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DETERMINATION OF THE INTESTINAL MICROBIOME COMPOSITION IN PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS OF DIFFERENT AGE CATEGORIES AND SEX

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The study of the interconnection between the quantitative and qualitative composition violation of the gastrointestinal tract microbiome and the development of inflammatory bowel diseases requires constant monitoring and detailed study due to the worldwide growing incidence and the inconsistency of data on gender and age-related differences in the course of diseases.

Material and methods. 72 samples of biological material from the colon were examined. Identification of microorganisms was carried out in accordance with Bergery's bacterial identification schemes and with the help of the chromogenic nutrient media "Graso Biotech".

Results and discussion. To establish the correlation between the intestinal microbiome and inflammatory bowel diseases, taking into account age and gender characteristics, an analysis of the intestinal microbiome composition was made in 72 patients with IBD of different age and gender: patients with Crohn's disease (n = 23) and ulcerative colitis (n = 49).

According to the analysis, significant changes were found in the microbial landscape of the digestive system in patients with Crohn's disease and ulcerative colitis. It was shown that in middle-aged patients, the detection frequency of a decompensated form of dysbiotic disorders was higher compared with young patients. In the analysis of symbiotic microbiota, significant deviations in the composition of *Bifidobacterium spp.* were found in middle-aged patients with Crohn's disease. Decrease in titers of typical *Escherichia coli* was usually observed in patients with ulcerative colitis. In the analysis of opportunistic microbiota, it was

shown that among all patients with inflammatory bowel diseases, a high frequency of *Staphylococcus spp.* and fungi of the genus *Candida* detection was recorded in middle-aged people. Indicators for identifying opportunistic enterobacteria were highest in patients with ulcerative colitis in middle-aged men and women, while in patients with Crohn's disease, the incidence rate of opportunistic enterobacteria was higher in young people.

Conclusion. Due to examination results of patients with inflammatory bowel diseases of various age categories and sex it has been revealed significant imbalances in the gastrointestinal tract microbiome in 100% of patients. The decompensated form of dysbiotic disorders in patients with Crohn's disease prevailed among both sex – 34.8% of women and 30.45% of men, while among patients with ulcerative colitis a subcompensated form of dysbiosis was found mainly in young women – 20.4%. In both Crohn's disease and ulcerative colitis, the frequency of decompensated dysbiotic form dominated in middle-aged patients.

The largest changes in the analysis of symbiotic microbiota representatives were observed in the composition of *Lactobacillus spp.*, the concentrations of those were reduced in 100% of patients with inflammatory bowel diseases. In the analysis of opportunistic microbiocenosis, it was found that among all patients with inflammatory bowel diseases, a high detection frequency of bacteria of the genus *Staphylococcus* and fungi of the genus *Candida* was observed in middle-aged people.

Keywords: intestinal dysbiosis, Crohn's disease, ulcerative colitis, opportunistic microbiota, associative symbiotic microbiota.

Research relation to the programs, plans, and department themes. The work is carried out within the framework of the initiative topic "The biological basis of the functioning of the microbiocenoses of the environment and the human body", the State Registration Number is 0119U100097.

Introduction. The intestinal tract is a habitat for a huge number of various bacterial species, viruses and fungi, collectively called the gut microbiome. The intestinal microbiota carries out important functions for the host's metabolism as well as for the development and functioning of the immune system [1]. However, in recent years, intestinal microbiota has been considered as the main causative agent of inflammatory bowel diseases (IBD) due to growing evidence that it can function as a "self-antigen" in Crohn's disease (CD) and ulcerative colitis (UC) [2]. For unknown reasons, the incidence of UC and CD is increasing worldwide. At the international level, the incidence of IBD is 0.5-24.5 cases per 100,000 population for UC and 0.1-16 cases per 100,000 population for CD. Overall, the prevalence of IBD is 396 cases per 100,000 people / year. Nearly 1.5 million people in the United States and over 2 million in Europe suffer from these diseases [3]. It is estimated that in 1 out of 5 people with UC, the disease is proceeding extremely difficult, the general state of the patients is not improving with the help of medicines, due to which the removal of the inflamed colon becomes a necessity. Around 60-75% of people with CD are in need of surgery to repair damage to their digestive system and to treat complications of CD [4]. Unfortunately, the metabolic biomarkers of the gut microbiome, which are probably involved in pathogenesis of IBD remain unclear. Therefore, the question of whether changes in the composition of the intestinal microbiota the cause or consequence in inflammatory bowel diseases are, is still under discussion. Considering this, research of intestinal microbiocenosis quantitative and qualitative composition in patients with CD and UC are relevant since the use of chemotherapy and standard treatment methods are usually not successful due to a contradictory etiology. Insufficient attention to age and gender characteristics of the diseases course leads to late diagnostics and possible complications with prolonged exacerbations. Thus, a deeper understanding of these differences is an important step that will provide an individual approach to the treatment of a particular patient.

The purpose and objectives of the study. *The purpose of the work* was to analyze the intestinal mi-

crobiome composition in patients with CD and UC of different ages and sex.

To achieve the goal, the following tasks were set:

- to determine the intestinal microbiome composition of SI "Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine" patients;
- to conduct a comparative analysis of the intestinal microbiocenosis composition to identify the links between the eubiosis violation and IBD;
- to identify differences in quantitative and qualitative indicators in the intestinal microbiome composition in people with CD and UC of different age categories and sex.

Material and methods. The researches were conducted on the basis of the SI "Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine" in the research sector.

All enrolled patients signed patient informed consent, the study conducted in accordance to the Helsinki Declaration of the World Medical Association, the Statute of the Ukrainian Bioethics Association, the standard provisions on ethics of the Ministry of Health of Ukraine No. 66 dated February 13, 2006.

From 2017 to 2019, 72 patients of different sex and age with IBD: CD and UC were examined. Diseases diagnosis was carried *via* laboratory and instrumental research methods.

Anamnesis collecting included information analysis of disease dynamics, symptoms and the presence of inflammatory bowel diseases in relatives.

An endoscopic examination was conducted in order to identify the inflammatory process, evaluate its activity, collect biopsy samples of the intestinal mucosa for their further histological examination. With the help of histological researches the lymphocytic transmural infiltration with intermittent spread was revealed.

Laboratory methods were used to evaluate inflammatory processes and nutritional status. Blood analysis of patients with inflammatory bowel diseases had showed anemia, leukocytosis, thrombocytosis, accelerated erythrocyte sedimentation rate (ESR), and an increase in the level of C-reactive protein (CRP), which is a marker of the inflammation presence in the body. In patients with inflammatory bowel diseases, hypergammaglobulinemia (elevated IgG concentrations) was observed, and in some cases, selective IgA deficiency (IgA < 7 mg / dl) was manifested. By immunochromatographic method the level of calprotectin in feces was calculated, the indicators of which were increased (> 50 µg / g) in inflammatory bowel diseases.

The study of the intestinal microbiome composition and the degree of dysbiotic disorders by qualitative and quantitative indicators was carried out by

microbiological analysis methods; species identification of clinical isolates – representatives of normal, opportunistic aerobic and anaerobic microbiota was identified according to Bergey's bacterial identification schemes [5]. The chromogenic nutrient media "Graso Biotech" were being used during the research.

Descriptive statistic was used for statistical analysis of the data; the comparison of the mean values of variables was carried out using parametric methods (Student's t-test) for the normal distribution of the data measured on the interval scale. Differences between the two indicators were considered significant at $p < 0.05$. The correspondence of the characteristics of distribution to the normal distribution law was verified using Mann-Whitney U-test. To compare the particle distribution of two or more variables, the χ^2 test was used. Correlation analysis was performed by Pearson. All calculations were performed in SPSS 9.0 for Windows.

Results and discussion. To establish the correlation between the intestinal microbiome and IBD, taking into account age and gender characteristics, an analysis of the intestinal microbiome composition was made in 72 patients with IBD of different age and gender: patients with CD (n = 23) and UC (n = 49). Most patients were young – 68%, middle-aged patients accounted 32%. All patients were divided according to the world health organization (WHO) classification from 25 to 44 years old – young-aged, 44-60 years old – middle-aged. The average age of patients with IBD was 36 years. Among the examined patients there were 37 women (51.4%) and 35 men (48.6%). Both young men and young women prevailed among patients with CD and UC (Fig. 1). CD was found in 7% of middle-aged women and 4.2% of men, while among middle-aged patients with UC, men predominated – 11.1% versus 9.7% of women. Among the young patients with CD, the dominant group was men (11.1%), and among patients with UC – women (25%).

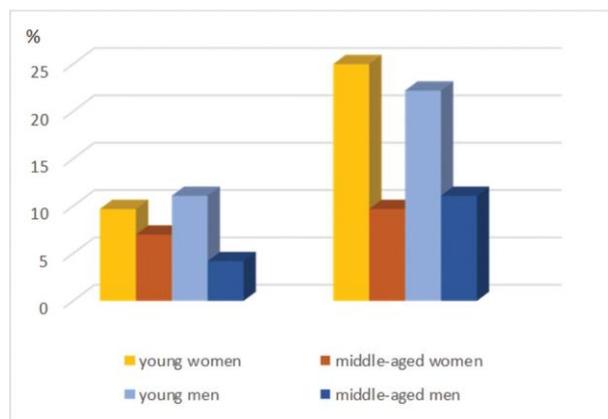


Fig. 1. The incidence rate of Crohn's disease (left) and ulcerative colitis (right) in patients of different ages and sex

During the colon microbiocenosis state analysis profound changes were found in the qualitative and quantitative microbiota composition in 100% of patients with IBD. According to the degree of dysbiotic disorders, dysbacteriosis of second and third degree was established. Among patients with CD, third degree dysbiosis was found in 21.7% of young men, 17.4% of young and middle-aged women, and 8.75% of middle-aged men (Fig. 2). Second degree dysbacteriosis was found mainly in young people (about 13%), and only two middle-aged patients had second degree dysbacteriosis.

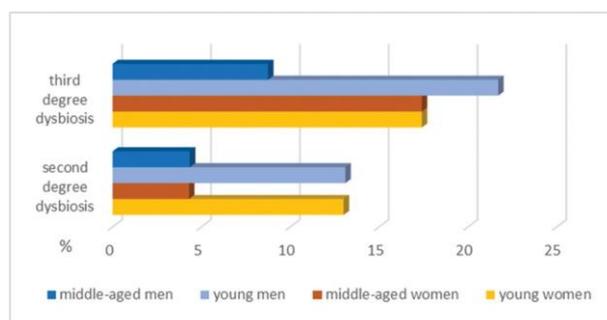


Fig. 2. The frequency of the dysbiosis degree detecting in patients of different ages and sex with Crohn's disease

In the examined patients with UC, the intestinal dysbiosis was also found mainly with a third severity degree – in 59.1% of people, of which 32.6% were young women and men, 14.3% and 12.2% of middle-aged men and women respectively (Fig. 3). A sub-compensated form of dysbiosis was most often diagnosed in young women (20.4%) and men (16.3%), and least often among representatives of the middle-aged group (2.1% of each gender patients).

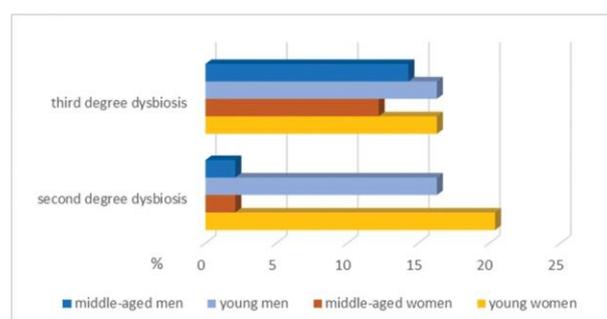


Fig. 3. The frequency of the dysbiosis degree detecting in patients of different ages and sex with ulcerative colitis

Using the connection between the condition, function of intestinal microbiota and IBD phenotypes, the great progress has been made in the last decade in diagnostic sphere. A lot of studies have proposed to introduce the concept of a microbial dysbiosis index for diagnosing diseases. The microbial dysbiosis

index, calculated on the basis of fecal samples before treatment, in addition to what differs in patients with inflammatory diseases from control healthy people, can be used to differentiate CD from UC [6]. The data about interconnection between the patients' age and the characteristics of IBD in the literature are contradictory and often depend on local clinical practice, which makes comparison between population groups more challenging [7]. However, age differences in gender distribution increase the likelihood that the cumulative effects of sex hormones, such as estrogen, after puberty may play a role in the development of IBD. Garcia-Rodriguez et al reported that both long-term hormone replacement therapy and oral contraceptives were associated with an increased risk of developing CD [8]. Different sex steroids concentrations can contribute to differences in the immune system of patients, since sex steroids can affect the immune cells function by binding to specific receptors that are expressed on these cells. Certainly, IBD in patients of different ages have specific pathogenetic features, clinical and morphological manifestations. Thus, in elderly patients, diseases often develop on the background of concomitant pathology, for instant, hypertension, coronary heart disease, diabetes mellitus, chronic non-specific lung diseases. It is noted that in elderly patients the classic symptomatology of both long-term chronic and recent diseases has changed, the symptoms are smoothed out or even inverted, which often disorients the doctor. The asymptomatic IBD clinical picture in the middle-aged patients can be combined with a more severe, complicated disease course. Moreover, in the middle-aged patients, quantitative changes in the intestinal microbiota also occur, namely, a decrease in the number of bacterial genera such as *Bifidobacterium*, *Bacteroides* and *Lactobacillus* is observed, which also contributes to a more severe course of the disease.

A ratio quantitative indicators analysis of various intestinal microbiocenosis representatives in patients with CD revealed significant deviations in the titers of certain symbionts – representatives of the genera *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and bacteria of the *Escherichia coli* species with normal enzymatic activity (NEA) (Fig. 4). In all patients with CD, there was a decrease in the number of *Lactobacillus spp.* to 10^0 - 10^5 CFU / g at normal rates of 10^7 - 10^8 CFU / g. A decrease in *Bifidobacterium spp.* titers to 10^4 - 10^7 CFU / g was observed in all studied groups, except of young female patients, who had the normal range of the *Bifidobacterium* genus members – 10^9 - 10^{10} CFU / g. 66.6% of middle-aged men were diagnosed with *Bifidobacterium spp.* imbalance, while only 12.5% of young men had reduced concentrations of these bacteria in the intestinal microbiome. Among

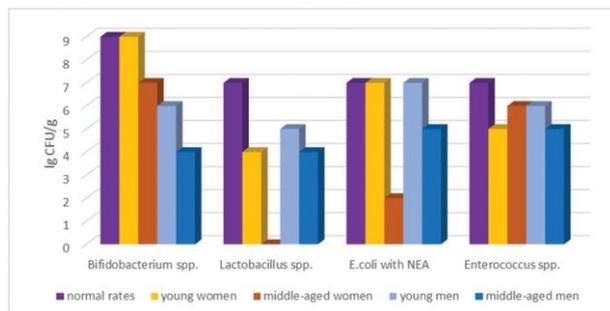


Fig. 4. Comparative analysis of symbiotic intestinal microbiota quantitative indicators in patients with Crohn's disease

middle-aged women, quantitative changes were observed in 40% of the studied patients. The drop in the *Enterococcus spp.* titers had slightly less pronounced character. At a normal 10^7 - 10^8 CFU / g, a decrease in concentrations of *Enterococcus* species to 10^5 - 10^6 CFU / g was diagnosed in 66.6% of middle-aged men, 12.5% of young men, 14.3% and 20% of young and middle-aged women, respectively. There was also a decrease in the bacteria titers of the *E. coli* with NEA to 10^2 - 10^5 CFU / g against the standard indicators of 10^7 - 10^8 CFU / g in middle-aged patients – men (33.3%) and women (20%), while the quantitative content of typical *E. coli* in the intestinal microbiome remained within the normal range in young patients.

In 100% of patients with UC, a drop in the *Lactobacillus spp.* titers to 10^4 - 10^5 CFU / g was observed (Fig. 5). The largest deviations in the bacteria of the genus *Lactobacillus* composition were noticed in middle-aged men – a decrease in concentrations to 10^4 CFU / g. Unlike patients with CD, in those who suffer from UC, the titers of *Bifidobacterium spp.* remained within the normal range for all young patients, nevertheless, in 14.3% of middle-aged women and 37.5% of middle-aged men, the values ranged from 10^6 to 10^7 CFU / g. The largest deviations in the concentrations of *E. coli* bacteria with NEA were recorded in the group of middle-aged men (decrease to 10^2 CFU / g),

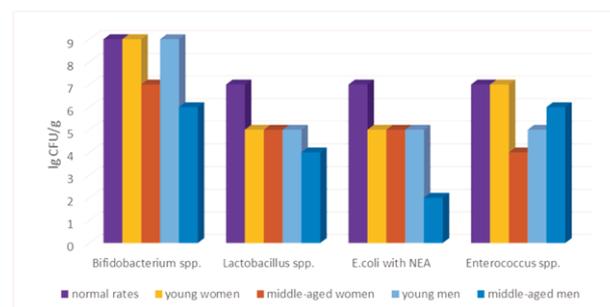


Fig. 5. Comparative analysis of symbiotic intestinal microbiota quantitative indicators in patients with ulcerative colitis

whereas in CD, such clinical picture was observed in the group of middle-aged women. In 42.9% of middle-aged women, 11.1% and 31.25% of young women and men, respectively, the concentrations of typical *E. coli* reached an average of 10^5 CFU / g. The titer of bacteria of the genus *Enterococcus* dropped to 10^4 - 10^6 CFU / g in almost all representatives of various categories: 18.75% of young men, 14.3% of middle-aged women and 12.5% of middle-aged men and only in young women *Enterococcus spp.* levels in the intestine remained within normal limits.

During the quantitative indicators analysis of opportunistic representatives in examined patients' intestinal microbiota, significant deviations of their concentrations were revealed in comparison with the permissible values. Among opportunistic microorganisms, bacteria of the genera *Staphylococcus*, *Proteus*, opportunistic enterobacteria (OE) of the genera *Enterobacter*, *Klebsiella* and *Citrobacter*, as well as fungi of the genus *Candida*, were most often distinguished.

In 20% of middle-aged women and 12.5% of young men with CD, bacteria of the genus *Proteus* were isolated at concentrations of 10^6 - 10^7 CFU / g, at a norm of $\leq 10^4$ CFU / g (Fig. 6), but microorganisms of this genus in individuals of other studied groups were not detected at all. OE in patients with CD was found in amounts of 10^7 - 10^8 CFU / g, which significantly exceeded the established norm. Most often OE was detected in young men – 62.5%, as well as in 42.85% of young women, 20% of middle-aged women and 33.3% of middle-aged men. Hemolytic strains of *E. coli* were isolated in 28.6% of young women, 37.5% of young men and 60% of middle-aged women in the amount of 10^7 - 10^8 CFU / g, with a norm of $\leq 10^4$ CFU / g. On the other hand, in middle-aged men, hemolytic *Escherichia* was not revealed at all. *Staphylococcus spp.* more often were stood out from middle-aged patients – in 50% of women and 66.6% of men, while bacteria of the genus *Staphylococcus* were less common in young patients (in 42.85% of women and 25%

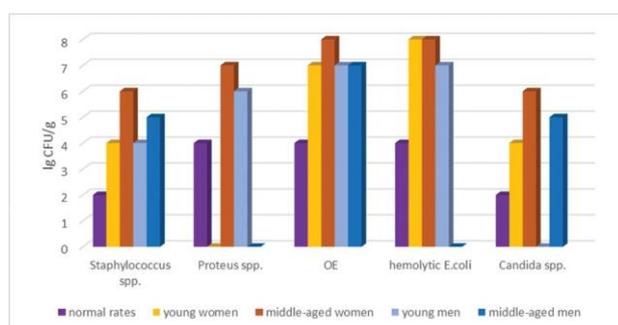


Fig. 6. Comparative analysis of opportunistic intestinal microbiota quantitative indicators in patients with Crohn's disease

of men). The highest titers of *Staphylococcus spp.* were recorded in middle-aged female patients – 10^4 CFU / g, with a norm of $\leq 10^2$ CFU / g. Significant deviations were shown in the content of *Candida spp.* in the intestinal microbiocenosis: in 28.6% of young women, as well as in 60% and 33.3% of middle-aged women and men, respectively, titers reached 10^4 - 10^6 CFU / g compared with normative indicators $\leq 10^2$ CFU / g, and it is important to note that more significant changes in quantitative indicators were shown in people of the middle-aged category.

Certain differences were also revealed among patients with UC of different sex and age in relation to opportunistic microbiota (Fig. 7).

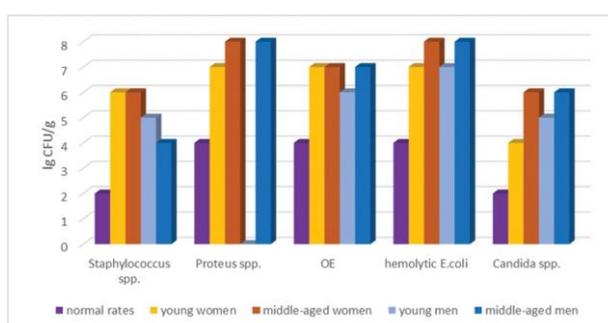


Fig. 7. Comparative analysis of opportunistic intestinal microbiota quantitative indicators in patients with ulcerative colitis

An increase in titers of hemolytic *E. coli* to 10^7 - 10^8 CFU / g while the normal range is $\leq 10^4$ CFU / g was observed in 37.5% of men and 28.6% of middle-aged women, against 12.5% and 11.1% of young patients respectively. In middle-aged individuals, a significant increase in *Candida spp.* titers was detected up to 10^4 - 10^6 CFU / g with a norm of $\leq 10^2$ CFU / g. Fungi of the genus *Candida* were isolated in 57.14% of middle-aged women, 37.5% of middle-aged men, 44.4% of young women and 25% of young men. In contrast to patients with CD, bacteria of the genus *Staphylococcus* in patients with UC were found in higher concentrations (10^5 CFU / g) *videre licet* in young men, but in a smaller number of people (25% against 75% of middle-aged men). In representatives of the female sex *Staphylococcus spp.* were also isolated more often in middle-aged patients (57.14%) than in young (5.5%) ones. Certain differences in the *Proteus spp.* levels were observed in men with UC compared with men with CD: bacteria were not isolated from young patients, while in the middle-aged group, significant increases in titers up to 10^8 CFU / g were observed in 12.5% cases. Moreover, bacteria of the genus *Proteus* were identified in young women with UC (11.1%), when in young women with

CD *Proteus spp.* were not found. OE was detected in titers of 10^6 - 10^7 CFU / g with a norm of $\leq 10^4$ CFU / g. 1.6% of young women, 31.25% of young men, as well as 50% of men and 42.85% of middle-aged women showed a high degree of the intestine colonization by opportunistic enterobacteria.

A tendency towards an increase in the quantitative and qualitative indicators of the opportunistic microbiota content with parallel decrease in symbiotic microorganisms' titers that was found in the study confirms the interconnection between the intestinal microbiome and the development of IBD.

Inflammatory bowel diseases, which include ulcerative colitis and Crohn's disease, constitute a group of autoimmune multisystem diseases that are characterized by certain clinical and pathological signs, the existence of nonspecific inflammation in the intestine and possible extraintestinal manifestations and complications.

Despite the fact that etiopathogenesis of CD and UC has not yet been established, recent studies have shown that a deregulated immune response against commensal microbiota components plays a decisive role in the pathophysiology of IBD, since compared with healthy people, patients with UC and CD have a reduced amount in the variety of useful symbionts and an increased number of pathogenic and opportunistic microorganisms. The influence of the intestinal microbiome in the diseases' development is associated with a deterioration in barrier function and an increase in intestinal permeability [9]. The balance of Th17 and Treg cells, which are characterized by pro-inflammatory and anti-inflammatory cytokines, is critical for the host's intestinal homeostasis and is directly affected by the normal gut microbiota content [1]. It was also found that a deficiency of anti-inflammatory immunoglobulin A, which inhibits the adhesion and invasion of bacteria in the intestine, correlates with a high level of *Escherichia coli* colonies [10]. All of the above gives reasons to consider dysbiosis as a modulator of the aberrant immune response in Crohn's disease and ulcerative colitis. Thus, the inflammatory bowel diseases are widespread in the world and pretty often lead to disability of patients. A lot of studies have established that intestinal microbiocenosis takes part in the pathogenesis of these diseases and has been exposed to significant changes in its composition. This assumption was established in a series of experiments conducted on animal models, and it was found that intestinal inflammation does not develop in sterile animals, since in the absence of intraluminal microorganisms the mucous membrane immune system is free from antigenic material which responsible for its activation [11].

The nature of dysbiosis, which is associated with IBD, characterized by reducing the commensal microbiota diversity, especially bacteria of the genera *Bifidobacterium*, *Lactobacillus* and the comparative increasing in the number of bacterial species belonging to the *Enterobacteriaceae* family.

The genus of *Bifidobacterium* includes gram-positive bacteria, which are presented in significant numbers in healthy people and make up about 95-99% of the total resident intestinal microbiota. To date, preclinical studies have shown that various strains of the *Bifidobacterium* genus have a positive effect on the prevention and treatment of colitis, namely: these bacteria demonstrate immunoregulatory effects, improve the intestinal epithelium barrier function and contribute to the normalization of the intestinal microbiota. *Bifidobacterium longum subsp. infantis 35624* selectively stimulates the specialization of Treg FoxP3 cells and / or induces the production of IL-10 in animal models and people with IBD. In addition, clinical studies of patients with UC and other inflammatory diseases have shown that the injection of this immunobiotic strain led to a marked decrease in the C-reactive protein level in plasma [12].

Several strains belonging to the genus of *Lactobacillus* are also representatives of the commensal microbiome and are constantly located in the human gastrointestinal tract. Nowadays, a lot of studies have depicted that strains belonging to the *Lactobacillus* genus regulate the gastrointestinal tract inflammation in a favorable way through strain-specific and health-beneficial mechanisms. Moreover, clinical studies conducted to date have shown that probiotic mixtures that contain four bacteria types of the *Lactobacillus* genus (VSL # 3) and *Lactobacillus reuteri* ATCC 55730 have a positive effect in the treatment of acute UC [12,13].

Some other studies have reported that certain bacterial antigenic signals, such as the retinoic acid of *Clostridium cluster IV and XIVa*, and polysaccharide A of *Bacteroides fragilis* and *Faecalibacterium prausnitzii*, are involved in triggering an immune response and the accumulation of Treg cells [1]. Animal model studies suggest that intestinal microbiota plays an important role in mucosal immune homeostasis and especially in the balance of Th17 / Treg cells, which is considered to be the dominant factor in the induction and inhibition of colon inflammation.

Recently, Gevers and colleagues demonstrated a correlation between changes in the species' diversity of microbiocenosis and disease activity index in patients with Crohn's disease [14]. To assess the colitogenic potential of certain types of bacteria that cause chronic inflammation, animal models infected with

intestinal pathogens, were examined. For example, *Bacteroides fragilis*, which is part of the human resident microbiota, causes chronic intestinal inflammation in mice. Similarly, an increase in the number of opportunistic species *Klebsiella pneumoniae* and *Proteus mirabilis* correlated with the severity of colitis in animal models [1].

Another microorganism often associated with inflammatory bowel disease is *Mycobacterium avium subsp. MAP (Mycobacterium avium subspecies paratuberculosis)* is a strictly aerobic pathogenic bacterium, which is now considered as a potential trigger in the etiopathogenesis of inflammatory bowel diseases. It has been found that MAP induces chronic colitis in cattle and other species, including primates and dogs. MAP-induced intestinal inflammation in cattle is called John's disease, and it almost imitates the clinical and histological features of human Crohn's disease. Evidence of the involvement of MAP in intestinal inflammation has been obtained in many studies of patients with CD who had higher levels of MAP DNA in the mucous tissue compared to healthy controls. Moreover, the participation of MAP in the pathology of inflammatory bowel diseases is confirmed by the presence of antibodies and reactive T-cells against MAP in patients with CD [15].

The current believing is that there is no well-defined evidence to prove that *Clostridium difficile* could be considered as a causing factor in IBD, however, its frequent association with IBD patients more likely shows that *Clostridium difficile* infection is secondary, and contributes to exacerbation in IBD pathogenesis [1, 16].

The intestinal microbiota also metabolizes nonabsorbent dietary fibers and produces short chain fatty acids such as butyrate and propionate. Butyrate is an important energy source for intestinal epithelial cells and led to increase the production of mucin and antimicrobial peptides. Therefore, in patients with inflammatory bowel diseases and dysbiosis, a decrease in the concentration of butyrate is observed in the analysis of feces microbiome. In accordance with this, in inflammatory bowel diseases, a slump in bacteria such as *Faecalibacterium prausnitzii*, which produce butyrate is also observed [17].

To conclude, a deeper understanding and constant monitoring of the interconnection between the intestinal microbiome and the clinical manifestations of Crohn's disease and ulcerative colitis can provide important information regarding the pathophysiology of inflammatory bowel diseases.

Conclusion

1. Due to examination results of patients with IBD of various age categories and sex it has been re-

vealed significant imbalances in the gastrointestinal tract microbiome in 100% of patients.

2. The decompensated form of dysbiotic disorders in patients with CD prevailed among both sex – 34.8% of women and 30.45% of men, while among patients with UC a subcompensated form of dysbiosis was found mainly in young women – 20.4%. In both CD and UC, the frequency of decompensated dysbiotic form dominated in middle-aged patients.
3. The largest changes in the analysis of symbiotic microbiota representatives were observed in the composition of *Lactobacillus spp.*, the concentrations of those were reduced in 100% of patients with inflammatory bowel diseases. Significant deviations in the *Bifidobacterium spp.* composition to 10^4 - 10^7 CFU / g were mainly detected in middle-aged patients with CD: 66.6% of men and 40% of women. Significant decrease in titers of *E. coli* with NEA was observed in patients with UC: in 42.9% of middle-aged women and 31.25% of young men.
4. In the analysis of opportunistic microbiocenosis, it was found that among all patients with IBD, a high detection frequency of bacteria of the genus *Staphylococcus* and fungi of the genus *Candida* was observed in middle-aged people. Hemolytic biovars of *Escherichia coli* were predominantly allocated from middle-aged male (37.5%) and female (60%) patients with IBD. Indicators of opportunistic enterobacteria were highest in patients with UC: in middle-aged men and women (50% and 42.85% respectively), while in patients with CD, the detection frequency of OE was higher in young people (42.85 % of women and 62.5% of men).

Prospects of further researches. Inflammatory bowel diseases are a global emerging challenge in the health sector. Among the potential causative agents of these diseases can be the intestinal microbiota, which was proposed as a new factor, since the obvious role of the intestinal microbiota in the host's physiology was confirmed by the interconnection between the imbalance of the intestinal microbiome (dysbiosis state) and host's metabolic disorders. It should be noted that in addition to bacteria, a significant number of viruses and fungi colonize the gastrointestinal tract. Insufficient research and conflicting data require more clinical trials to better understanding this ecosystem and clarifying the dysbiosis concept in inflammatory bowel diseases as a causative factor, as well as to identifying potential bacterial strains that are involved in the pathogenesis of these diseases. In addition, a better understanding of the dysbiotic changes characteristics in patients of different age categories and sex with inflammatory bowel diseases will allow us to choose effective probiotics, which are now considered as an alternative or adjuvant approach to traditional therapy which is aimed at correcting intestinal microbiocenosis and modulating the host's immune system.

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ВИЗНАЧЕННЯ СКЛАДУ МІКРОБІОМУ КИШЕЧНИКА У ПАЦІЄНТІВ РІЗНОГО ВІКУ ТА СТАТІ З ХВОРОБОЮ КРОНА ТА НЕСПЕЦИФІЧНИМ ВИРАЗКОВИМ КОЛІТОМ

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Резюме. Дослідження взаємозв'язку між порушенням кількісного та якісного складу мікробіому шлунково-кишкового тракту і розвитком запальних захворювань кишечника потребує постійного моніторингу та більш детального вивчення через зростання захворюваності в усьому світі й суперечливість даних стосовно гендерних і вікових відмінностей перебігу захворювань.

Було досліджено 72 зразка біологічного матеріалу з товстої кишки. Ідентифікацію мікроорганізмів проводили згідно з Визначником бактерій Берджі та за допомогою хромогенних поживних середовищ "Graso Biotech". Для статистичного аналізу даних використовували описову та індуктивну статистику. Всі розрахунки виконували у програмі SPSS 9.0 for Windows.

За результатами аналізу було виявлено суттєві зміни в мікробному пейзажі травної системи в пацієнтів з хворобою Крона та неспецифічним виразковим колітом. Було показано, що у хворих середнього віку

частота виявлення декомпенсованої форми дисбіотичних порушень була вищою порівняно з молодими пацієнтами. При аналізі симбіотичної мікробіоти значні відхилення у складі *Bifidobacterium spp.* було встановлено у пацієнтів середніх років із хворобою Крона. Зменшення титрів типових *Escherichia coli* зазвичай спостерігалось у хворих на неспецифічний виразковий коліт. При аналізі умовно-патогенної мікробіоти було показано, що серед усіх хворих на запальні захворювання кишечника найвища частота виявлення *Staphylococcus spp.* та грибів роду *Candida* була зареєстрована в осіб середнього віку. Показники виявлення умовно-патогенних ентеробактерій були найвищими у хворих на виразковий коліт чоловіків та жінок середнього віку, в той час як у хворих на хворобу Крона частота виділення умовно-патогенних ентеробактерій була вищою в осіб молодого віку.

Ключові слова: дисбіоз кишечника, хвороба Крона, неспецифічний виразковий коліт, умовно-патогенна мікробіота, асоціативна симбіотична мікробіота.

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ОПРЕДЕЛЕНИЕ СОСТАВА МИКРОБИОМА КИШЕЧНИКА У ПАЦИЕНТОВ РАЗНОГО ВОЗРАСТА И ПОЛА С БОЛЕЗНЬЮ КРОНА И НЕСПЕЦИФИЧЕСКИМ ЯЗВЕННЫМ КОЛИТОМ

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Резюме. Исследование взаимосвязи между нарушением количественного и качественного состава микробиома желудочно-кишечного тракта и развитием воспалительных заболеваний кишечника требует постоянного мониторинга и более детального изучения из-за роста заболеваемости во всем мире и противоречивость данных о гендерных и возрастных различиях течения заболеваний.

Было исследовано 72 образца биологического материала из толстой кишки. Идентификацию микроорганизмов проводили согласно с Определителем бактерий Берджи и с помощью хромогенных питательных сред "Graso Biotech". Для статистического анализа данных использовали дескриптивную и индуктивную статистику. Все расчеты выполняли в программе SPSS 9.0 for Windows.

По результатам анализа были обнаружены существенные изменения в микробном пейзаже пищеварительной системы у пациентов с болезнью Крона и неспецифическим язвенным колитом. Было показано, что у больных среднего возраста частота выявления декомпенсированной формы дисбиотических нарушений была выше в сравнении с молодыми пациентами. При анализе симбиотической микробиоты значительное отклонения в составе *Bifidobacterium spp.* было установлено у пациентов средних лет с болезнью Крона. Уменьшение титров типичных *Escherichia coli* обычно наблюдалось у больных неспецифическим язвенным колитом. При анализе условно-патогенной микробиоты было показано, что среди всех больных воспалительными заболеваниями кишечника высокая частота выявления *Staphylococcus spp.* и грибов рода *Candida* была зарегистрирована у лиц среднего возраста. Показатели выявления условно-патогенных энтеробактерий были самыми высокими у больных язвенным колитом мужчин и женщин среднего возраста, в то время как у больных болезнью Крона частота выделения условно-патогенных энтеробактерий была выше у лиц молодого возраста.

Ключевые слова: дисбиоз кишечника, болезнь Крона, неспецифический язвенный колит, условно-патогенная микробиота, ассоциативная симбиотическая микробиота.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

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