ORIGINAL ARTICLE

CONTENTS

Specifics of barrier function impairment of the large intestine in patients with ulcerative collitis and joint damage

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ABSTRACT

Aim: To study the characteristics of barrier function impairments of the large intestine in patients with UC and concomitant joint damage.

Materials and Methods: At the clinical base of the Department of Therapy and Family Medicine, 80 patients with inflammatory bowel disease (IBD) were examined. Patients with IBD were divided into two groups. Group 1 (n=78) included patients with IBD and diagnosed with joint damage, namely spondylarthritis (SPA), and group 2 (n=40) included patients with IBD without joint damage. Patients were tested to determine the level of fecal calprotectin (FC), a1-antirpsin (a1-AT) and zonulin. Changes in the quantitative and qualitative composition of the colon microflora were assessed.

Results: Patients with UC have been diagnosed with large intestine dysbiosis (LID), which is more pronounced in patients of the 1st group. Stage 3 dysbiosis was 17,3 % ($p<0.01$) more likely to be found in patient of the 1st group, and stage 4 dysbiosis - 12,9 % ($p<0.05$) more likely. The level of zonulin, both in blood serum and in feces, was significantly higher in patients with UC compared to controls. In group I patients we found a significant increase in zonulin compared to group II patients (by 1.5 times in blood serum and by 1.4 times in feces, $p<0.01$). Identical results were observed when analyzing the levels of α1-AT in blood, feces, as well as its clearance (1.6 times ($p<0.01$), 1.3 times ($p<0.05$) and 1.4 times ($p<0.01$) accordingly).

Conclusions: Increased levels of zonulin and a1-AT in blood serum and feces was found in patients with UC, which indicates an increased permeability of the intestinal wall in these patients. At the same time, more pronounced changes indicating intestinal barrier function impairment were found in patients with UC and SPA. In patients with UC and SPA, a direct correlation between the change in intestinal permeability and LID severity was established (fecal zonulin levels correlate with LID stages III and IV- $r=0.94$; $p<0.01$ та $r=0.88$; $p<0.01$ accordingly).

KEY WORDS: inflammatory bowel disease, ulcerative colitis, joint damage, spondylarthritis, diagnostics, calprotectin, a1-antitrypsin, zonulin, dysbiosis

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INTRODUCTION

Inflammatory bowel disease (IBD) - is a chronic inflammatory disease of the gastrointestinal tract (GI tract), which includes two clinical manifestations: Crohn's disease (CD) ulcerative colitis (UC). A recent study, conducted by the Center of disease prevention and control according to the 2015 National Health Survey has shown that IBD is actually a more widespread disease than was previously reported. Their analysis showed that IBD affects 3.1 million or 1.3% of the US adult population, approximately three times higher than previous estimates [1].

IBD is often associated with extraintestinal manifestations (EIMs). EIMs during IBD are so widespread that these diseases should be considered as systemic and not limited to the GI tract alone [2]. EIMs are a common phenomenon both in Crohn's disease and UC. Almost any organ system can be affected, including musculoskeletal, dermatological, renal, hepatopancreatobiliary, and pulmonary systems [3]. In general, peripheral arthritis, aphthous ulcers in the oral cavity, episcleritis, or erythema nodosum may be associated with active intestinal inflammation and may improve with standard treatment of intestinal inflammation. However, anterior uveitis, ankylosing spondylitis, and primary sclerosing cholangitis usually occur independently of disease outbreaks [4].

Arthropathy is common feature among patients with IBD, and these disorders are known as spondylarthritis (SPA). Depending on the initial symptoms, spondylarthritis is divided into axial and peripheral. Radiological signs of sacroiliitis are observed in approximately 15-27% of patients with UC [5].

The pathogenesis of EIMs in IBD is unclear, but is believed to involve immune and genetic factors. It is believed that the immune response can be induced at extraintestinal areas by the diseased mucosa of the GI tract due to common epitopes at different areas. Bacteria translocating across the permeable intestinal barrier elicit an adaptive immune response that ultimately fails to distinguish between bacterial epitopes and epitopes of joints and skin [1].

Thus, the study of the characteristics of barrier function impairments of the large intestine and its relation to joint damage in UC is currently extremely relevant.

AIM

The aim of the research to study the characteristics of barrier function impairments of the large intestine in patients with UC and concomitant joint damage.

MATERIALS AND METHODS

Complex evaluation and treatment of patients were conducted at the clinical base of the Department of Therapy and Family Medicine Faculty of postgraduate education of Uzhhorod National University. The clinical study included 118 patients with UC. Patients with UC were divided into 2 groups depending on joint damage or the lack of it:

- group I included of 78 patients with UC who have been diagnosed with joint damage, namely SPA. Group I consisted of 63 males (80.8 %) and 15 (19,2 %) females. Average age was 51.3±4.7 years.

- group II included 40 patients with UC without joint damage. Group I consisted of 11 males (27.5 %) and 29 (72.5 %) females. Average age was 48.9±5.3 years.

The control group included 30 practically healthy individuals (18 male (60.0%) and 12 (40.0%) female). Average age was 50.7±5.5 years.

All studies were conducted with patients consent. Written informed consent for appropriate diagnosis and treatment was obtained from all patients and controls, and all measures were taken to ensure data depersonalization. The research methodology complied with the Helsinki Declaration of Human Rights of 1975 and its 1983 revision, the Council of Europe Convention on Human Rights and Biomedicine, as well as Ukraine's legislation.

Exclusion criteria were age under 18 and over 75; Crohn's disease; lactose intolerance, gluten intolerance; celiac disease; surgical interventions on the intestines, including appendectomy for up to 6 months prior; colon cancer; doligosigma; diverticulosis of the colon; type 1 diabetes; type 2 diabetes (decompensated stage); mental illnesses that do not allow an adequate assessment of the patient's state of health and the signing of consent for diagnosis and treatment; pregnancy and lactation; HIV infection; oncological diseases.

UC was diagnosed according to IBD diagnostic standards. The diagnosis of UC was verified using endoscopic (rectomanoscopy, sigmoscopy, colonoscopy) and morphological testing. The activity of fecal calprotectin (FC) was determined by enzyme-linked immunosorbent assay (ELISA) using the Tecan Sunrise test system from ImmunDiagnostic (Germany).

SPA with predominant damage to the spine was diagnosed based on physical and clinical examination methods, as well as the results of imaging methods (radiological examination, computed tomography or magnetic resonance imaging) of the spine. Functional assessment of spinal mobility and muscle strength of the back and abdominal core muscles was performed to study the mobility of the spine (Shober test, spine extension, fingertip to floor distance test, functional tests to determine the strength endurance of the back extensor muscles and the abdominal core muscles), as well as the Harris test and Lekens index according to the recommendations of the American Academy of Orthopaedic Surgeons (AAOS, 2018), American College of Rheumatology (ACR), 2018, European League Against Rheumatism (EULAR), 2018. Examination of the spine included inspection, palpation, and objective pain assessment.

In order to determine the barrier function of the intestine, the level of α1-antitrypsin (α1-AT) in blood serum and feces was assessed by ELISA using the test system of Immuniagnostic AG (Germany), and its clearance was calculated based on the obtained values. The level of zonulin was also determined in blood serum and feces by ELISA using the test system of the company "Elabscience" (USA).

The qualitive and quantitative composition of the microflora of the large intestine was studied. For this purpose, feces from patients were collected in dry, sterile dishes and delivered to the bacteriological laboratory no later than 2 hours after collection. The material was placed on a standard set of selective and differential diagnostic nutrient media for growth and isolation of aerobic and anaerobic microorganisms using a tenfold dilution method. Changes in the quantitative and qualitative composition of the microflora of the large intestine were determined using the unified practical classification of intestinal dysbiosis by Kuvaeva-Ladodo (1991), according to which 4 degrees of dysbiotic disorders are distinguished.

The analysis and processing of the results of the examination of patients was carried out with the help of the STATISTICA 10.0 computer program (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the obtained results.

RESULTS

Clinical symptoms indicating intestinal damage in examined patients with UC were evaluated (Table 1).

Table 1. Clinical manifestations of intestinal damage in examined patients

Clinical manifestation	Patients with IBD			
	Group 1 (n=78)		Group 2 (n=40)	
	Abs. number	%	Abs. number	%
Frequency of bowel movements:				
- 1-5 times a day	49	62.8 %**	15	37.5 %
- 6-10 times a day	22	28.2 %	18	45.0 %**
- more than 10 times a day	7	9.0 %	7	17.0 %*
Pain along the colon	32	41.0 %	20	50.0 %
Meteorism	38	48.7 %*	12	30.0 %
Feeling of incomplete bowel movement	20	25.6 %*	5	12.5 %
Impurities in the stool:				
- blood	38	48.7 %	32	80.0 %**
- mucus	68	87.2 %	38	95.0 %

Note: the difference between the indicators in patients by groups is significant: * - $p<0.05$; ** - $p<0.01$.

The results show that clinical symptoms indicating UC are more pronounced in patients of the II group (without joint damage), namely, the frequency of diarrhea in the patients of the II group was on average 6-10 times a day, while in the patients of the I group the frequency of defecation was increased up to 5 times a day. Also, blood was significantly more often detected in the feces of group II patients (80.0% of patients - $p<0.01$).

After assessing the quantitative and qualitative composition of microorganisms in the feces of examined patients with UC in combination with joint damage, the degree of severity of colon dysbiosis in these patients was determined – Fig. 1.

As per the obtained results, it has been shown that patients with UC of the I group have more pronounced dysbiotic changes in the colon. At the same time, 17.3% ($p<0.01$) of patients of the I group were more often diagnosed with dysbiosis of the III degree, as well as 12.9% - with dysbiosis of the IV degree ($p<0.05$). In patients with UC of the II group (without SPA), when assessing the severity of dysbiotic changes, the II degree of severity was more often diagnosed.

The degree of activity of the pathological process was evaluated using the fecal calprotectin indicator in patients with UC – Fig. 2.

A significant increase in the level of FC in patients with UC of both groups was observed - up to 524.71±5.26 mkg/l in patients of the I group and up to 648.77±6.43 mkg/l in the examined subjects of the II group, while the normal range (23.15±0.66 mkg/l) was observed in the control group ($p<0.001$). It should be noted that a greater degree of activity was observed in patients of the II group with UC (Table 2).

The level of zonulin, both in blood serum and in feces, was significantly higher in patients with UC compared to controls (Table 2). Additionally, in patients with UC and SPA, a significant increase of this biomarker of intestinal lesions was established compared to the data in patients of the II group (by 1.5 times in blood serum and by 1.4 times in feces - $p<0.01$). Identical results were also observed when analyzing α1-AT levels in blood, feces, as well as its clearance (by 1.6 times ($p<0.01$), 1.3 times ($p<0.05$) and 1.4 times ($p<0.01$), respectively). The obtained results point at impairments in the intestinal barrier function in examined UC patients. At the same time, more pronounced changes were found in patients with UC and EIMS, namely SPA.

A correlational analysis was performed to determine the relationship between the degree of disease activity according to the FC level, the severity of large intestine dysbiosis (LID) and indicators of impaired intestinal permeability in these patients – Tables 3, 4.

Correlation analysis indicates a relationship between the change in the zonulin level in blood and feces and the severity of LID and the degree of inflammation according to the level of FC mainly in patients with UC and SPA. At the same time, the obtained results indicate a high specificity and sensitivity to changes in the level of zonulin in feces, for determining the impairment of the intestinal barrier function in patients with IBD. In patients of the II group, a correlation was established mainly between the change in the level of zonulin in feces and the studied parameters.

The a1-AT indicator, which also indicates increased permeability of the intestinal wall and impaired barrier function, correlates with the degree of activity of the pathological process in the intestine in patients with UC according to the FC level. A relationship between the severity of LID and the level of α1-AT mainly in feces was also observed, especially in cases of UC and concomitant SPA.

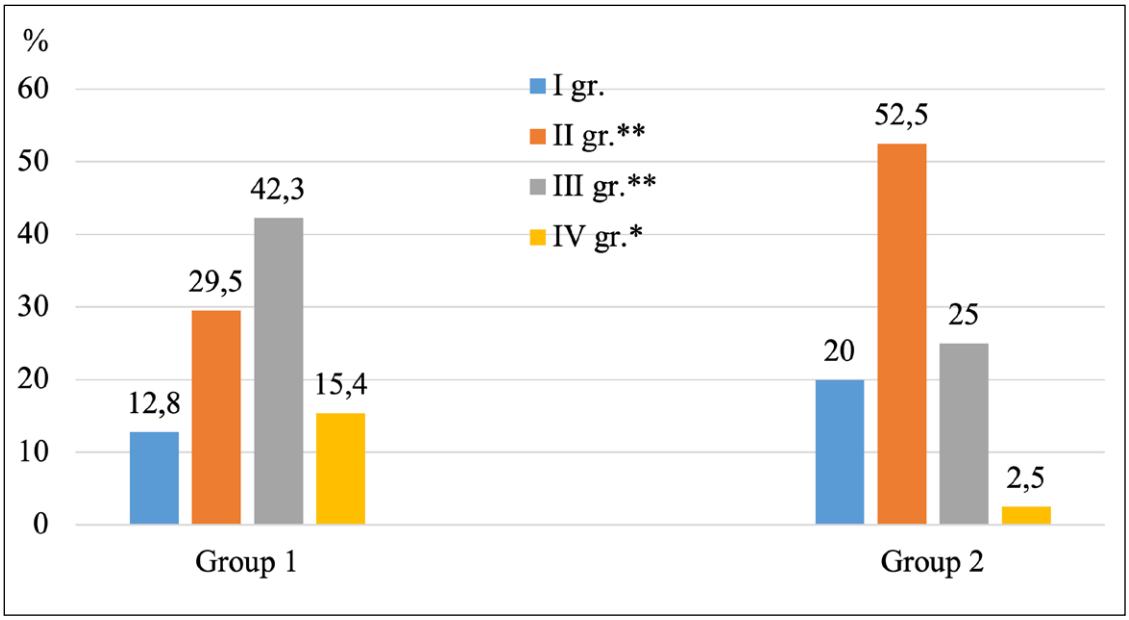

Fig. 1. Dysbiosis manifestation of the large intestine in examined patients with UC.
Note: the difference between the indicators in patients by groups is significant: * - $p<0.05$; ** - $p<0.01$.

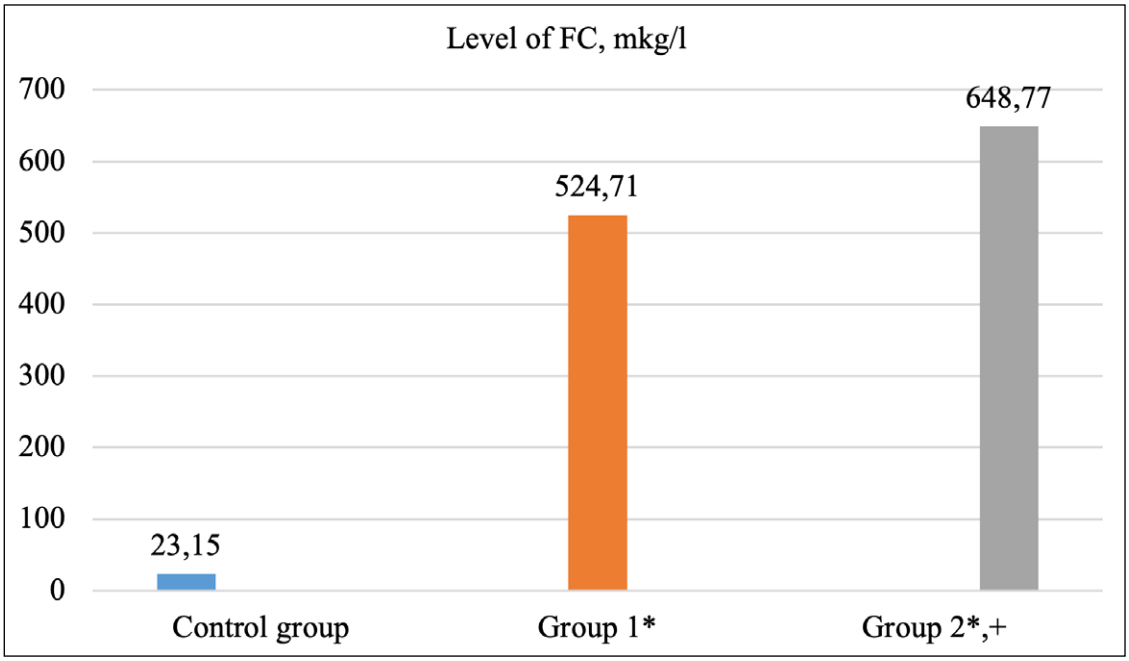

Fig. 2. The level of fecal calprotectin in the examined subjects.
Note: the difference between the values in patients with UC and the control group is significant: * - $p<0.001$;
the difference between the indicators in patients by groups is significant: + - $p<0.01$.

Therefore, an impairment of the intestinal barrier function is indicated by increased levels of zonulin and α1-AT in the blood and feces of patients with UC. The analysis of the obtained data makes it possible to assert the role of increased intestinal permeability in the progression of EIMS in UC, namely joint damage. At the same time, both the severity of LID and impaired intestinal permeability directly affect EIMS in patients with IBD, which should be taken into account in the diagnosis and treatment of these patients.

DISCUSSION

Several pathways by which the microbiota may contribute to EIMS in UC have been previously discussed. Due to increased permeability of the intestinal barrier, components of the microbiota, such as lipopolysaccharides, bacterial antigens or metabolites, can move from the intestine to extraintestinal organs or cause systemic inflammatory reactions [6]. Dysbacteriosis can lead to the activation of intestinal immune cell populations

Table 2. Indicators of biomarkers of intestinal barrier impairment in the examined subjects

Indicator	Control group (n=30)	Examined patients	
		Group 1 (n=78)	Group 2 (n=40)
zonulin:			
in blood serum, mg/dl	13.44±0.21	129.17±1.88***++	87.91±1.15**
in faeces, mg/dl	16.23±0.44	194.56±2.41***++	138.44±1.75**
α1-AT:			
in blood serum, mg/dl	118.77±2.08	388.12±3.46**++	244.17±2.26**
in faeces, mg/dl	13.69±0.15	39.55±0.38**+	29.56±1.29**
clearance α1-AT, ml/day	16.32±0.51	81.69±1.74***++	57.84±1.07**

Note: the difference between the indicators of the control group and the examined patients is statistically significant: * - p<0.05; ** - p<0.01; *** - p<0.001; between the indicators of patients in group 1 and group 2, the difference is statistically significant: + - p<0.05; ++ - p<0.01.

Table 3. Comparison of zonulin levels with the level of FC and severity of large intestine dysbiosis in examined patients

Indicator	Examined patients			
	I group		II group	
	Indicator			
	zonulin		zonulin	
	serum	feces	serum	кал
FC	r=0,90; p<0,01	r=0,96; p<0,01	_	r=0,96; p<0,01
LID II	r=0,76; p<0,01	r=0,78; p<0,01	r=0,80; p<0,01	r=0,82; p<0,01
LID III	r=0,82; p<0,01	r=0,94; p<0,01	_	r=0,84; p<0,01
LID IV	_	r=0,88; p<0,01	_	_

Table 4. Comparison of a1-AT levels with FC level and severity of large intestine dysbiosis in examined patients

Indicator	Examined patients		
	I group		II group
	Indicator		
	a1-AT		a1-AT
	serum	feces	feces
FC	r=0,80; p<0,01	r=0,86; p<0,01	r=0,78; p<0,01
LID II	_	r=0,80; p<0,01	r=0,70; p<0,05
LID III	_	r=0,84; p<0,05	_

that eventually migrate to other organs. Preliminary data suggest an increase in clostridia in patients with IBD and arthritis. Patients with SPA had decreased faecal microbial diversity (increased Ruminococcus gnavus and Dialister), but these patients did not suffer from IBD [7-9].

Arthritis is the most common EIMS of IBD, affecting axial joints, peripheral joints, or both. SPA affects both genders equally, with a higher prevalence in cases of Crohn's disease involving the colon than in cases of UC, and may occur before, concurrently with, or after the onset of IBD [10]. Our results indicate that UC is more often combined with SPA in males.

Currently the only biomarker approved and recommended by the European Crohn's and Colitis Organization (ECCO), is the commonly used fecal calprotectin. Inflammation in IBD leads to an acute phase response, detectable in serum and blood, characterized by increased concentrations of proteins involved in coagulation and fibrinolysis, such as fibrinogen and plasminogen; components of the complement system; proteinase inhibitors, including α1-antitrypsin, α1-antichymotrypsin; transport proteins such as haptoglobin and ceruloplasmin; and a number of other proteins, such as C-reactive protein (CRP) or ferritin. A Czech study examining 40 IBD patients for fecal and serum zonulin levels showed that both were elevated in patients with active Crohn's disease, which may be explained by the fact that zonulin is considered the best marker of increased permeability of the intestine [11].

Therefore, one of the important steps in understanding the role of intestinal permeability was the discovery

of zonulin [12]. Zonulin regulates intestinal permeability by modulating intracellular tight junctions. Zonulin, as an analogue of Vibrio cholerae toxin - zonula occludens (47 kDa protein), increases intestinal permeability in the small intestine and participates in the development of innate intestinal immunity. Circulating zonulin is considered as a potential biomarker of intestinal permeability [13]. A decrease in the intestinal barrier function may be related to the secondary activation of zonulin [14].

Various research is being conducted to study the relationship between zonulin and microbiocenosis, systemic inflammation and intestinal barrier dysfunction. A study by Zak-Golab A. et al. (2013) indicates a relationship between the level of zonulin and changes in the intestinal microbiocenosis, as well as systemic microcellular inflammation in patients with diabetes [13]. With the presence of inflamation, intestinal dysbacteriosis can "influence" epithelial cells to produce increased amounts of zonulin in the intestinal lumen and in the bloodstream which can lead to an impairment of the intestinal barrier function, that enables external antigens to enter the bloodstream and cause an excessive immune response, which, in in turn, leads to further permeability [15]. Results of a study by Caviglia GP et al. (2019) show, that serum zonulin levels are highly sensitive for assessing intestinal permeability in patients with IBD [16]. An increase in the concentration of zonulin in blood serum is associated with an increase in the functional activity of circulating neutrophils and an increase in the number of CD3+CD8+ cells, NK cells and a decrease in the number of CD19+ cells in patients with UC [17].

Therefore, our studies has shown that intestinal barrier function impairment is present in patients with UC, which is more pronounced in patients with EIMS and depends on the severity of LID.

CONCLUSIONS

1. In patients with UC, an increase in the level of zonulin and a1-AT in blood serum and feces was found, which indicates an increased permeability of the intestinal wall in these patients. At the same time, more pronounced changes indicating disturbances in the barrier function of the pocket were found in patients with UC and SPA. 2. In patients with UC and SPA, a direct correlation between the change in intestinal permeability and the severity of LID was established (the level of zonulin in feces correlates with LID III and IV - $r=0.94$; $p<0.01$ and $r=0.88$; $p<0.01$ respectively).

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CONFLICT OF INTEREST
The Author declare no conflict of interest

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