes and blood serum in sinusitis polyposa patients after conventional treatment

Indicator, unit of measure	Control (n-34)	Patients		Deviations of obtained indicators in % from their level before treatment
		before	after treatment	
Hydroperoxide, mol/l	1,372	1,592	1,575	-1,1
MDA in plasma, mmol/L	0,374	0,481	0,463	-3,7
MDA in erythrocytes, mmol/L	7,406	8,693	7,962	-8,4

Table II. Indicators of antioxidant activity of erythrocyte membranes in sinusitis polyposa patients after conventional treatment

Indicator, unit of measure	Control (n-34)	Patients		Deviations of obtained indicators in % from their level before treatment
		before	after treatment	
Reduced glutathione, mmol/L	4,208	3,753	3,832	2,1
Activity of glutathione-peroxidase, mol/l Er.	11,996	8,306	8,332	0,3
Activity of glutathione-reductase, mol.	9,55	7,497	7,269	3
Catalase, mol/L.M.	5,23	6,089	6,343	4,2
SOD, c.u.	506,25	600,91	612,19	1,9

Table III. Indicators of lipid peroxidation of erythrocyte membranes and blood serum in sinusitis polyposa patients after treatment with a complex method

Indicator, unit of measure	Control (n-34)	Patients		Deviations of obtained indicators in % from their level before treatment
		before	after treatment	
Hydroperoxide, mol/l	1,372	1,592	1,367	-14,1
MDA in plasma, mmol/L	0,374	0,481	0,44	-8,5
MDA in erythrocytes, mmol/L	7,406	8,693	7,402	-14,9

Table IV. Indicators of antioxidant activity of erythrocyte membranes in sinusitis polyposa patients after treatment with a complex method

Indicator, unit of measure	Control (n-34)	Patients		Deviations of obtained indicators in % from their level before treatment
		before	after treatment	
Reduced glutathione, mmol/L	4,208	3,753	4,214	12,3
Activity of glutathione-peroxidase, mol/l Er.	11,996	8,306	10,761	29,6
Activity of glutathione-reductase, mol.	9,55	7,497	8,824	17,7
Catalase, mol/L.M.	5,23	6,089	5,41	-11,,2
SOD, c.u.	506,25	600,91	543,05	-9,2

ed with the respiratory organs were selected according to the rules for choosing acupuncture points [10; 13, 14-16]. In particular, the points of general action were used 014 (he-gu), 0111 (qui-chi), E36 (Tszu-san-li) and local – 0119 (he-liao), 0120 – (in-xiang), E3 – (ju-liao). In addition, the impact was also exerted on the extrameridianal points EM3 (yin-tang), EM15 (jia-bi).

Laser puncture always started with distant points of general action, which were changed daily to avoid adaptation.

One month after the treatment, nasal breathing was restored in all the patients of the 2nd group, in 86.7% of patients olfaction improved, working efficiency increased, and sleep returned to normal.

On examination, none of them had polyps in the nasal cavity, the mucous membrane acquired a pink color in 93.3% of cases, in 6.7% of cases its edema remained or it had a cyanotic hue.

The indicators of the main functions of the nasal mucosa returned to normal.

When studying the processes of LPO and AOA in individuals of this group, a significant decrease in the products of peroxidation was found. Their indicators approached the control values (the content of hydroperoxide decreased by 14.1%, MDA in plasma – by 8.5%, in erythrocytes – by 14.9%). An increase in the indicators of antioxidant protection was revealed, which approached the control values (the level of reduced glutathione increased by 12.3%, glutathione peroxidase – by 29.3%, glutathione reductase – by 17.7%, and a decrease in the catalase content by 11, 2%, SOD – by 9.2% of their value before treatment (Table III. IV).

In the long-term period (1 year after), 29 individuals were examined. A positive result of treatment, regarded as a "significant improvement" was established in 25 (85.7%) of them, improvement – in 3 (10.7%), no effect was noted – in 1 (3.6%).

CONCLUSIONS

Thus, it can be stated that complex treatment with the use of endonasal laser therapy and laser acupuncture in the postoperative period is effective, significantly improves the general condition of patients, helps to restore nasal breathing, lengthening the period of remission. This complex method of treatment helps to normalize the impaired physiological functions of the nose, leads to a subsidence of inflammation in the mucous membrane of the nasal cavity and paranasal sinuses, activates antioxidant protection and decreases the peroxide activity of lipids in the membranes of erythrocytes and blood serum. The proposed method of therapy can be recommended for widespread use in medical institutions.

REFERENCES

1. Hong P., Pereyra C.A., Guo U., Breslin A., Melville L. Evaluating Complications of Chronic Sinusitis. Case Rep Emerg Med. 2017. 8743828
2. Bergmark R.W., Pynnonen M. Diagnosis and First-Line Treatment of Chronic Sinusitis. JAMA.2017. Dec 19; 318 (23): 2344–2345.
3. Rosenfeld R.M., Piccirillo J.F., Chandrasekhar S. S., Brook I., Ashok Kumar K., Kramper M., Orlandi R.R., Palmer J.N., Patel Z.M., Peters A., Walsh S.A., Corrigan M.D. Clinical practice guideline (update): adult sinusitis. Otolaryngol Head Neck Surg. 2015. Apr; 152 (2): 1–39
4. Palamar B.I., Gruzieva T.S. The estimation of economic effectiveness of preventive measures of non-infectious diseases. «Wiadomości Lekarskie». 2019. 72 (8): 1532-1541.
5. Iskhaki Yu.B. Allergy in otorhinolaryngology. Dushanbe: Irfon; 1980, p. 92.
6. Mikhireva M.M., Portenko G.M. Electropuncture in combination with surgical intervention in the treatment of patients with polyposis rhinosinusitis. Vestnik Otorinolaryngologii. 1990; 2: 40-45.
7. Dunaivitser B.I., Gyulkhasyan A.A., Minasyan 0.3. et al. On some neuroreflex reactions of the brain in patients with chronic paranasal sinuitis. Zhurn. ushnyh, nocovyh i gorlovyh boleznei. 1983; 3: 8-12.
8. Arefieva N.A., Bikbaeva A.I., Prozorovskaya K.N. Pathogenesis and treatment of patients with recurrent polyposis ethmoiditis. Zhurn. ushnyh, nocovyh i gorlovyh boleznei. 1991; 1: 14-20.
9. Lantsov A.A., Lavrenova G.V., Shlopov V.G., Shevchenko T.I. Morphological aspects of recurrent sinuitis. Zhurn. ushnyh, nocovyh i gorlovyh boleznei. 1991; 2: 38-41.
10. Skvirskaya I.V, Gicheva TN. The state of immune reactions in patients with chronic polypous rhinosinuitis. Materialy region. nauch.-prak. konf. Otorinolaringologov i rasshir Plenuma RNOLO; 20-21 June 1990, Irkutsk. M.; 1990, p. 46-47.
11. Filatov V.F., Zhuravlev A.S., Filatov S.V. Pathophysiological mechanisms of development and course of productive sinuitis. Neirogumoralnaya regulatsiya v patologi: Sb. nauchn.tr. Kharkov: KGMU; 1984, p. 123-1 27.
12. Lupir A.V. Clinical and epidemiological characteristics of the contingent of examined patients with polypoid rhinosinitis. Zhurnal ushnyh, nocovyh i gorlovyh boleznei. 2011; 5: 8-16.
13. Tabeeva D.M. Acupuncture guide. M .: Medicina; 1980.
14. Macheret E.L., Samasyuk I.Z. Reflexology Guide. K .: Visch. Shk., 1984.
15. Lopatin A.S. Modern theory of the pathogenesis of polyposis rhinosinusitis. Vestnik otorinolaringologii. 2003; 3: 110-114.
16. Mitin Yu.V., Shiyan S.P. Particularities of the pathogenesis and treatment of polypous rhinosinus. Zhurnal ushnyh, nocovyh i gorlovyh boleznei. 2012; 3: 152-153.

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REVIEW ARTICLE

NON-SURGICAL TREATMENT OF PEYRONIE'S DISEASE: A COMPREHENSIVE REVIEW

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ABSTRACT

This review article focuses on conservative treatment options, topical, intralesional therapy, traction and vacuum therapy. A PubMed database search was performed for studies that were published between 1948 and 2019. Search keywords included "Peyronie's disease," "conservative therapy," "traction treatment," "extracorporeal shock wave therapy," "topical and oral therapies," and "vaccum therapy." Clinical trials in men with Peyronie's disease and scientific articles relating to pharmacologic data were included in the review. When possible, large, randomized, and well-designed trials were selected. Non-English-language articles were excluded.

KEY WORDS: Peyronie's disease, Intralesional treatment, penile curvature, Oral therapy, Penile traction, extracorporeal shock wave therapy

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INTRODUCTION

Peyronie's disease (PD) is a fibrotic condition of the tunica albuginea (TA) characterized by excessive accumulation collagen fibers and other components extracellular matrix (ECM), avoiding of apoptosis myofibroblasts during the abnormal wound healing process, cytokine dysregulation and local inflammatory changes with formation inelastic penile plaques [1,2]. François Gigot de la Peyronie first described this condition in 1743, but the pathogenesis is remaining unclear and enigmatic [3]. This disease has an acute (active) and chronic stable phases with different clinical course. In the acute phase, with duration, up to 18 months after the onset of PD occurs the inflammatory changes in TA due to repeated microtrauma during sexual intercourse. Insufficient activation of fibrinolysis and the fibroblast activation leads to a rapid growth plaque. In this period, the patient with PD notes the pain in flaccid state and painful erection, palpable nodes, and development of penile deformation. In the chronic phase, the pain generally disappears, and the formed plaques are stabilized, causing a penile curvature, penile deformities (such as hourglass deformity, hinge effect), and the shortening length of the penis [4-5,8].

THE AIM

This review article focuses on conservative treatment options, topical, intralesional therapy, traction and vacuum therapy. A PubMed database search was performed for studies that were published between 1948 and 2019. Search keywords included "Peyronie's disease," "conservative therapy," "traction treatment," "extracorporeal shock wave therapy," "topical and oral therapies," and "vaccum therapy." Clinical trials in men with Peyronie's disease and scientific articles relating to pharmacologic data were included in the review. When possible, large, randomized, and well-designed trials were selected. Non-English-language articles were excluded.

MATERIALS AND METHODS

The prevalence of PD ranges from 0.4 to 20.3 %, considering men with concomitant diabetes mellitus and erectile dysfunction. Peyronie's disease remains an under-diagnosed problem due to a paucity of epidemiological data of prevalence [4,6,10]. In the active inflammatory stage of PD should use oral, intralesional, and topical therapies despite controversial results and weak or moderate strength of recommendation in EAU and AUA guidelines. This is especially relevant in patients who have contraindications for surgical treatment due to severe concomitant somatic diseases.[7,9]. The oral medications available to treat Peyronie's disease are relatively inexpensive and are well-tolerated without adverse events. However, conservative treatment, when prescribed alone is not as effective as other modalities, such as intralesional injection and mechanical therapies. For any oral agent proposed for treatment of Peyronie's, the dose or tissue concentration that must reach in the tunica albuginea have a crucial role. The therapy with traction or vacuum devices may represent a viable therapeutic option for treating PD.

REVIEW AND DISCUSSION

ORAL MEDICATIONS

VITAMIN E (TOCOPHEROL)

Vitamin E is a fat-soluble compound with potent antioxidant action, which was first suggested for the treatment of Peyronie's disease in 1948 by Scott and Scardino[11]. It is assumed that vitamin E decreases the collagen deposition by reducing free oxygen radicals, inhibition of TGF-β1, and have an anti-inflammatory and anti-cyclooxygenase-2 (COX-2) property [12,13]. Most urologists commonly prescribe it in dose 600 mg per day because of its low cost, safety, and full availability. There are some clinical trials of vitamin E with conflicting results concerning the reduction of penile pain and deformity. In one study, Safarinejad et al. did not show significant improvement in pain, curvature, or plaque size in patients with PD treated with vitamin E compared with placebo[14]. Paulis et al. showed that vitamin E in combination therapy could lead to a significant improvement of penile curvature and reduction plaque size[15].

TAMOXIFEN

Tamoxifen is a nonsteroidal estrogen receptor antagonist that can block TGF-β1-signaling through Smad- dependent and Smad-independent manner[16,17]. Its anti-fibrotic activity has confirmed in several studies. El-Shazly et al. showed that tamoxifen effectively prevents the recurrence of urethral stricture after internal urethrotomy and improvement of Qmax and IPSS score compared with the control group [18].In the rat model of Peyronie's disease, Ilg MM, et al demonstrated that the combination of vardenafil and tamoxifen inhibited the myofibroblast transformation and ECM production[19].

Given this fact, Ralph et al. first demonstrated that tamoxifen is effective for the treatment of Peyronie's disease in 1992, and by now, there are few clinical trials about of effectiveness of tamoxifen as a part of combination therapy. In one study, Park et al. showed that the tamoxifen, together with acetyl-L-carnitine and phosphodiesterase type 5 inhibitor, significantly improved penile curvature [20,21]. The monotherapy tamoxifen will not lead to the significant improvement of penile deformity. This fact has demonstrated in a small placebo-controlled randomized study in 25 patients, which compared tamoxifen with placebo; no significant changes in penile curvature noted in either group [22]. The long-term use of tamoxifen can lead to such adverse events as constipation, hot flashes, atrial dysrhythmia, hypotension, superficial phlebitis, bone pain, decreased libido[23].

POTASSIUM PARA-AMINOBENZOATE (POTABA)

Potassium para-aminobenzoate is the agent with the antifibrotic activity, which first used for treatment PD by Zarafonetis and Horrax in 1953 [24]. It is believed that this substance exerts their antifibrotic properties through the improvement of tissue oxygenation, an increase of the secretion of glycosaminoglycans, and enhancement of the activity of monoamine oxidases with further decreasing local levels of serotonin[25]. In a prospective, randomized, double-blind, placebo-controlled, multicentre study in 103 patients with Peyronie's disease, Weidner et al. showed that Potaba could lead to a significant reduction in plaque size, but not in penile curvature or pain [26]. However, the treatment with Potaba can cause gastrointestinal adverse effects and skin photosensitization. Roy et al. reported a case report of acute hepatitis associated with the treatment with Potaba, and after 4 months of discontinuation of this agent, the patient noted a full biochemical recovery [27].

PHOSPHODIESTERASE-5 INHIBITORS (PDE-5I)

As known, nitric oxide (NO) and cyclic GMP have an anti-fibrotic activity by preventing excessive ECM deposition and myofibroblast transformation through inhibition TGF-β1-signaling. The anti-fibrotic effect of PDE5 inhibitors on PD plaque is associated with increasing the levels of circulating NO caused by inhibition of cGMP degradation to GMP[28,29]. This fact has confirmed in several studies of penile fibrosis in the animal model. Ferrini et al. demonstrated that the long-term use of a high dose of the vardenafil more effectively reduced the collagen/smooth muscle and collagen III/I ratios and the numbers of myofibroblasts in experimental PD-like plaque in the rat [30]. In another study in the diabetic rat, Li et al. showed that concomitant administration of P144-antagonist peptide of TGF-β1 and sildenafil improved the cavernosal fibrosis[31]. Gonzalez-Cadavid et al. have repeatedly proposed that the continuous long-term administration of PDE5 inhibitors may improve Peyronie's disease[28,32]. In one study, Ozturk et al. showed that ongoing administration of sildenafil 50 mg significantly reduced the size of the plaque, and penile pain was in 33.3 % and 66.6 % cases, respectively[33]. In another study, Palmieri et al. reported that extracorporeal shock wave therapy neither alone nor in combination with tadalafil 5 mg once daily was able to significantly improve plaque size and curvature degree in patients with Peyronie's disease[34]. Recently, Cocci et al. demonstrated that the combination of sildenafil 25 mg and collagenase of Clostridium hystoliticum (CCH) significantly improved the penile curvature compared with CCH alone[35].

COLCHICINE

Colchicine is most commonly used for treating acute attacks of gout with anti-microtubule and anti-inflammatory properties. It is proposed that colchicine treat Peyronie's disease through inhibition of microtubule polymerization with a subsequent reduction in collagen synthesis, down-regulating of TGF-β expression, and diminishing of inflammatory mediators[12,36]. Prieto Castro et al. reported that combined use of vitamin E and colchicine improved

the penile curvature and plaque size in the early stage of Peyronie's disease[37].In one study, Akman demonstrated that lateral curvature is the most commonly changed deformity that mostly shifts to the dorsal side of the penis after treatment with colchicine[38]. In another prospective, randomized, single-blind study, Toscano et al. showed the absence of any significant differences between treatment with thiocolchicine and verapamil in an improvement of the penile curvature and reducing plaque size [39].

L-CARNITINE

L-carnitine is a derivate of 6-N-trimethyl-lysine with antioxidant, anti-inflammatory, and anti-proliferative activity. It is assumed that carnitine reduces intracellular calcium levels in endothelial cells and suppresses fibroblast proliferation and ECM production via stimulation of NO-synthase [40,41]. In one study, Safarinejad et al showed that propionyl-L-carnitine alone or in combination with vitamin E had no significant improvement in penile curvature, plaque size, and pain in early stages of Peyronie's disease[42]. Recently, Park et al in another study showed that combined therapy with tamoxifen (20 mg), acetyl-L-carnitine (300 mg) twice daily, and phosphodiesterase type 5 inhibitor had a better response in men with penile curvature less than 30° [43]. Ciociola et al. reported case report of a 20-years-old Caucasian male where along with tadalafil, pentoxifylline, and L-arginine the propionyl-L-carnitine decreased PD plaque size compared with intracavernosal verapamil alone [44].

PENTOXIFYLLINE

Pentoxifylline (PTX) is a nonspecific cAMP-PDE inhibitor with anti-inflammatory and anti-fibrotic properties. Several studies demonstrated that the pentoxifylline can inhibit fibroblast proliferation, TGF-β1 expression, ECM deposition, and increases fibrinolytic activity [45,46]. In the culture of tunica albuginea-derived fibroblasts, Lin et al showed that pentoxifylline decreased the expression of Smad1/5 proteins and enhanced phosphorylation of the inhibitory Smad 6 protein [47]. Smith et al found that pentoxifylline diminished or stabilized the calcium content in PD plaque in 91.9% cases [48]. In one study, Alizadeh et al. demonstrated that combined therapy significantly improved penile curvature, plaque size, and the recovery rate of erectile dysfunction compared with pentoxifylline or verapamil alone[49].

TOPICAL TREATMENT

TOPICAL VERAPAMIL

Verapamil is one of the calcium channel blockers, which is widely used in cardiology for the treatment of hypertension, atrial fibrillation, and other cardiac rhythm disturbances [50]. Several studies showed that verapamil can inhibit the synthesis and secretion of the extracellular matrix and induces synthesis of procollagenase of fibroblasts. The verapamil also inhibits pro-inflammatory cytokines, TGF-β1, and cellular proliferation of fibroblasts with further depolymerization of actin filaments. Eventually, it leads to alteration of cell shape, apoptosis, and reduction of ECM production in fibrous tissue[51,52]. Despite these useful properties of verapamil, there is no evidence that topical administration can reach high levels in tunica albuginea with enough concentration for clinically meaningful effect[53]. However, in one placebo-controlled pilot study, Fitch et al. showed that topical verapamil significantly improved the penile curvature, decreased the PD plaque size, reduced pain syndrome and increased the quality of erection in 61.1%, 84.7%, 100% and 81.8% cases, respectively at nine-month of treatment[54].

EXTRACORPOREAL SHOCK WAVE THERAPY

Extracorporeal shock wave therapy (ESWT) is a non-invasive method that uses acoustic waves in a pulsatile manner to treat Peyronie's disease. The exact mechanism of action is still not well understood. It is proposed that ESWT works by remodeling the penile plaque, resulting in a local enhancement of macrophage activity through stimulation of inflammatory reaction with subsequent lysis of fibrotic tissue and increasing of angiogenesis around PD plaque. Eventually, these effects may lead to reducing plaque size, diminishing penile deformity, and alleviating pain[55-57].In one study, Chitale et al. demonstrated that there were no significant differences between ESWT and sham therapy in an improvement of penile curvature and pain[58].In another placebo-controlled, randomized study, Hatzichristodoulou et al. showed that in 40% cases, ESWT had led to worsening of penile deviation, and there were not any differences in plaque size between the intervention group and placebo(p=0.33)[59]. In one meta-analysis, Gao et al. demonstrated that ESWT reduces penile plaque size and relieves pain syndrome but not improve penile curvature and sexual function[60].However, Shimpi et al. reported that ESWT improved pain and penile curvature and reduced PD plaque[61]. In another recent study, Di Mauro et al. demonstrated that ESWT decreased plaque size, penile curvature, and pain assessed by visual analogue scale ($p<0.001$). They also showed that ESWT might improve an erectile function measured by IIEF and increase a penile length in erection ($p<0.001$) [62].

MECHANICAL THERAPY

TRACTION DEVICES

The mechanotransduction or gradual extension of tissue by traction in patients with PD increases the activity of collagenases and metalloproteinases. Ultimately, it leads to the formation of new connective tissue by cellular proliferation and softening of PD plaque. One of the chief complaints in patients with PD is the loss of penile length; thus, penile traction therapy is a reasonable treatment option [63,64].In

Table 1. Intralesional therapies for treatment of Peyronie's disease

Intralesional therapy	Mechanisms of action	Side effects
Interferon alpha 2b	Antifibrotic activity, Immune system stimulation	Flu-like symptoms, sinusitis, Penile swelling
Hyaluronic acid	Anti-inflammatory properties Antioxidant activity	Small ecchymoses
Collagenase, Clostridium, Histolyticum	Degradation of collagen	Penile bruishing, Penile swelling

one study, Ziegelmann et al. demonstrated that penile traction therapy with the RestoreX device for 30 to 90 minutes per day significantly improved the penile curvature and length in men with PD without serious adverse effects[65]. Moncada et al. demonstrated that the use of the Penimaster PRO significantly reduced the penile curvature in ranging from 200 to 500, with 51.4% improvement compared with a non-intervention group[66].In a nonrandomized prospective controlled trial, Martínez-Salamanca et al. showed that penile traction therapy decreased the penile curvature from 33° at baseline to 15° at six months and 13° at nine months of treatment in patients with PD in the acute phase [67](Table 1). Recently, Ibrahim et al. reported that penile traction therapy combined with pentoxifylline or colchicine significantly decreased penile curvature and PD plaque size[68].

VACUUM DEVICES

Vacuum therapy is a non-invasive method of treatment Peyronie's disease with a similar mechanism of action as for penile traction therapy. It based on the reordering of disordered collagen in the inelastic scar through mechanotransduction with the further active remodeling of tissue [69,70].In a rat model, Li et al. showed that the vacuum erection device can reduce Peyronie's-like plaque size and decreased the expression of TGF-β1 and SMAD2/3 proteins [71].In one study, Raheem et al. demonstrated that the vacuum pump therapy reduced penile curvature by 5-25 degrees and may reduce the number of patients going on to surgery [72]. In another randomized,open-label study, Ralph et al. showed that vacuum-pump therapy both alone or in combination with collagenase Clostridium histolyticum (CCH) treatment might improve the penile curvature[73].

INTRALESIONAL TREATMENT

INTRALESIONAL INTERFERON A2B

Interferon α-2b (IFN-α-2b) have been shown to decrease fibroblast proliferation and produce collagen and other ECM proteins, through increasing the levels of collagenase and inhibiting of metalloproteinases, which inhibit collagenase[74]. Under these properties of IFN-α-2b, it is widely applied in the treatment of hypertrophic scars, liver fibrosis, and other fibrotic conditions related to fibroblast dysregulation[75,76].In one study, Stewart et al. showed that intralesional interferon-α2b provided greater than 20% reduction in penile curvature with a total response rate of 91% independently from the localization of PD-plaque[77]. Trost et al. reported similar findings after intralesional interferon-α2b injections in patients with penile curvature less than 30-degree and without impacting penile vascular parameters[78]. In a prospective study, Sokhal et al. reported a significant improvement of plaque volume and penile curvature after treatment with intralesional IFN-α2b[79].

HYALURONIC ACID (HA)

Hyaluronic acid (HA) plays an essential role in the regeneration of the cellular components during the wound healing process. Hyaluronan (HA) is also active in the ECM reorganization, cytokine, and growth factor adhesion as well as recruitment of matrix metalloproteinases (MMPs). Hyaluronic acid (HA) exists in two forms: high molecular weight hyaluronan with anti-inflammatory properties and HA fragments with low molecular weight, which promote inflammation. The matrix enriched with high molecular weight hyaluronan acts as an alternate protective target for reactive oxygen and nitrogen species (ROS/RNS) generated during inflammation and limits penetration of those species to the cell membrane[80].

Based on these data, it was proposed the intralesional administration of HA with promising effects in improvement of Peyronie's disease.In one prospective,double-blinded, randomized study, Favilla et demonstrated that treatment with intralesional hyaluronic acid (HA) had greater efficacy in terms of penile curvature compared with intralesional verapamil. Gennaro et al. reported that intralesional HA significantly reduced the plaque volume and penile curvature compared with the control group.In a prospective, interventional, multicenter pilot study, Zucchi et al. showed that intralesional administration of hyaluronic acid (HA) significantly improved plaque size, penile curvature, and overall sexual satisfaction.

INTRALESIONAL COLLAGENASE CLOSTRIDIUM HISTOLYTICUM (CCH)

The proteolytic properties of collagenase Clostridium histolyticum (CCH) isolated from bacteria C.histolyticum were first described in the early 1950s. Gelbard is considered the first researcher who used the collagenase Clostridium histolyticum for the treatment of Peyronie's

disease in the 1980s. In IMPRESS (Investigation for Maximal Peyronie's Reduction Efficacy and Safety Studies) I and II clinical trials, Gelbard et al. demonstrated that men treated with collagenase Clostridium histolyticum (CCH) had a mean 34% improvement in penile curvature compared with a mean 18.2% improvement in the placebo group. These findings in further allowed to establish the current FDA-approved intralesional collagenase plus modeling protocol for stable PD.In one study, Yang et al. showed that treatment with collagenase Clostridium histolyticum (CCH) reduced penile curvature on 15.4 degrees compared with baseline but without significant changes in penile pain. Nguyen et al. conducted a retrospective analysis of patients in acute phase Peyronie's disease treated with CCH and found that there was no significant difference in final change in curvature 16.7° vs. 15.6° between the acute and stable phases of PD, respectively. The results of IMPRESS I and II trials are replicated in some other studies. In the multi-institutional analysis, Hellstrom et al. showed that the 502 patients who completed 4 or more cycles curvature improved from a mean of 49.7 degrees before to 32.7 degrees after treatment, a 33% improvement from baseline ($p < 0.0001$) with a low rate of complications. The treatment is effective in atypical cases of Peyronie's disease. In prospective study, Alom et al. demonstrated that after treatment with collagenase Clostridium histolyticum (CCH), ventral and lateral curvatures experienced greater relative improvements in curvature compared to dorsal (ventral 29.5 degrees [49%], lateral 11.4 [38%], dorsal 15.0 [25%], respectively). In one retrospective analysis of 65 patients with PD, Cocci et al. demonstrated that CCH improved penile curvature (PC) on 15^0-20^0 degree compared with baseline regardless of the form of penile deformity (ventral, hourglass and shortening) without significant differences between groups. Fernández-Pascual et al. showed that combination of percutaneous needle tunneling (PNT) and CCH significantly improved the penile curvature compared with CCH alone 36.2 ± 12.5% vs. 28.1 ± 14.5 %, respectively ($p=0.001$). However, the rate of success of CCH therapy may be associated with a degree of severity of plaque calcification. Wymer et al. showed that the patients with a noncalcified plaque and penile curvature $\geq 60^0$ had a good response on treatment with CCH compared with moderate or severe (>1 cm) calcination.Despite the enormous clinical effect of collagenase Clostridium Histolyticum (CCH) in improving penile curvature, it is impossible not to take into account possible serious complications that may require surgical intervention. In one study, Beilan et al. reported that of the 102 patients with Peyronie's disease, 12(11,8%) had a hematoma and 5(4,9%) penile fracture after treatment with CCH. Using the modified injection procedure, termed the «fan» technique, Amighi et al. demonstrated the absence of corporeal ruptures and minimal percentage of hematomas, but bruising or swelling was seen in 54.6% and 27.0% of cases, respectively. Intralesional therapies are summarized in table 1.

CONCLUSIONS

PD remains a challenge for urologists. Evidence suggests that, for oral therapy, a combination of drugs produces better results but current evidences from AUA and EAU guidelines are weak. Although on the improvement of pain syndrome, extracorporeal shock wave therapy is still unproven and necessary more RCT. Clostridium histolyticum(CCH) is the only FDA-approved treatment option available. Penile traction and vacuum therapy may improve penile curvature.

REFERENCES

1. Garaffa G., Trost L.W., Serefoglu E.C. et al. Understanding the course of Peyronie's disease. Int J Clin Pract. 2013 ;67(8):781-8. doi: 10.1111/ijcp.12129.
2. Aliperti L.A. , Mehta A. Peyronie's Disease: Intralesional Therapy and Surgical Intervention Curr Urol Rep. 2016 ;17(9):60. doi:10.1007/s11934-016-0622-2.
3. De Young L.X., Bella A.J., O'Gorman D.B. et al Protein biomarker analysis of primary Peyronie's disease cells. J Sex Med 2010;7:99–106. DOI: 10.1111/j.1743-6109.2009.01556.x
4. Langston J.P., Carson I.I.I.C.C. Peyronie's disease: Review and recent advances. Maturitas 2014. http://dx.doi.org/10.1016/j.ma...
5. Levine L.A., Larsen S.M. Surgical correction of persistent Peyronie's disease following collagenase clostridium histolyticum treatment. J Sex Med. 2015 ;12(1):259-64. doi: 10.1111/jsm.12721.
6. Chung E., Ralph D., Kagioglu A. et al. Evidence-Based Management Guidelines on Peyronie's Disease. J Sex Med. 2016;13:905-923. doi: 10.1016/j.jsxm.2016.04.062.
7. Carson C.C., Levine L.A. Outcomes of surgical treatment of Peyronie's disease. BJU Int. 2014;113(5):704-13. doi: 10.1111/bju.12565.
8. Levine L.A., Larsen S.M. Surgery for Peyronie's disease. Asian J Androl. 2013;15(1):27-34. doi:10.1038 /aja. 2012.92.
9. Twidwell J., Levine L. Topical treatment for acute phase Peyronie's disease utilizing a new gel, H-100: a randomized, prospective, placebo-controlled pilot study. Int J Impot Res. 2016 ;28(2):41-5. doi:10.1038 / ijir.2015.22.
10. Nehra A., Alterowitz R., Culkin D.J. et al. Peyronie's Disease: AUA Guideline. The Journal of Urology® .2015. doi: 10.1016/j.juro.2015.05.098 .
11. Scott W.W., Scardino P.L. A new concept in the treatment of Peyronie's disease. South Med J. 1948;41:173-7.
12. Paulis G., Brancato T. Inflammatory mechanisms and oxidative stress in Peyronie's disease: therapeutic «rationale» and related emerging treatment strategies.Inflamm Allergy Drug Targets. 2012;11(1):48-57.
13. Tasanarong A., Kongkham S., Duangchana S. et al Vitamin E ameliorates renal fibrosis by inhibition of TGF-beta/Smad2/3 signaling pathway in UUO mice.J Med Assoc Thai. 2011;94(7):S1-9.
14. Safarinejad M.R., Hosseini S.Y., Kolahi A.A. Comparison of Vitamin E and Propionyl-L-Carnitine, Separately or in Combination, in Patients With Early Chronic Peyronie's Disease: A Double-Blind, Placebo Controlled, Randomized Study. Journal of Urology. 2007;178(4):1398–1403. doi: 10.1016/j.juro.2007.05.162.
15. Paulis G., Brancato T., D'Ascenzo R. et al. Efficacy of vitamin E in the conservative treatment of Peyronie's disease: legend or reality? A controlled study of 70 cases. Andrology. 2013;1(1):120–128.
16. Yan P., Tang H., Chen X. et al.Tamoxifen attenuates dialysate-induced peritoneal fibrosis by inhibiting GSK-3β/β-catenin axis activation.Biosci Rep. 2018;38(6). doi: 10.1042/BSR20180240.

17. Kim D., Lee A.S., Jung Y.J. et al.Tamoxifen ameliorates renal tubulointerstitial fibrosis by modulation of estrogen receptor α-mediated transforming growth factor-β1/Smad signaling pathway.Nephrol Dial Transplant. 2014 ;29(11):2043-53. doi: 10.1093/ndt/gfu240.
18. El-Shazly M., Hodhod A., Selim M. et al.The Effectiveness of Tamoxifen in the Prevention of Recurrent Urethral Strictures Following Internal Urethrotomy.Urol Int. 2018;101(4):472-477. doi: 10.1159/000493173.
19. Ilg M.M. et al. Antifibrotic Synergy Between Phosphodiesterase Type 5 Inhibitors and Selective Oestrogen Receptor Modulators in Peyronie's Disease Models. Eur Urol. 2018. https://doi.org/10.1016/j.euru...
20. Ralph D.J., Brooks M.D., Bottazzo G.F. et al. The treatment of Peyronie's disease with tamoxifen. Br J Urol. 1992;70:648–651.
21. Park T.Y., Jeong H.G., Park J.J. et al. The Efficacy of Medical Treatment of Peyronie's Disease: Potassium Para-Aminobenzoate Monotherapy vs. Combination Therapy with Tamoxifen, L-Carnitine, and Phosphodiesterase Type 5 Inhibitor. World J Mens Health. 2016;34(1):40-6. doi: 10.5534/wjmh.2016.34.1.40.
22. Teloken C., Rhoden E.L., Grazziotin T.M. et al. Tamoxifen versus placebo in the treatment of Peyronie's disease. J Urol. 1999;162:2003–5.
23. Wibowo E., Pollock P.A., Hollis N. et al. Tamoxifen in men: a review of adverse events.Andrology. 2016;4(5):776-88. doi: 10.1111/andr.12197.
24. Zarafonetis C.J., Horrax T.M. Treatment of Peyronie's disease with potassium para-aminobenzoate (potaba). J. Urol. 1959;81: 770–772.
25. Hauck E.W., Diemer T., Schmelz H.U. et al. Critical analysis of nonsurgical treatment of Peyronie's disease. Eur Urol. 2006;49:987–97.
26. Weidner W., Hauck E.W., Schnitker J. Potassium para-aminobenzoate (POTABA) in the treatment of Peyronie's disease: a prospective, placebo-controlled, randomized study. Eur Urol 2005: 47(4):530-535.
27. Roy J., Carrier S. Acute hepatitis associated with treatment of Peyronie's disease with potassium para-aminobenzoate (Potaba).J Sex Med. 2008;5(12):2967-9. doi: 10.1111/j.1743-6109.2008.00918.x.
28. Gonzalez-Cadavid N.F., Rajfer J. Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy. Nat. Rev. Urol. 2010;7:215–221.
29. Iacono F., Prezioso D., Somma P. et al Histopathologically proven prevention of post-prostatectomy cavernosal fibrosis with sildenafil. Urol Int. 2008;80(3):249-52. doi: 10.1159/000127335.
30. Ferrini M.G., Kovanecz I., Nolazco E. et al. Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. BJU Int. 2006;97: 625–633.
31. Li W.J., Wang H., Zhou J., Li B. et al. P144, A TGF-β1 antagonist peptide, synergizes with sildenafil and enhances erectile response via amelioration of cavernosal fibrosis in diabetic rats.J Sex Med. 2013;10(12):2942-51. doi: 10.1111/jsm.12325.
32. Gonzalez-Cadavid N.F., Rajfer J. The two phases of the clinical validation of preclinical translational mechanistic research on PDE5 inhibitors since Viagra's advent. A personal perspective.Int J Impot Res. 2019;31(2):57-60. doi: 10.1038/s41443-018-0076-9.
33. Ozturk U., Yesil S., Goktug H.N. et al. Effects of sildenafil treatment on patients with Peyronie's disease and erectile dysfunction.Ir J Med Sci. 2014;183(3):449-53. doi: 10.1007/s11845-013-1036-5.
34. Palmieri A., Imbimbo C., Creta M. et al Tadalafil once daily and extracorporeal shock wave therapy in the management of patients with Peyronie's disease and erectile dysfunction: results from a prospective randomized trial.Int J Androl. 2012;35(2):190-5. doi: 10.1111/j.1365-2605.2011.01226.x.
35. Cocci A., Cito G., Urzì D. et al. Sildenafil 25 mg ODT + collagenase Clostridium hystoliticum vs collagenase Clostridium hystoliticum alone for the management of Peyronie's disease: A matched-pair comparison analysis. J Sex Med. 2018;15:1472-1477. doi: 10.1016/j.jsxm.2018.08.012.
36. Tan R.B., Sangkum P., Mitchell G.C. et al Update on medical management of Peyronie's disease.Curr Urol Rep. 2014;15(6):415. doi: 10.1007/s11934-014-0415-4.
37. Prieto Castro R.M., Leva Vallejo M.E., Regueiro Lopez J.C. et al. Combined treatment with vitamin E and colchicine in the early stages of Peyronie's disease. BJU Int. 2003;91:522–4.
38. Akman T., Sanli O., Uluocak N. et al The most commonly altered type of Peyronie's disease deformity under oral colchicine treatment is lateral curvature that mostly shifts to the dorsal side. Andrologia. 2011;43(1):28-33. doi: 10.1111/j.1439-0272.2009.01004.x.
39. Toscano L.Jr., Rezende M.V., Mello L.F. et al prospective, randomized, single – blind study comparing intraplaque injection of thiocolchicine and verapamil in Peyronie's Disease: a pilot study.Int Braz J Urol. 2016 ;42(5):1005-1009. doi: 10.1590/S1677-5538.IBJU.2015.0598.
40. Adeva-Andany M.M., Calvo-Castro I., Fernández-Fernández C. et al. Significance of l-carnitine for human health.IUBMB Life. 2017;69(8):578-594. doi: 10.1002/iub.1646.
41. Cavallini G., Biagiotti G. Oral propionyl-L-carnitine and intraplaque verapamil in the therapy of advanced and resistant Peyronie's disease. BJU Int. 2002;89:895.
42. Safarinejad M.R., Hosseini S. Comparison of vitamin E and propionyl-L-carnitine, separately or in combination, in patients with early chronic Peyronie's disease: a double-blind, placebo controlled, randomized study. J Urol. 2007;178:1398–403.
43. Park T.Y., Jeong H.G., Park J.J. et al.The Efficacy of Medical Treatment of Peyronie's Disease: Potassium Para-Aminobenzoate Monotherapy vs. Combination Therapy with Tamoxifen, L-Carnitine, and Phosphodiesterase Type 5 Inhibitor.World J Mens Health. 2016;34(1):40-6. doi: 10.5534/wjmh.2016.34.1.40.
44. Ciociola F., Colpi G.M. Peyronie's disease: a «triple oxygenant therapy». Arch Ital Urol Androl. 2013 ;85(1):36-40. doi: 10.4081/aiua.2013.1.36.
45. Safarinejad M.R., Asgari M. A double-blind placebo-controlled study of the efficacy and safety of pentoxyfylline in early chronic Peyronie's disease. BJU Int. 2010;106(2):240–8.
46. Shindel A.W., Lin G. Pentoxifylline attenuates transforming growth factor- beta-1-stimulated collagen deposition and elastogenesis in human tunica albuginea-derived fibroblasts part 1: impact on extracellular matrix. J Sex Med. 2010;7(6):2077–85.
47. Lin G., Shindel A.W., Banie L. et al. Pentoxifylline attenuates transforming growth factor-beta1-stimulated elastogenesis in human tunica albuginea-derived fibroblasts part 2: Interference in a TGF-beta1/ Smad-dependent mechanism and downregulation of AAT1. J Sex Med 2010;7:1787-97
48. Smith, J. F. et al. Pentoxifylline treatment and penile calcifications in men with Peyronie's disease. Asian J. Androl. 2011;13: 322–325.
49. Alizadeh M., Karimi F., Fallah M.R. Evaluation of verapamil efficacy in Peyronie's disease comparing with pentoxifylline.Glob J Health Sci. 2014;6(7):23-30. doi: 10.5539/gjhs.v6n7p23.
50. Wang X., Lin Y., Chen L. et al.Treatment with verapamil for restoration of sinus rhythm in atrial fibrillation with rapid ventricular response: A case report.Medicine (Baltimore). 2019;98(23):e15892. doi: 10.1097/MD.0000000000015892.
51. Wang R., Mao Y., Zhang Z. et al.Role of verapamil in preventing and treating hypertrophic scars and keloids.Int Wound J. 2016 ;13(4):461-8. doi: 10.1111/iwj.12455.
52. Boggio R.F., Boggio L.F., Galvão B.L. et al Topical verapamil as a scar modulator. Aesthetic Plast Surg. 2014;38:968–75.
53. Martin D.J., Badwan K., Parker M. et al Transdermal application of verapamil gel to the penile shaft fails to infiltrate the tunica albuginea. J. Urol. 2002;168: 2483–2485.

54. Fitch W.P. 3rd, Easterling W.J., Talbert R.L. et al. Topical verapamil HCl, topical trifluoperazine, and topical magnesium sulfate for the treatment of Peyronie's disease — a placebo-controlled pilot study. J. Sex. Med. 2007; 4: 477–484.
55. Krieger J.R., Rizk P.J., Kohn T.P. et al. A Shockwave Therapy in Sex Med Rev. the Treatment of Peyronie's Disease. 2019;7(3):499-507. doi: 10.1016/j.sxmr.2019.02.001.
56. Hatzimouratidis K., Eardley I., Giuliano F. et al.EAU guidelines on penile curvature. Eur Urol. 2012;62(3):543-52. doi: 10.1016/j.eururo.2012.05.040.
57. Husain J., Lynn N.N., Jones D.K. et al. Extracorporeal shock wave therapy in the management of Peyronie's disease: initial experience. BJU Int. 2000;86:466–468.
58. Chitale S., Morsey M., Swift L. et al.Limited shock wave therapy vs sham treatment in men with Peyronie's disease: results of a prospective randomized controlled double-blind trial.BJU Int. 2010;106(9):1352-6. doi: 10.1111/j.1464-410X.2010.09331.x.
59. Hatzichristodoulou G. et al. Extracorporeal shock wave therapy in Peyronie's disease: results of a placebo-controlled, prospective, randomized, single-blind study. J. Sex. Med. 2013;10: 2815–2821.
60. Gao L., Qian S., Tang Z. et al. A meta-analysis of extracorporeal shock wave therapy for Peyronie's disease.Int J Impot Res. 2016;28(5):161-6. doi: 10.1038/ijir.2016.24.
61. Shimpi R.K., Jain R.J. Role of extracorporeal shock wave therapy in management of Peyronie's disease: A preliminary report.Urol Ann. 2016;8(4):409-417. doi: 10.4103/0974-7796.192100.
62. Di Mauro M., Russo G.I., Della Camera P.A. et al. Extracorporeal Shock Wave Therapy in Peyronie's Disease: Clinical Efficacy and Safety from a Single-Arm Observational Study.World J Mens Health. 2019;37(3):339-346. doi: 10.5534/wjmh.180100.
63. Chung E., De Young L., Solomon M., Brock G.B. Peyronie's disease and mechanotransduction: an in vitro analysis of the cellular changes to Peyronie's disease in a cell-culture strain system. J Sex Med. 2013; 10(5):1259-67. doi: 10.1111/jsm.12082.
64. Schaeffer A.J., Burnett A.L. Nonsurgical interventions for Peyronie disease: 2011 update. J Androl. 2012 ;33(1):3-14. doi: 10.2164/jandrol.110.010561.
65. Ziegelmann M., Savage J., Toussi A., Alom M. et al. Outcomes of a Novel Penile Traction Device in Men with Peyronie's Disease: A Randomized, Single-Blind, Controlled Trial.J Urol. 2019. doi: 10.1097/JU.0000000000000245.
66. Moncada I., Krishnappa P., Romero J. et al. Penile traction therapy with the new device 'Penimaster PRO' is effective and safe in the stable phase of Peyronie's disease: a controlled multicentre study.BJU Int. 2019 ;123(4):694-702. doi: 10.1111/bju.14602.
67. Martínez-Salamanca J.I., Egui A., Moncada I. et al. Acute phase Peyronie's disease management with traction device: a nonrandomized prospective controlled trial with ultrasound correlation. J Sex Med. 2014 ;11(2):506-15. doi: 10.1111/jsm.12400.
68. Ibrahim A., Gazzard L., Alharbi M. et al. Evaluation of Oral Pentoxifylline, Colchicine, and Penile Traction for the Management of Peyronie's Disease.Sex Med. 2019. doi: 10.1016/j.esxm.2019.07.003.
69. Lin H., Liu C., Wang R. Effect of Penile Traction and Vacuum Erectile Device for Peyronie's Disease in an Animal Model.J Sex Med. 2017;14(10):1270-1276. doi: 10.1016/j.jsxm.2017.08.011.
70. Chung E., De Young L., Solomon M. et al Peyronie's disease and mechanotransduction: an in vitro analysis of the cellular changes to Peyronie's disease in a cell-culture strain system.J Sex Med. 2013;10(5):1259-67. doi: 10.1111/jsm.12082.
71. Li J., Wang S., Qin F. et al.Reduction in Peyronie's-like plaque size using a vacuum erection device in a rat model of Peyronie's disease via the TGF-β/SMAD signalling pathway.Andrologia. 2018;50(7):e13051. doi: 10.1111/and.13051.
72. Raheem A.A., Garaffa G., Raheem T.A. et al.The role of vacuum pump therapy to mechanically straighten the penis in Peyronie's disease. BJU Int. 2010;106(8):1178-80. doi:10.1111/j.1464-410X.2010.09365.x.
73. Ralph D.J., Abdel Raheem A., Liu G. Treatment of Peyronie's Disease With Collagenase Clostridium histolyticum and Vacuum Therapy: A Randomized, Open-Label Pilot Study. J Sex Med. 2017;14(11):1430-1437. doi: 10.1016/j.jsxm.2017.08.015.
74. Chong W., Tan R.B. Injectable therapy for Peyronie's disease.Transl Androl Urol. 2016;5(3):310-7. doi: 10.21037/tau.2016.03.15.
75. Berman B., Maderal A., Raphael B. Keloids and Hypertrophic Scars: Pathophysiology, Classification, and Treatment.Dermatol Surg. 2017;43(1):S3-S18. doi: 10.1097/DSS.0000000000000819.
76. Tamai H., Shingaki N., Mori Y. et al. Low-Dose Pegylated Interferon α-2b Plus Ribavirin for Elderly and/or Cirrhotic Patients with Genotype 2 Hepatitis C Virus.Gut Liver. 2016 ;10(4):617-23. doi: 10.5009/gnl15193.
77. Stewart C.A., Yafi F.A., Knoedler M.M. et al. Intralesional Injection of Interferon-α2b Improves Penile Curvature in Men with Peyronie's Disease Independent of Plaque Location.J Urol. 2015;194(6):1704-7. doi: 10.1016/j.juro.2015.06.096.
78. Trost L.W., Ates E., Powers M. et al.Outcomes of intralesional interferon-α2B for the treatment of Peyronie disease.J Urol. 2013 ;190(6):2194-9. doi: 10.1016/j.juro.2013.05.022.
79. Sokhal A.K., Jain N.K., Jhanwar A. et al. Prospective study to evaluate the clinical outcome of intralesional interferon-α2b in the management of Peyronie's disease.Urol Ann. 2018;10(2):154-158. doi: 10.4103/UA.UA_65_17.
80. Isık S., Taşkapılıoğlu M.Ö., Atalay F.O. et al Effects of cross-linked high-molecular-weight hyaluronic acid on epidural fibrosis: experimental study.J Neurosurg Spine. 2015;22(1):94-100. doi: 10.3171/2014.10.SPINE131147.

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REVIEW ARTICLE

DIAGNOSTIC AND PREDICTIVE VALUE OF RIGHT HEART CATHETERIZATION-DERIVED MEASUREMENTS IN PULMONARY HYPERTENSION

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ABSTRACT

Right heart catheterization is a unique tool not only in the diagnosis but also in the management of patients with a wide range of cardiovascular diseases. The technique dates back to the 18th century, but the biggest advances were made in the 20th century. This review focuses on pulmonary hypertension for which right heart catheterization remains the diagnostic gold standard. Right heart catheterization-derived parameters help classify pulmonary hypertension into several subgroups, assess risk of adverse events or mortality and make therapeutic decisions. According to the European Society of Cardiology guidelines pulmonary hypertension (PH) is defined as an increase in mean pulmonary artery pressure (PAPm) > 25 mmHg, whereas a distinction between pre- and post-capillary PH is made based on levels of pulmonary artery wedge pressure (PAWP). Moreover, right atrial pressure (RAP), cardiac index (CI) and mixed venous oxygen saturation (SvO_2) are the only parameters recommended to assess prognosis and only in patients with pulmonary arterial hypertension (PAH). Patients with RAP > 14 mmHg, CI < 2.0 l/min/m^2 and SvO_2 < 60% are at high (> 10%) risk of death within the next year.

The purpose of this paper is to show that RHC-derived parameters can be used on a considerably larger scale than currently recommended. Several prognostic parameters, with specific thresholds have been identified for each subtype of pulmonary hypertension and can be helpful in everyday practice for treatment of PH.

KEY WORDS: pulmonary hypertension, right heart catheterization, pulmonary artery hypertension

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INTRODUCTION

Right heart catheterization (RHC) is a medical procedure that has been used for decades to evaluate heart function and to diagnose a wide spectrum of cardiovascular diseases. The history of catheterization began in 1711 when Stephen Hale performed the first catheterization of equine vessels using brass pipes to measure blood pressure and cardiac output. In 1844, physiologist Claude Bernard was the first to catheterize right and left ventricle in a horse using glass tubes. The 20th century witnessed rapid developments in cardiac catheterization beginning with self-cannulation using a urethral catheter by dr. Forssmann in 1929, through advances in the procedure made by dr. André Cournand and dr. Dickinson Richards in 1940, followed by the Nobel Prize in 1956 for dr. Cournand, dr. Richards and dr. Werner, and finally the development of a 'Swan–Ganz' catheter in 1970 by Jeremy Swan and William Ganz, from Cedars-Sinai Medical Center [1].

For decades RHC has been used in many clinical situations, to monitor patients in the Intensive Care Units and assess cardiac output, to perform pulmonary angiography, to detect and evaluate shunts between the systemic and pulmonary circulation, to diagnose pulmonary hypertension and examine patients before cardiac transplantation, to assess the heart function in valvular or congenital diseases, to distinguish between restrictive cardiomyopathy and constrictive pericarditis or to provide an access for endomyocardial biopsy heart defect closure (since 1976) or balloon pulmonary angioplasty [2]. RHC is part of a mandatory and comprehensive evaluation of PH patients. The RHC findings are used not only to confirm the diagnosis but also to predict survival or mortality in patients with specific PH subtypes.

THE AIM

The aim of this paper is to establish the cut-off values of several pulmonary hemodynamic parameters which can be useful in everyday practice. According to the current guidelines only some RHC parameters (RAP, CI and SvO_2) are relevant for estimating prognosis and only in PAH patients. PAH is a relatively rare disease, according to the Polish Registry of PH the average prevalence is 30.8 per million adults [3]. This entails difficulties in selecting a sufficiently large sample size to perform a reliable statistical analysis, nevertheless numerous studies have demonstrated the prognostic value of various RHC-derived parameters, not only in PAH, but also in other PH subgroups. Patients with

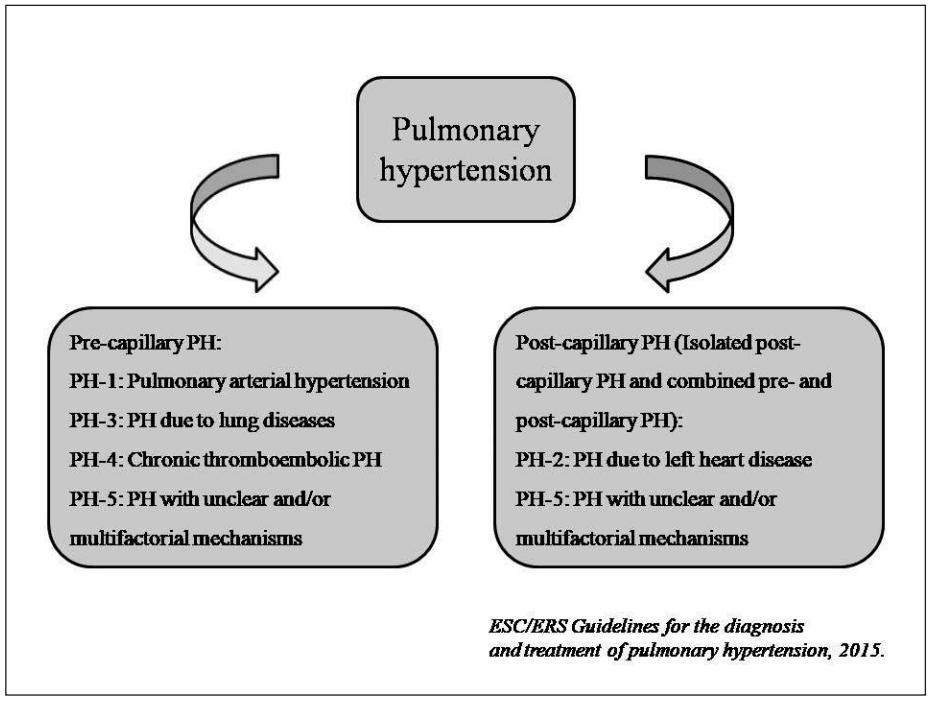

Fig. 1. Haemodynamic classification of pulmonary hypertension.

PH are a heterogeneous group of individuals, and although the current guidelines are quite narrowly applicable to RHC for prognosis, in our opinion the data presented below will show that RHC is essential for therapeutic decision-making and provides important prognostic information for each patient with PH.

REVIEW AND DISCUSSION

RHC remains the gold standard in the diagnosis of pulmonary hypertension (PH), which is defined as an increase in mean pulmonary artery pressure (PAPm) ≥ 25 mmHg, and further classified into pre-capillary and post-capillary PH (with two subgroups: isolated post-capillary PH and combined post-capillary and pre-capillary PH) (Fig. 1, Table 1) [4].

RHC is usually performed through puncture of the subclavian or jugular vein under local anaesthetic. Optimally, a balloon-tipped catheter is inserted under fluoroscopic guidance through a central vein, right atrium and right ventricle to the pulmonary artery.

It is recommended to measure several hemodynamic parameters during RHC i.e. right atrial pressure (RAP), right ventricular pressure (RVP), pulmonary artery pressure (PAP), pulmonary artery wedge pressure (PAWP) and cardiac output (CO). The measurements are then used to calculate cardiac index (CI), diastolic pressure gradient (DPG), transpulmonary pressure gradient (TPG) or pulmonary vascular resistance (PVR) (Table 2). In addition, blood is drawn from intracardiac chambers, great vessels and pulmonary artery to detect and quantify shunts between the systemic and pulmonary circulation, to measure mixed venous oxygen saturation (SvO_2) and to calculate cardiac output using the Fick method [5].

During RHC, when the diagnosis of pulmonary hypertension is confirmed, pulmonary vasoreactivity (vasodilator) testing should be performed for Group 1 PH and Group 2 PH, to identify patients with a reversible form of PH. Current guidelines recognize the use of four medications for assessment of pulmonary vasoreactivity, i.e. inhaled nitric oxide, inhaled iloprost or epoprostenol and intravenous adenosine (Table 3). In patients with pulmonary arterial hypertension (PAH, PH-1), a positive acute response (responders) is defined as a reduction of the PAPm ≥10 mmHg to reach an absolute value of PAPm ≤ 40 mmHg with an increased or unchanged CO [4]. A decrease of TPG below 15 mmHg, during vasoreactivity testing with sodium nitroprusside or nitric oxide, is mandatory for PH-2 patients with severe heart failure to be eligible for a heart transplant [6].

The current cut-off value of PAPm for a diagnosis of PH was established in 1973, but available data suggests that normal resting PAPm is 14.0 ± 3.3 mmHg, therefore, new definitions of PH and PH in left heart disease (LHD) were proposed during the 6th World Symposium on Pulmonary Hypertension in Nice in 2018 (Table 4) [7]

Regarding the new PH definition evidence supporting its usefulness has been available in the literature for years. Elevated PAPm ≥ 20 mmHg is associated with a significantly worse prognosis in patients with connective tissue disease (log-rank test p = 0.005) [8] and idiopathic pulmonary fibrosis (IPF) (log-rank test p = 0.001), compared with PAPm < 20 mmHg [9]. Patients with PAPm = 19–24 mmHg show a 23% (95% CI [12–36%], P < 0.0001) and a 7% (95% CI [1–12%], P = 0.0149) increase of the adjusted hazard for mortality and hospitalization, respectively, compared to patients with PAPm ≤ 18 mmHg [10].

PULMONARY ARTERIAL HYPERTENSION (PH-1)

According to the current PH guidelines the RHC-derived measurements are one of the variables used to estimate 1-year mortality in patients with PAH. RAP, CI and SvO_2

$$PCa = \frac{SV}{PP}$$

PCa (PAC) = pulmonary vascular compliance (pulmonary arterial capacitance)
SV = stroke volume
PP = pulse pressure

Fig. 2. Formula of the pulmonary vascular compliance.

$$Rup = \frac{100 \times (mPpa - Poccl)}{(mPpa - Ppao)}$$

Ppao = pulmonary artery occluded pressure,
mPpa = mean pulmonary artery pressure,
Poccl = occlusion pressure

Fig. 3. Formula of the upstream resistance.

are used to classify patients into one of the three groups according to mortality risk: low risk < 5% (RAP < 8 mmHg; CI ≥ 2.5 l/min/m^2; SvO_2 > 65%), intermediate risk 5 – 10% (RAP 8 –14 mmHg; CI 2.0 – 2.4 l/min/m^2; SvO_2 60 – 65%) and high risk > 10% (RAP > 14 mmHg; CI < 2.0 l/min/m^2; SvO_2 < 60%) [4]. The French PAH registry shows that the initially measured hemodynamic parameters have no prognostic value, but RAP and SVI measured in patients receiving treatment (PDE-5 I, ERB, prostanoids) are predictive of poor prognosis. The risk of death or lung transplantation increases by 5% per each 1 mmHg of RAP increase (HR = 1.05; 95% confidence interval: 1.02 – 1.09; p <0.001) and by 28% per each 10 mml/m^2 of SVI decrease (HR = 1.28; 95% confidence interval: 1.11 – 1.49; p <0.001). The optimal cut-off value is 9 mm Hg for RAP (AUC = 0.62; 95% confidence interval: 0.57 – 0.67; p<0.01) and 38 ml/m^2 for SVI (AUC = 0.68; 95% confidence interval: 0.64–0.72; p< 0.01). It is noteworthy that this negative impact of low SVI is seen even among those considered to be at lower risk (e.g. low NYHA class or CI ≥2.5 l/min/m^2) [11]. In 2007, a study carried out in a small group of patients (64 vs. 981 patients) demonstrated that SVI ≤ 25 ml/m^2 was a negative prognostic factor (log-rank test, p=0.010) [12]. Another parameter which is significantly associated with poor outcomes is increasing DPG (Hazard ratio [HR] 1.29 per 10 mmHg increase) [13].

In the REVEAL registry PVR was included as one of the risk factors in patients with PAH. PVR < 5 Wood units (WU) was associated with better survival outcomes. Previously diagnosed patients with a PVR < 5 WU had a 5-year survival of 73.9% ± 2.3%, compared with 66.0% ± 1.8% in patients with a PVR of 5 to 10 WU. Newly diagnosed patients with a PVR < 5 WU had a 5-year survival of 70.1% ± 4.9%, compared with 57.3% ± 3.2% in patients with a PVR of 5 to 10 WU [14]. The REVEAL Registry Risk Score Calculator has been developed to predict 1-year survival in patients newly diagnosed with PAH. The risk calculator is based on 15 variables, including echocardiographic findings, blood test results, clinical characteristics and classification, as well as such RHC-derived parameters as RAPm > 20 mmHg and PVR > 32 WU [15].

In patients with systemic sclerosis-related pulmonary arterial hypertension (SSc-PAH) several hemodynamic measurements are also predictors of increased mortality. A study by Mukherjee et al. demonstrated that raised RAPm, raised PAPm and low CI were related to survival. The Cox multivariate regression analysis revealed that RAPm was the strongest independent factor for a poor outcome, with hazard ratio 20.7 and a p value of 0.0001. As there was a relative correlation between RAPm, PAPm and CI, the latter two values could not be considered as independent predictors [16]. In addition, data from the REVEAL study showed that RAPm > 20 mm Hg and PVR > 32 WU were predictors of mortality in the SSc-PAH group [17]. Campo et al. proved that PVR > 7.2 WU (HR = 3.13, 95% confidence interval: 1.50 – 6.52; p < 0.01), SVI < 30 ml (HR = 2.34, 95% confidence interval: 1.11 – 4.96; p = 0.03) and PCa < 1.25 ml/mm Hg (HR = 3.06, 95% confidence interval: 1.41 – 6.65; p < 0.01) were strong predictors of mortality [18].

Patient selection for liver transplantation due to portopulmonary hypertension (PP-PH) requires careful hemodynamic assessment. Studies in patients with PP-PH at Mayo Clinic provided the following results: PAPm > 50 mmHg was associated with 100% cardiopulmonary mortality, whereas values of PAPm 35-50 mmHg and PVR > 250 dynes·s·cm^2 were linked to the mortality rate around 50%. No cardiopulmonary mortality was reported in patients with PAPm <35 mmHg or TPG <15 mmHg [19].

Investigators are still searching for new RHC-derived parameters. One of them is pulmonary vascular compliance (PCa), defined as SV divided by pulse pressure – the difference between systolic pulmonary artery pressure and diastolic pulmonary artery pressure (PAPs – PAPd), which describes elastic properties of the pulmonary arterial system (Fig. 2). In adult patients with PAH-related congenital heart disease, the Kaplan-Meier survival curves show that PCa < 1.04 ml/mmHg is a risk factor for mortality (Log rank: P < 0.001). ROC curve analysis for PCa = 1.04 ml/mmHg shows 87% sensitivity and 64% specificity (AUC = 0.746, 95% confidence interval: 0.657 – 0.836, P < 0.001) [20]. Also in patients with idiopathic PAH (IPAH), PCa has been recognized as an indicator of mortality in univariate analysis (HR=17.0 per ml/mmHg decrease; 95% confidence interval: 13.0 – 22.0; p < 0.0001). Patients with PCa = 0.40-0.81 ml/mm Hg have a 61% 4-year mortality [21].

Surgical correction of congenital heart disease causing Eisenmenger syndrome is considered useful in patients with PVRi < 4 WU.m^2 and PVR < 2.3 WU. Surgery is not the

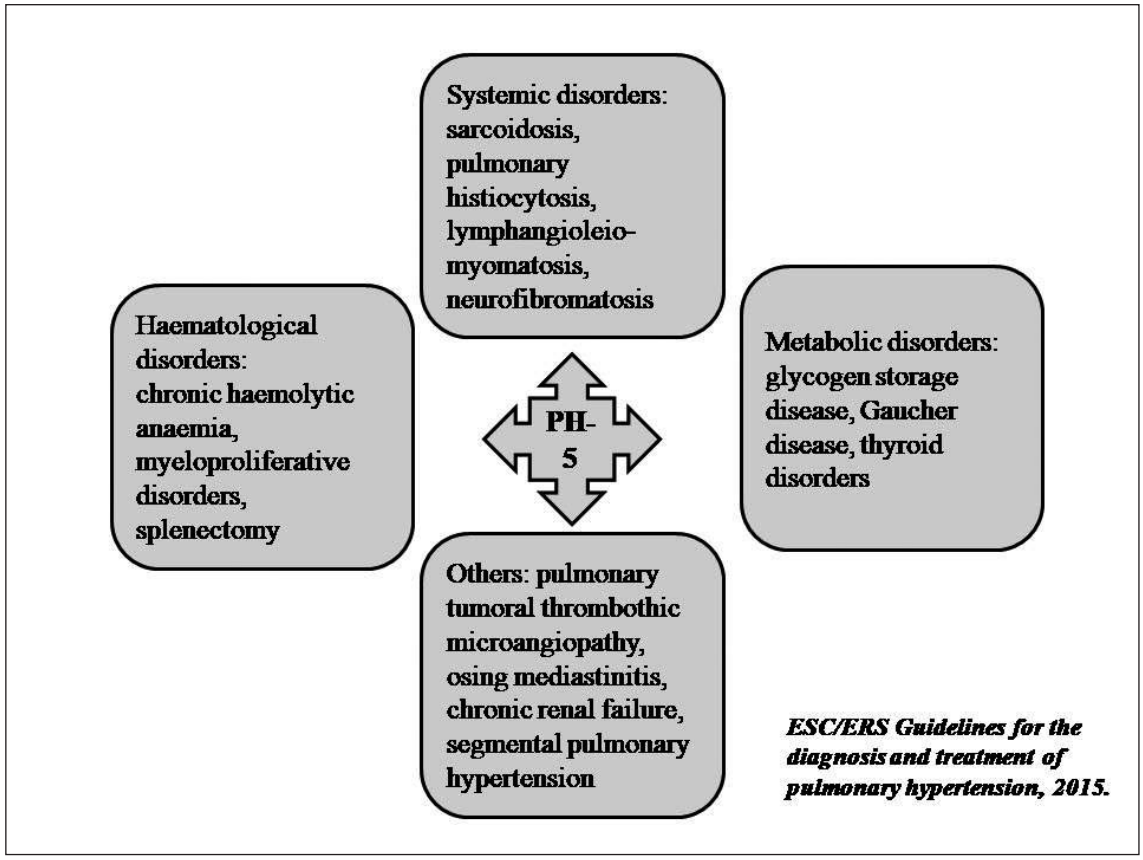

Fig. 4. Clinical classification of the group 5 pulmonary hypertension.

best option in subjects with PVRi > 8 WU.m^2 and PVR > 4.6 WU. Patients with an intermediate PVRi of 4-8 WU.m^2 and PVR of 2.3-4.6 WU require individualized approach [4]. In univariate analysis, SVi (HR = 1.058; 95% confidence interval: 1.02 – 1.10; p = 0.002), PVRi (HR = 1.024; 95% confidence interval: 1.00-1.05; p = 0.01) and ΔPVRi after vasoreactivity testing with intravenous epoprostenol (HR = 0.972; 95% confidence interval: 0.95-0.99; p = 0.02) were found to be significant predictors of poor outcomes in patients with PAH associated with congenital heart disease and Eisenmenger syndrome and receiving bosentan therapy. ΔPVRi was demonstrated as the only independent predictor of clinical worsening (HR = 0.973; 95% confidence interval: 0.95 – 0.99; p = 0.01), and ΔPVRi < 25%, with 56% sensitivity and 100%, specificity could predict clinical worsening. The area under the ROC curve was 0.773 (95% confidence interval: 0.608 – 0.892) [22].

PULMONARY HYPERTENSION DUE TO LEFT HEART DISEASE (PH-2)

Prognostic factors have also been identified in pulmonary hypertension due to left heart disease (PH-LHD), including subsets of patients with an LVEF ≥ 50% (PH-HFpEF) and LVEF < 50% (PH-HFrEF). Despite significantly higher DPG values in the PH-HFpEF compared to the PH-HFrEF group, the variable was not a significant predictor of survival. On the other hand, both groups had similar PCa values, but PCa < 1.1 ml/mmHg in PH-HFpEF [23] and PCa < 2.15 ml/mmHg in PH-HFrEF [24] were established as significant predictors of survival.

The interpretation of DPG values appears more complex. An elevated DPG ≥ 7 mmHg is associated with increased mortality in patients with PH due to LHD, but the correlation seems to be weak, and other factors e.g. levels of *N*-terminal pro-brain natriuretic peptide (NT-proBNP) or NYHA class have better prognostic values [25]. On the other hand, available evidence suggests that DPG ≥ 7 mmHg does not have a significant effect on survival in PH-LHD, but elevated PVR > 3WU and TPG > 9 mmHg can be a predictor of death [26].

RHC is mandatory at all stages of the qualification process of heart failure patients for a heart transplant (HT). It should be performed not only prior to an adult patient being listed for cardiac transplantation but also repeated periodically until transplantation. The 2016 guidelines for HT recommend to adjust the assessment process to specific clinical circumstances [6]. The risk of death due to right heart failure after heart transplantation is significantly increased in some clinical situations: if PVR > 5 WU or PVRI > 6 WU.m^2 (children), or TPG exceeding 16-20 mmHg; if PAPs > 60 mmHg in combination with one of the former values or if PVR cannot be reduced to < 2.5 after a vasodilator challenge without a drop in SBP < 85 mmHg. Vasodilator testing should be performed in subjects with PAPs > 50 mmHg and either TPG > 15 mmHg

or pulmonary PVR > 3WU (with SBP > 85 mmHg). The diagnosis of irreversible pulmonary hypertension should be suspected in patients with unsuccessful acute vasodilator testing and medical treatment failure [27].

A left ventricular assist device (LVAD) can significantly reduce PVR in heart transplant candidates. Regardless of pre-implantation PVR: low (< 5 WU) or high (≥ 5 WU), a 3-year survival after transplantation is similar between groups (85.0% and 79.0%, respectively), however posttransplant in-hospital mortality remains significantly increased among patients with the initially high PVR (P<0.05) [28]. Even PVR ≥3 WU vs. PVR < 3 WU places patients with HF and LVAD at higher risk of death (HR 1.55; P = 0.026), whereas elevated DPG is only associated with the development of RV failure (HR: 3.30; P = 0.004 for DPG ≥ 7 versus DPG < 7) [29].

There are other parameters, not included in the current HT guidelines, which can help evaluate patients before and after HT. One of them is relative pulmonary hypertension calculated from mean artery pressure (MAP) and PAPm (MAP/PAPm). Preoperative value < 3 in transplant candidates > 60 years of age is associated with lower survival rates after transplantation (HR 5.39; 95% confidence interval: 1.64 – 17.74; p=0.006) [30]. Postoperative PAPm > 20 mmHg, compared to PAPm ≤ 20 mmHg, is linked to a significantly increased mortality rate within one year after transplantation (11.5 ± 0.7 vs. 15.6 ± 0.6 years, p <0.001) [31].

Aortic stenosis is one of the most common valve diseases and its prevalence is expected to rise with an aging population. In some clinical situations RHC can help with patient evaluation and qualification for surgical treatment or transcatheter aortic valve implantation (TAVI). There is a significant association between PH and reduced survival after surgical aortic valve replacement (p=0.006) or TAVI (1-year mortality unadjusted: HR: 2.03; 95% confidence interval: 1.07 – 3.85; p = 0.030; after adjustment: HR: 1.95; 95% confidence interval: 1.01 – 3.76; p = 0.046) [32]. Of all PH subgroups, Cpc-PH remains the strongest predictor of death both for surgical (HR 4.39, 95% confidence interval: 2.40 – 8.03; p < 0.001) [33] and TAVI patients (adjusted HR: 3.28; 95% confidence interval: 1.43 – 7.53; p = 0.005 at 1 year) [32]. In addition, among patients with severe aortic stenosis (AVA < 1 cm^2) lack of postoperative reduction in PAPm ≥ 10 mmHg is an independent predictor of mortality (HR: 0.93; 95% confidence interval: 1.2 – 12.5; p = 0.048), whereas preoperatively elevated PAWP is a significant predictor of reduced PAPm (OR, 1.26; 95% confidence interval: 1.13 – 1.41; p < 0.0001) [34]. In aortic regurgitation, PH has been less studied.

The relationship between prognosis in mitral regurgitation and parameters related to pulmonary hypertension has been described in the literature, however, these are forecasts based mainly on PAPs values from echocardiographic measurements, which is beyond the scope of this study.

Rheumatic heart disease, especially mitral stenosis (MS) remains a major health problem in developing countries, and percutaneous balloon mitral valvotomy (PBMV) is a safe and effective procedure in symptomatic patients. Baseline PVR> 1.81 WU has been shown to be an independent predictor of persistent elevation of RVSP > 50 mmHg immediately after PBMV with 69% sensitivity and 86% specificity (95% confidence interval: 64–95; p = 0.002; AUC = 0.79) [35]. Furthermore, post-PBMV PAPm has been established as an independent predictor of all-cause mortality (per mmHg – HR: 1.045; 95% confidence interval: 1.015 – 1.077; p = 0.003) and mitral valve reintervention (per mmHg – HR: 1.055; 95% confidence interval: 1.024 – 1.087; p < 0.001) [36].

PULMONARY HYPERTENSION DUE TO LUNG DISEASES AND/OR HYPOXIA (PH-3)

Although RHC is not recommended in conventional evaluation of PH-3 patients, it may offer clues that help narrow the differential diagnosis and make therapeutic decisions regarding lung transplantation. PH was found to have a negative effect on survival after lung transplantation (LT) within 90 days of follow-up, compared to non-PH patients (p = 0.043 and p = 0.003, respectively), but at one year after LT only pre-capillary PH versus post-capillary PH remained a negative prognostic factor (p = 0.037 and p = 0.447, respectively) [37]. In patients with end-stage lung disease awaiting LT, PAPm ≥ 30 mmHg, PAPd ≥ 20 mmHg and PAPs ≥ 44 mmHg are associated with worse prognosis with sensitivity = 70%, 70%, 73%, specificity = 76%, 69%, 72% and AUC = 0.67, 0.68, 0.72, respectively [38]. Despite the correlation between mortality in patients awaiting LT and exacerbation of PH, PAH-approved drugs are not recommended due to inhibition of hypoxic pulmonary vasoconstriction [4].

CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (PH-4)

Not only direct RHC-derived measurements can be used to predict outcome in PH patients. Evidence shows that analysis of pulmonary artery occlusion pressure waveform might identify CTEPH patients with persistent PH after pulmonary thromboendarterectomy (PTE), which contributes to poor outcome. Pulmonary vascular resistance can be divided into large arterial (upstream, Rup) and small arterial plus venous (downstream) components (Fig 3). Lower Rup can be seen in patients with CTEPH with small vessel disease and embolic material so that it makes it impossible to perform an effective PTE. Patients with Rup <60% seem to be at the highest risk of persistent PH after PTE [39]. Different cut-off points may influence the sensitivity and specificity of the Rup in distinguishing operable from inoperable CTEPH. The cut-off point of 79.3% gives a 100% sensitivity and 57.1% specificity, whereas the cut-off value of 83.8% decreases sensitivity to 83.3% but specificity is increased to 71.4% [40].

The level of preoperative PVR, with a threshold > 800 dyn.sec.cm-5, was found to be a risk factor for increased mortality in patients after PTE, with sensitivity and specificity of 77% and 60%, respectively [41]. PAPm ≥ 38mmHg

and PVR ≥ 425 dyn.sec.cm-5 were predictors of poor outcome at 3-6 months post PTE [42].

Recent years have seen major advances in the CTEPH treatment. Balloon pulmonary angioplasty (BPA) has become a very useful alternative and complementary therapy to PTE, especially in inoperable patients with distal lesions. It is very important because at the moment of CTEPH diagnosis, almost 40% patients are considered inoperable [43]. In the biggest Japan BPA registry after a series of BPA procedures PAPm decreased from 43.2 ± 11.0 to 22.5 ± 5.4 mmHg [44], whereas in Polish patients with residual PH after PTE, a series of BAP reduced PAPm from 44.7 ± 6.4 to 30.8 ± 7.5 mm Hg (31% decline; $p < 0.001$) [45]. Substantial evidence shows that several RHC-derived measurements can be helpful in patient assessment before and after BPA. In multivariate analysis, PAPd at baseline was found to be an independent predictor of residual PH (PAPm > 30mmHg at follow-up) after BPA (OR: 2.04; 95% confidence interval: 1.06 – 5.76; $p = 0.029$) [46] and a relative increase in SvO2 > 125.4% over the baseline value significantly correlated with increased eGFR one year after BPA, with 100% specificity and 24.1% sensitivity [47].

It is essential to bear in mind that there are several potential risks related to the BPA procedure, with reperfusion pulmonary injury (RPI) being most important. Post-BPA pulmonary arterial pressure distal (Pd) to the site of stenosis is associated with RPI occurrence (OR: 1.139, 95% confidence interval: 1.053 – 1.231, $p = 0.001$) and post-BPA Pd > 19.5 mmHg can predict RPI with 79.6% sensitivity and 75.4% specificity, whereas the area under ROC curve is 0.814 [48].

PULMONARY HYPERTENSION WITH UNCLEAR AND/OR MULTIFACTORIAL MECHANISMS (PH-5)

PH-5 is a heterogeneous group of diseases with unclear or multifactorial mechanisms, requiring an individual approach to patients, for whom no universal treatment is available (Fig. 4) [4]. This category includes PH associated with sickle cell disease (SCD), where average life expectancy is 25.6 months, whereas a 119-month survival of 70% has been reported in patients with SCD without PH. RHC-derived measurements that affect outcome include PAPm, where each increase of 10 mmHg is associated with an approximately 2-fold increase in the rate of death [49], and TPG ≥12mmHg is deemed to be an independent predictor of increased mortality [50].

CONCLUSIONS

Despite the availability of a wide range of non-invasive diagnostic tests RHC remains the gold standard for assessing pulmonary artery pressure. RHC-derived parameters are useful not only for PH diagnosis but also for prognostic purposes in numerous PH subtypes, facilitating the decision-making process. Although they are not included in the current ESC guidelines, they may be extremely useful in everyday practice for evaluation of a heterogeneous group of patients with PH. Comprehensive PH patient assessment is the cornerstone of individualized care with a significantly better outcome.

REFERENCES

1. Nossaman BD, Scruggs BA, Nossaman VE, Murthy SN, Kadowitz PJ. History of right heart catheterization: 100 years of experimentation and methodology development. Cardiol Rev. 2010;18(2):94–101. doi:10.1097/CRD.0b013e3181ceff67
2. Callan P, Clark AL. Right heart catheterisation: indications and interpretation. Heart. 2016;102(2):147–157. doi:10.1136/heartjnl-2015-307786
3. Kopeć G, Kurzyna M, Mroczek E, et al. Characterization of patients with pulmonary arterial hypertension: Data from the Polish Registry of Pulmonary Hypertension (BNP-PL). J Clin Med. 2020;9(1):173. Published 2020 Jan 8. doi:10.3390/jcm9010173
4. Galiè N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Rev Esp Cardiol (Engl Ed). 2016;69(2):177. doi:10.1016/j.rec.2016.01.002
5. Callan P, Clark AL. Right heart catheterisation: indications and interpretation. Heart. 2016;102(2):147–157. doi:10.1136/heartjnl-2015-307786
6. Mehra MR, Canter CE, Hannan MM, et al. The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: A 10-year update. J Heart Lung Transplant. 2016;35(1):1–23. doi:10.1016/j.healun.2015.10.023
7. Simonneau G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1):1801913. doi:10.1183/13993003.01913-2018
8. Suzuki A, Taniguchi H, Watanabe N, et al. Significance of pulmonary arterial pressure as a prognostic indicator in lung-dominant connective tissue disease. PLoS One. 2014;9(9):e108339. doi:10.1371/journal.pone.0108339
9. Kimura M, Taniguchi H, Kondoh Y, et al. Pulmonary hypertension as a prognostic indicator at the initial evaluation in idiopathic pulmonary fibrosis. Respiration. 2013;85(6):456–463. doi:10.1159/000345221
10. Maron BA, Hess E, Maddox TM, et al. Association of Borderline Pulmonary hypertension with mortality and hospitalization in a large patient cohort: insights from the veterans affairs clinical assessment, reporting, and tracking program. Circulation. 2016;133(13):1240–1248. doi:10.1161/CIRCULATIONAHA.115.020207
11. Weatherald J, Boucly A, Chemla D, et al. Prognostic value of follow-up hemodynamic variables after initial management in pulmonary arterial Hypertension. Circulation. 2018;137(7):693–704. doi:10.1161/CIRCULATIONAHA.117.029254
12. van Wolferen SA, Marcus JT, Boonstra A, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007;28(10):1250–1257. doi:10.1093/eurheartj/ehl477
13. Mazimba S, Mejia-Lopez E, Black G, et al. Diastolic pulmonary gradient predicts outcomes in group 1 pulmonary hypertension (analysis of the NIH primary pulmonary hypertension registry). Respir Med. 2016;119:81–86. doi:10.1016/j.rmed.2016.08.024
14. Farber HW, Miller DP, Poms AD, et al. Five-Year outcomes of patients enrolled in the REVEAL Registry. Chest. 2015;148(4):1043–1054. doi:10.1378/chest.15-0300
15. Benza RL, Gomberg-Maitland M, Miller DP, et al. The REVEAL Registry risk score calculator in patients newly diagnosed with pulmonary arterial hypertension. Chest. 2012;141(2):354–362. doi:10.1378/chest.11-0676

16. Mukerjee D, St George D, Coleiro B, et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension: application of a registry approach. Ann Rheum Dis. 2003;62(11):1088–1093. doi:10.1136/ard.62.11.1088
17. Chung L, Farber HW, Benza R, et al. Unique predictors of mortality in patients with pulmonary arterial hypertension associated with systemic sclerosis in the REVEAL registry. Chest. 2014;146(6):1494–1504. doi:10.1378/chest.13-3014
18. Campo A, Mathai SC, Le Pavec J, et al. Hemodynamic predictors of survival in scleroderma-related pulmonary arterial hypertension. Am J Respir Crit Care Med. 2010;182(2):252-260. doi:10.1164/rccm.200912-1820OC
19. Krowka MJ, Plevak DJ, Findlay JY, Rosen CB, Wiesner RH, Krom RA. Pulmonary hemodynamics and perioperative cardiopulmonary-related mortality in patients with portopulmonary hypertension undergoing liver transplantation. Liver Transpl. 2000;6(4):443–450. doi:10.1053/jlts.2000.6356
20. Cheng XL, Liu ZH, Gu Q, et al. Prognostic value of pulmonary artery compliance in patients with pulmonary arterial hypertension associated with adult congenital heart disease. Int Heart J. 2017;58(5):731–738. doi:10.1536/ihj.16-449
21. Mahapatra S, Nishimura RA, Sorajja P, Cha S, McGoon MD. Relationship of pulmonary arterial capacitance and mortality in idiopathic pulmonary arterial hypertension. J Am Coll Cardiol. 2006;47(4):799-803. doi:10.1016/j.jacc.2005.09.054
22. D'Alto M, Romeo E, Argiento P, et al. Pulmonary vasoreactivity predicts long-term outcome in patients with Eisenmenger syndrome receiving bosentan therapy. Heart. 2010;96(18):1475–1479. doi:10.1136/hrt.2010.199661
23. Al-Naamani N, Preston IR, Paulus JK, Hill NS, Roberts KE. Pulmonary Arterial Capacitance Is an Important Predictor of Mortality in Heart Failure With a Preserved Ejection Fraction. JACC Heart Fail. 2015;3(6):467–474. doi:10.1016/j.jchf.2015.01.013
24. Pellegrini P, Rossi A, Pasotti M, et al. Prognostic relevance of pulmonary arterial compliance in patients with chronic heart failure. Chest. 2014;145(5):1064–1070. doi:10.1378/chest.13-1510
25. Yamabe S, Dohi Y, Fujisaki S, et al. Prognostic factors for survival in pulmonary hypertension due to left heart disease. Circ J. 2016;80(1):243–249. doi:10.1253/circj.CJ-15-0708
26. Tampakakis E, Leary PJ, Selby VN, et al. The diastolic pulmonary gradient does not predict survival in patients with pulmonary hypertension due to left heart disease. JACC Heart Fail. 2015;3(1):9–16. doi:10.1016/j.jchf.2014.07.010
27. Mehra MR, Kobashigawa J, Starling R, et al. Listing criteria for heart transplantation: International Society for Heart and Lung Transplantation guidelines for the care of cardiac transplant candidates – 2006. J Heart Lung Transplant. 2006;25(9):1024–1042. doi:10.1016/j.healun.2006.06.008
28. Tsukashita M, Takayama H, Takeda K, et al. Effect of pulmonary vascular resistance before left ventricular assist device implantation on short- and long-term post-transplant survival. J Thorac Cardiovasc Surg. 2015;150(5):1352–1361.e13612. doi:10.1016/j.jtcvs.2015.07.012
29. Alnsasra H, Asleh R, Schettle SD, et al. Diastolic pulmonary gradient as a predictor of right ventricular failure after left ventricular assist device implantation. J Am Heart Assoc. 2019;8(16):e012073. doi:10.1161/JAHA.119.012073
30. Bianco JC, Mc Loughlin S, Denault AY, Marenchino RG, Rojas JI, Bonofiglio FC. Heart transplantation in patients >60 years: Importance of relative pulmonary hypertension and right ventricular failure on midterm survival. J Cardiothorac Vasc Anesth. 2018;32(1):32–40. doi:10.1053/j.jvca.2017.09.017
31. Gude E, Simonsen S, Geiran OR, et al. Pulmonary hypertension in heart transplantation: discrepant prognostic impact of pre-operative compared with 1-year post-operative right heart hemodynamics. J Heart Lung Transplant. 2010;29(2):216–223. doi:10.1016/j.healun.2009.08.021
32. O'Sullivan CJ, Wenaweser P, Ceylan O, et al. Effect of pulmonary hypertension hemodynamic presentation on clinical outcomes in patients with severe symptomatic aortic valve stenosis undergoing transcatheter aortic valve implantation: Insights from the new proposed pulmonary hypertension classification. Circ Cardiovasc Interv. 2015;8(7):e002358. doi:10.1161/CIRCINTERVENTIONS.114.002358
33. Weber L, Rickli H, Haager PK, et al. Haemodynamic mechanisms and long-term prognostic impact of pulmonary hypertension in patients with severe aortic stenosis undergoing valve replacement. Eur J Heart Fail. 2019;21(2):172–181. doi:10.1002/ejhf.1322
34. Cam A, Goel SS, Agarwal S, et al. Prognostic implications of pulmonary hypertension in patients with severe aortic stenosis. J Thorac Cardiovasc Surg. 2011;142(4):800–808. doi:10.1016/j.jtcvs.2010.12.024
35. Elmaghawry LM, El-Dosouky II, Kandil NT, Sayyid-Ahmad AMS. Pulmonary vascular resistance and proper timing of percutaneous balloon mitral valvotomy. Int J Cardiovasc Imaging. 2018;34(4):523–529. doi:10.1007/s10554-017-1255-3
36. Jorge E, Pan M, Baptista R, et al. Predictors of very late events after percutaneous mitral valvuloplasty in patients with mitral stenosis. Am J Cardiol. 2016;117(12):1978–1984. doi:10.1016/j.amjcard.2016.03.051
37. Andersen KH, Schultz HH, Nyholm B, Iversen MP, Gustafsson F, Carlsen J. Pulmonary hypertension as a risk factor of mortality after lung transplantation. Clin Transplant. 2016;30(4):357–364. doi:10.1111/ctr.12692
38. Nowak J, Hudzik B, Przybyłowski P, et al. Prognostic value of mean, diastolic, and systolic pulmonary artery pressure in patients with end-stage lung disease referred for lung transplantation. Transplant Proc. 2018;50(7):2048–2052. doi:10.1016/j.transproceed.2018.02.152
39. Kim NH, Fesler P, Channick RN, et al. Preoperative partitioning of pulmonary vascular resistance correlates with early outcome after thromboendarterectomy for chronic thromboembolic pulmonary hypertension. Circulation. 2004;109(1):18–22. doi:10.1161/01.CIR.0000111841.28126.D4
40. Toshner M, Suntharalingam J, Fesler P, et al. Occlusion pressure analysis role in partitioning of pulmonary vascular resistance in CTEPH. Eur Respir J. 2012;40(3):612–617. doi:10.1183/09031936.00134111
41. Tromeur C, Jaïs X, Mercier O, et al. Factors predicting outcome after pulmonary endarterectomy. PLoS One. 2018;13(6):e0198198. doi:10.1371/journal.pone.0198198
42. Cannon JE, Su L, Kiely DG, et al. Dynamic risk stratification of patient long-term outcome after pulmonary endarterectomy: Results from the United Kingdom national cohort. Circulation. 2016;133(18):1761–1771. doi:10.1161/CIRCULATIONAHA.115.019470
43. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): Results from an international prospective registry. Circulation. 2011;124(18):1973–1981. doi:10.1161/CIRCULATIONAHA.110.015008
44. Ogawa A, Satoh T, Fukuda T, et al. Balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension: Results of a multicenter registry. Circ Cardiovasc Qual Outcomes. 2017;10(11):e004029. doi:10.1161/CIRCOUTCOMES.117.004029
45. Araszkiewicz A, Darocha S, Pietrasik A, et al. Balloon pulmonary angioplasty for the treatment of residual or recurrent pulmonary hypertension after pulmonary endarterectomy. Int J Cardiol. 2019;278:232–237. doi:10.1016/j.ijcard.2018.10.066

46. Tsuji A, Ogo T, Ueda J, et al. Predictors of residual pulmonary hypertension after balloon pulmonary angioplasty in patients with chronic thromboembolic pulmonary hypertension. Int J Cardiol. 2017;226:118–120. doi:10.1016/j.ijcard.2016.09.132
47. Isobe S, Itabashi Y, Kawakami T, et al. Increasing mixed venous oxygen saturation is a predictor of improved renal function after balloon pulmonary angioplasty in patients with chronic thromboembolic pulmonary hypertension. Heart Vessels. 2019;34(4):688–697. doi:10.1007/s00380-018-1284-4
48. Kinutani H, Shinke T, Nakayama K, et al. High perfusion pressure as a predictor of reperfusion pulmonary injury after balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension. Int J Cardiol Heart Vasc. 2015;11:1–6. Published 2015 Nov 26. doi:10.1016/j.ijcha.2015.11.006
49. Castro O, Hoque M, Brown BD. Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. Blood. 2003;101(4):1257–1261. doi:10.1182/blood-2002-03-0948
50. Nguyen KL, Tian X, Alam S, et al. Elevated transpulmonary gradient and cardiac magnetic resonance-derived right ventricular remodeling predict poor outcomes in sickle cell disease. Haematologica. 2016;101(2):e40–e43. doi:10.3324/haematol.2015.125229

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REVIEW ARTICLE

PROBLEM OF GENITAL PROLAPSES AND THEIR CORRECTION BY NONSURGICAL METHODS

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ABSTRACT

The aim: to determine the most optimal method of genital prolapse correction among the available nonsurgical methods from the perspective of differentiated approach.

Materials and methods: using the methods of comparison, analysis and synthesis, we have compiled and processed the world's data from such major search databases as PubMed, EMBASE, Cochrane over the last ten years.

Conclusions: the detection of genital prolapses at an early stage and the early nonsurgical treatment can prevent the disease progression, eliminate the symptoms, especially urinary problems in most patients, and improve the life quality.

KEY WORDS: genital prolapse, fractional CO_2 laser, hyaluronic acid, platelet rich plasma, cystocele

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INTRODUCTION

Modern medicine aims to improve the life quality, minimize complications and improve the long-term outcomes of various therapies. Today, women's health has a great impact on the life quality of not only the woman but also her partner. Genital prolapse (GP) is a significant problem, as it makes women feel less attractive and undesirable. The complexity of this pathology on the one hand and the age and numerous extragenital diseases on the other exclude the possibility of surgical correction and require conservative treatment.

When choosing among a great number of various methods of conservative treatment of GP described today, there is no clear element of differentiation. At present, there are no common approaches to the management and choice of nonsurgical correction of prolapse and the conditions associated with it, and therefore this issue is perhaps the most controversial in the treatment of GP.

THE AIM

To determine the most optimal method of mild forms of genital prolapse correction among the available nonsurgical methods from the perspective of differentiated approach.

MATERIALS AND METHODS

We have obtained world's data from such major search databases as PubMed, EMBASE, Cochrane over the last ten years. The search was performed by the following keywords: genital prolapse, fractional CO_2 laser, pelvic floor dysfunction, hyaluronic acid, platelet rich plasma. Then using the methods of comparison, analysis, synthesis, generalization and scientific abstraction, we analyzed the obtained data in order to determine the most optimal method of prolapse correction among the available techniques taking into account the possibility of a differentiated approach to solving this problem.

REVIEW AND DISCUSSION

GP is a problem that is complicated by both the woman's unwillingness to discuss it even with the doctor and the inattention to the initial manifestations of the disease by gynecologists and related primary care professionals. If there are symptoms of GP, every fifth woman is at risk of undergoing surgery before the age of 80. Therefore, from the point of view of the development approaches to the GP nonsurgical correction, the choice of treatment option is determined by a number of factors, including the age, concomitant extragenital pathology, degree of risk of surgery and anesthetic management, degree of prolapse, anatomical and functional changes of the reproductive organs, presence and nature of concomitant gynecological pathology, possibility and necessity of preservation or sometimes restoration of fertility, features of adjacent organs dysfunction. For historical reasons, conservative methods are almost always in the sidelines when doctors have to deal with the issue of GP correction. However, in recent years, these treatment techniques have become increasingly popular. Each of the conservative methods of GP treatment had a long difficult evolutionary way, but as of today they are still of great significance in the correction of such an important medical and social problem as GP.

In nonsurgical correction of genital prolapses, the following techniques are of the most importance now:

- the use of fractional CO2 laser in patients with GP combined with neurogenic bladder dysfunction [1];
- the use of pessary as a temporary symptomatic treatment option (when surgical treatment is not possible) [2, 3];
- the use of such bulking agents as platelet rich plasma and hyaluronic acid preparations;
- electrical stimulation of pelvic floor muscles using anal, vaginal and urethral sensors;
- training the pelvic floor muscles using Kegel exercise.

PLATELET RICH PLASMA (PRP)

The use of biotechnologies to accelerate soft tissue regeneration has become one of the modern trends in reconstructive medicine. As a result, the possibility of using platelet rich plasma (PRP) is of great interest among the researchers now. The popularity of the technique is explained by the absence of allergic reactions, the simplicity and ease of use, as well as the increasing scope of application, and GP is no exception here.

Platelet rich plasma (PRP) also termed platelet rich growth factors, platelet rich fibrin, platelet concentrate, is essentially a plasma with an increased concentration of platelets [4]. Normally, the number of platelets in the blood is in the range of 150-350 thousand/μl and averages 200 thousand/μl. The studies have shown that the clinical effect of platelet rich plasma should be expected if the platelet concentration in it is 1,000,000/μl. At lower concentrations, the stimulating effect on the regenerative processes in the tissues is not manifested, at the same time, it has not been proven so far that an increase in platelet concentration of more than 1,000,000/μl leads to a further acceleration of regeneration. The use of platelet rich plasma in gynecology is recommended in the treatment of the initial presentation of genital prolapses, in particular the isolated manifestations of 1-2 degree cystocele or rectocele, as well as the correction of non-stress urinary incontinence associated with urethral hypermobility, which is one of the possible consequences of genital prolapse [5]. It is also claimed that PRP is effective in regeneration as well as in the injury remodeling phase. The remodeling phase involves the production of type III collagen by fibroblasts [6]. Despite the interest in the method and the obvious widespread use, there is no evidence of randomized clinical trials evaluating the effectiveness of PRP [7]. The presence of plasma clot formation and platelet growth factors in PRP, which provide the healing and remodeling processes of collagen and elastic fibers, is the basis for its use. PRP contains not only growth factors, but also adhesive molecules and cytokines that stimulate repair and anabolic processes in damaged tissues [8]. It is known that platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-b), epithelial growth factor (EGF) and vascular endothelial growth factor (VEGF) are released from alpha-granules.

HYALURONIC ACID PREPARATIONS

The mechanism of hyaluronic acid gel polymer action when eliminating initial presentation of genital prolapses involves compensation of soft tissue deficiency in the area of defeat due to the increase of their volume, as well as the mechanical compression [9]. The main requirements for modern bulking agents are, first of all, biocompatibility, the absence of inflammatory response and autoimmune systemic reaction, as well as the absence of fibrosis in the injection site. Also, the particles of these substances have to be of sufficient size (diameter not less than 80 microns) so as not to migrate from the injection site and not to disintegrate for some time [10 – 11]. The procedure is less traumatic, relatively cost effective and can be used in the outpatient setting under local anesthesia.

FRACTIONAL CO2 LASER IRRADIATION

A laser is a source of light, but unlike thermal light sources, lasers have a high degree of monochromaticity, spatial coherence, directionality and polarization of radiation at a considerable intensity and brightness, and can also be tuned over wavelengths and emit light pulses of short duration [12]. Absorption is a key physical process that ensures the effective interaction of laser radiation with biological tissues (Berger N., Eeg P. H., 2006). When absorbed by the tissue, the laser radiation is converted into thermal energy leading to the rapid tissue heating. CO_2 laser belongs to lasers that emit wavelength of 10 600 nm, and they interact very well even with a small amount of water (10-15%), which is in the tissues [13]. Numerous studies prove that the use of CO_2 laser for the rejuvenation of mucous membranes gives good prognostic results [1, 14-15]. It is believed that neocollagenesis is the cause of long-term improvement, which is to increase tissue density. Heating with laser irradiation leads to the denaturation of collagen, i.e. the destruction of hydrogen bonds in the tertiary helical structure of collagen fibers, and the subsequent formation of a random helix. As soon as a sufficient amount of collagen fiber denaturation occurs, the tissue is immediately tightened, because when heated, the tissue causes inflammatory mediators to initiate the process of tissue repair and stimulates the synthesis of type I and III collagen [16].

Laser treatment for women with dystrophic changes of the vaginal and vulvar mucosa has been developed in the modern world. This technique was approved by the FDA (USA) in 2015 for the treatment of genitourinary syndrome of menopause and vaginal relaxation syndrome [17]. Due to the stimulating effect of thermal laser exposure, the process of producing collagen and elastin is activated in the area where it is required. The action of CO_2 laser on the mucous membranes initiates an aseptic inflammation reaction, accompanied by the synthesis of proinflammatory cytokines IL-1β and TNF-α by tissue macrophages, which causes the induction of AP-1 transcription factor and leads to an increase in the amount of fragmented collagen and MMP-degradable. At the recovery stage, fibroblast proliferation, TGF-β growth factor transformation, and intercellular matrix renewal are observed due to type I and III neocollagenesis processes. There is also an increase in the secretion of transforming growth factor TGF-β1, which

regulates collagen synthesis and prevents excessive tissue fibrosis [18]. The result is a unique balance between maximum impact efficiency and minimal tissue damage. It is known that when heated the tissue activates the so-called heat shock proteins (HSP). This is one of the fundamental changes occurring at the molecular level, not taking into account changes in the regulation of other molecules, such as transforming growth factor β (TGF-β), hyaluronidase and hyaluronic acid [19 – 21]. Zerbinati et al. in their study also showed that using CO_2 laser for photorejuvenation of the vaginal epithelium activates heat shock proteins, increasing glycogen content in the cells of the vaginal epithelium, thereby increasing the thickness of the vaginal epithelium [22]. Some studies showed the activation or induction of TGF-β growth factors by laser treatment 2-14 days after irradiation. Growth factors contribute to the synthesis of matrix components, such as fibronectin and collagen, transformation of myofibroblasts, angiogenesis due to the reaction of heat shock [23-25]. Also, one of the important enzymes that catalyze the breakdown of collagen are matrix metalloproteinases (MMPs), which levels increase in the first three days after treatment, reaching a maximum by day 7. There is evidence that MMPs are involved not only in cleavage but also in remodeling of new collagen [26].

Currently, we have sufficient experience of the treatment of various pathological changes in the vulvovaginal area using fractional CO_2 laser systems. For vaginal exposure, the following parameters are used: wavelength 10 600 nm, tissue power 30 W (max), peak power 320 W (max), frequency from 5 to 100 Hz, pulse length from 0.2 to 80 ms, and seven-mirror articulated arm for radiation delivery. It is common practice to perform 3 treatment procedures with an interval of 1 month, and then to maintain the achieved effect with a single procedure once per 6-12 months. Complete tissue repair occurs by the 30th day after CO_2 exposure [27]. Laser thermolysis is one of the most important discoveries in laser technologies in the last two decades. The use of this technology for the treatment of genital prolapses in the early stages of their development acts as an incentive to further improvement of this technique. All of the above mentioned methods can be used both as monotherapy and in combination.

CONCLUSIONS

Having analyzed the main sources of literature devoted to modern ideas about treatment of patients with mild GP, its pathogenesis, clinical evidence and diagnostics, we can conclude about the diversity of this pathology and the lack of clear treatment algorithms, in particular conservative methods.

Thus, genital prolapse is a significant problem not only for women of perimenopausal or menopausal age, but also for those of reproductive age, both in the postpartum and in later life. The issues of conservative treatment of prolapse are still poorly understood and require further thorough study and improvement of the existing techniques. The detection of genital prolapse on the early stages of its development and timely conservative treatment can prevent the disease progression, minimize disease manifestations, especially in relation to the urinary bladder (incontinence) in most patients, and improve the life quality.

REFERENCES

1. Manuskiatti W., Fitzpatrick R.E., Goldman M.P. Longterm effectiveness and side effects of carbon dioxide laser resurfacing for photoaged facial skin. J Am Acad Dermatol. 1999; 40(3): 401–411.
2. Krizhanovsky A.N. Pathogenesis and early diagnosis of pelvic floor failure after physiological delivery: cand. med. sci. abstracts diss. M. 2012: 25p. (In Russia).
3. Rosenblum N. Pelvic organ prolaps: Considerations in surgical management. Annual Meeting Ana-heim. Course 10 EC.2007 . https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5610367/.
4. Pietrzak W., Eppley B.Scientific foundations platelet rich plasma: biology and new technology. J. Craniofac. Surg. 2005;16(6):1043–1054.
5. Zheleznaya A.A., Tsvyashko T.I., Knurov I.Yu. et al. The use of platelet-rich autoplasma in obstetric and gynecological practice. Medical and social problems of the family. 2016;21(1):76. (in Ukrainian).
6. Xian L., Roy Chowdhury S., Bin Saim A., Bt Hj Idrus R.Concentration-dependent effect of platelet-rich plasma on keratinocyte and fibroblast wound healing. Cytotherapy. 2015;17(3):293-300.
7. Paoloni J., De Vos R., Hamilton B. et al. Platelet-Rich Plasma Treatment for Ligament Injuries. Clinical Journal of Sport Medicine. 2011;21(1):37-45.
8. Everts P. Platelet-rich plasma and platelet gel: a review. JECT. 2006;38:174–187.
9. Makhmedzhanova F.N. Uroflowmetry with urinary dysfunction in women. Gynecology. 2013;15(1):76–78. (In Russian).
10. Gvozdev M.Y. Recurrent urinary incontinence. 2014; 3:80–87. (In Russian).
11. Rock J. A., Jones H. W., Rock J. A.Jr. TeLinde's Operative Gynecology. 11th ed. 2012; 1472 p.
12. Zvelto. O. Laser principles. St. Petersburg: «Lan». 4th edition. 2008; 720 p. (In Russian).
13. Roberts T.L. 3rd, Pozner J.N. Lasers, facelifting, and the future. Clin Plast Surg 2000; 27(2): 293–299.
14. Schwartz R.J., Burns A.J., Rohrich R.J. et al. Long-term assessment of CO2 facial laser resurfacing: aesthetic results and complications. Plast Reconstr Surg. 1999; 103(2): 592–601.
15. Weinstein C. Carbon dioxide laser resurfacing: longterm follow up in 2,123 patients. Clin Plast Surg. 1998; 25(1): 109–130.
16. Bernstein L.J., Kauvar A.N., Grossman M.C., Geronemus R.G. The short- and long-term side effects ofcarbon dioxide laser resurfacing. Dermatol S urg. 1997; 23(7): 519–525.
17. Palacios S., Castelo-Branco C., Currie H. et al. Update on management of genitourinary syndrome of menopause: A practical guide. Maturitas. 2015;82(3):308-313. https://doi.org/10.1016/j.maturitas.2015.07.020.
18. Apolikhina I.A., Gorbunova E.A., Odinokova V.A. Minimally invasive innovative laser technology in gynecological practice. Obstetrics and gynecology. 2014; 11: 17-22. (In Russian).
19. Arany P.R., Nayak R.S., Hallikerimath S. et al. Activation of latent TGF-beta1 by lowpower laser in vitro correlates with increased TGF-beta1 levels in laser-enhanced oral wound healing. Wound Repair Regen. 2007; 15(6): 866–874.
20. Hantash B.M., Bedi V.P., Kapadia B. et al. In vivo histological evaluation of a novel ablative fractional resurfacing device. Lasers Surg Med. 2007; 39(2): 96–107.

21. Ravanti L., Kähäri V.M. Matrix metalloproteinases in wound repair (review). Int J Mol Med. 2000; 6(4): 391–407.
22. Zerbinati N., Serati M., Origoni M. et al. Microscopic and ultrastructural modifications of postmenopausal atrophic vaginal mucosa after fractional carbon dioxide laser treatment. Lasers in medical science. 2015;30(1):429-436.
23. Gallucci R.M., Lee E.G., Tomasek J.J. IL-6 modulates alpha-smooth muscle actin expression in dermal fibroblasts from IL-6-deficient mice. J Invest Dermatol. 2006; 126(3): 561– 568. http://dx.doi.org/10.1038/sj.jid.5700109.54.
24. Haisa M., Okochi H., Grotendorst G.R. Elevated levels of PDGF alpha receptors in keloid fibroblasts contribute to an enhanced response to PDGF. J Invest Dermatol. 1994; 103(4): 560–563.
25. Kumar S., Millis A.J., Baglioni C. Expression of interleukin 1-inducible genes and production of interleukin 1by aging human fibroblasts. Proc Natl Acad Sci USA. 1992; 89(10): 4683–4687. http://dx.doi.org/10.1073/pnas.89.10.4683.
26. Orringer J.S., Kang S., Johnson T.M. et al. Connective tissue remodeling induced by carbon dioxide laser resurfacing of photodamaged human skin. Arch Dermatol. 2004; 140(11): 1326–1332. http://dx.doi.org/10.1001/archderm. 140.11.1326.
27. Manstein D., Herron G.S., Sink R.K. et al. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med. 2004; 34(5): 426–438. http://dx.doi.org/10.1002/lsm.20048.

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