ORIGINAL ARTICLE

CONTENTS

Differentiated approach to management of patients with irritable bowel syndrome and ulcerative colitis in non-alcoholic fatty liver disease

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ABSTRACT

Aim: To determine the main clinical and laboratory features and severity of colon dysbiosis in irritable bowel syndrome (IBS) and IBD in patients with NAFLD.

Materials and Methods: 80 patients with NAFLD were examined. Patients were divided into two groups. Group 1 (n=40) included patients with NAFLD in combination with ulcerative colitis (UC), and group 2 (n=40) included patients with NAFLD and IBS (clinically manifested by diarrhoea). At patients diagnosed the level of faecal calprotectin (FC) and a1-antirpsin (a1-AT). Changes in the quantitative and qualitative composition of the colon microflora were assessed.

Results: In both groups of examined patients, a decrease of *Bifidobacteria* and *Lactobacilli*, as well as *Enterococcus* and *E. coli* with normal enzymatic properties was found compared with the control group. In patients with NAFLD and IBD, an increase in the level of FC was found in 23.8 times compared with the control group. As expected, there was an increase in the level of a1-AT in the blood serum, faeces and its clearance in patients of group 1.

Conclusions: In patients with NAFLD, both UC and IBS have similar clinical symptoms. An effective biomarker for differentiating and choosing treatment tactics in patients with NAFLD and UC is the determination of the level of FC.

KEY WORDS: non-alcoholic fatty liver disease, inflammatory bowel disease, ulcerative colitis, irritable bowel syndrome, diagnostics, calprotectin, a1-antitrypsin, colon dysbiosis

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INTRODUCTION

Non-alcoholic steatohepatitis (NASH) and benign steatosis are on the histologic spectrum of non-alcoholic fatty liver disease (NAFLD), a clinicopathological condition [1]. Experts estimate that a quarter of the world's population has NAFLD. The incidence of non-alcoholic steatohepatitis (NASH) is projected to increase by 56% over the next 10 years [2-4]. Such a rapid increase in the number of people with NAFLD is associated with the pandemic of obesity and type 2 diabetes [5].

In the pathogenesis of NAFLD, a significant role is assigned to the disturbance of the functional state of the colon and, as a result, the accumulation of microbial waste products and endotoxins. NAFLD and steatohepatitis are associated with increased intestinal barrier permeability and translocation of bacteria or bacterial products into the bloodstream. An experimental study has shown that intestinal epithelial barrier and intestinal vascular barrier disorders are early events in the pathogenesis of NAFLD [6].

Numerous studies have shown a strong association between inflammatory bowel disease (IBD) and NAFLD. In the study by Bessissow et al, the prevalence of NAFLD was 33.6% in patients with IBD. Also, study found that the prevalence of NAFLD was 8.2% in patients with IBD compared to patients without NAFLD. Many possible pathophysiological hypotheses have been proposed to explain this relationship, including disease-specific risk factors such as chronic inflammation, steroid exposure, drug hepatotoxicity, malnutrition, and altered gut microbiota [7-9].

Another global challenge of the 21st century is the COVID-19 pandemic. Many patients, whose number is constantly growing, have persistent gastrointestinal symptoms that they attribute to COVID-19. SARS-CoV-2, the virus that causes COVID-19, replicates in the gut, and acute COVID-19 is associated with changes in the gut microbiome. Gastrointestinal symptoms are present in half of patients with acute COVID-19, persist 6 months after COVID-19 in 10-25% of patients, and are rated as

the most distressing symptom in 11% of all patients. These symptoms include heartburn, constipation, diarrhoea and abdominal pain and decrease over time. The cause of prolonged gastrointestinal symptoms of COVID-19 is unknown, and hypotheses include the SARS-CoV-2 virus itself infecting the gastrointestinal tract; COVID-19, which may be accompanied by changes in the gut microbiome, a profound systemic inflammatory response and critical illness; and/or the impact of pandemic stress on gastrointestinal function and symptom perception, which may be unrelated to either SARS-CoV-2 or COVID-19 [10].

Thus, the study of the peculiarities of early diagnosis of conditions manifested by bowel lesions (irritable bowel syndrome (IBS), IBD) in patients with NAFLD and a differentiated approach to the management of such patients is currently extremely relevant.

AIM

The aim of the research to determine the main clinical and laboratory features and severity of colon dysbiosis in IBS and IBD in patients with NAFLD.

MATERIALS AND METHODS

At the clinical base of the Department of Procedure of Internal Diseases, 80 patients with NAFLD were examined. The examined patients with NAFLD for the period 2020 to 2024 were treated in the gastroenterological and endocrinological departments of the Municipal Non-Profit Enterprise «Andriy Novak Transcarpathian Regional Clinical Hospital» of the Transcarpathian Regional Council. Among the examined patients, there were 46 (57.5%) men, with an average age of 40.7±5.5 years; there were 34 (42.5%) women, with an average age of 39.5±4.8 years. The control group included 30 healthy individuals (18 (60.0%) men and 12 (40.0%) women). The average age was 44.3±4.7 years.

All studies were conducted with patient consent. Written consent was obtained from all patients and control subjects for appropriate diagnosis and treatment, with all measures taken to ensure data anonymity, and the methodology was in line with the Helsinki Declaration of Human Rights of 1975 and its 1983 revision, the Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation.

The exclusion criteria were as follows: age under 18 years and over 75 years, liver damage due to viral (hepatitis B, C, D viruses), alcohol etiology; Wilson-Conovalov disease; haemochromatosis; lactose intolerance, gluten intolerance; intestinal surgery, including appendectomy for up to 6 months; colon cancer; doligosigma; colon diverticulosis; type 1 diabetes mellitus; type 2 diabetes mellitus (decompensation stage); pulmonary tuberculosis; psychiatric diseases that do not allow adequate assessment of the patient's health status and signing an informed consent for diagnosis and treatment; pregnancy and lactation; acute myocardial infarction, stroke (in the history of up to 6 months); systemic autoimmune diseases; HIV infection; oncological diseases.

Patients with NAFLD were divided into two groups. Group 1 (n=40) included patients with NAFLD in combination with ulcerative colitis (UC), and group 2 (n=40) included patients with NAFLD and IBS (clinically manifested by diarrhoea).

The diagnosis of NAFLD (metabolic-associated fatty liver disease (MAFLD) or steatotic liver disease associated with metabolic disorders) was made in accordance with the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine of 06.11.2014 No. 826) and the EASL-EASD-EASO clinical guidelines for the diagnosis and treatment of these patients. The degree of liver damage was determined using online calculators NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), fibrotest, FibroIndex, Forns, APRI, and liver elastometry results. All patients underwent an ultrasound examination of the abdominal cavity according to the generally accepted methodology.

The diagnosis of UC was established according to the standards for the diagnosis of IBD. In all patients, the diagnosis of UC was verified using endoscopic (rectoromanoscopy, sigmoscopy, colonoscopy) and morphological methods of investigation.

The diagnosis of IBS was made on the basis of the IV Rome criteria and the clinical guidelines of the Ukrainian Gastroenterological Association for the management of patients with irritable bowel syndrome.

The activity of faecal calprotectin (FC) was determined by ELISA using the Tecan Sunrise test system, ImmunDiagnostic (Germany). The level of α1-antitrypsin (α1-AT) was determined in blood serum and faeces by ELISA using a test system from Immundiagnostic AG (Germany), and its clearance was calculated based on the obtained values.

To study the species and quantitative composition of the colon microflora, faeces were collected in dry sterile dishes and delivered to the bacteriological laboratory no later than 2 hours after collection without the use of preservatives. The material was sown on a standard set of selective and differential diagnostic nutrient media for the isolation of aerobic and anaerobic microorganisms by the method of tenfold dilution

Table 1. Clinical manifestations of intestinal lesions in the examined patients

Clinical manifestation	Patients with NAFLD	
	Group 1 (n=40)	Group 2 (n=40)
Diarrhoea	100.0 %	100.0 %
Frequency of bowel movements:		
- 1-5 times a day	12.5 %	92.5 %***
- 6-10 times a day	70.0 %***	7.5 %
- more than 10 times a day	17.5 %	0
Meteorism	47.5 %	65.0 %*
Pain along the colon	40.0 %	70.0 %**
Feeling of incomplete bowel movement	30. 0 %	75.0 %**
Impurities in the stool:		
- blood	55.0 %	0
- mucus	90.0 %***	10.0 %

Note: the difference between the indicators in patients by groups is significant: * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$.

(10^{-1}-10^{-9}). Changes in the quantitative and qualitative composition of the colon microflora were determined using the unified working classification of intestinal dysbiosis by Kuvaeva-Ladodo (1991), according to which 4 phases of dysbiotic disorders are distinguished.

The analysis and processing of the results of the examined patients were performed with the help of the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluation of the results.

RESULTS

Clinical changes indicating intestinal lesions in patients with NAFLD were evaluated (Table 1).

At all patients with NAFLD were diagnosed with diarrhoea. However, in patients of group 2, the frequency of stools in the vast majority of patients did not exceed 5 per day, while in patients of group 1, diarrhoea occurred more often up to 10 times per day (in 70.0% of cases). Mucus and blood impurities in the faeces are mainly diagnosed in patients with NAFLD and UC. However, flatulence is 27.5% more common in patients with NAFLD and IBS. Pain along the colon, as well as a feeling of incomplete emptying, were significantly more often diagnosed in patients with IBS in group 2.

A microbiological examination of the faeces was conducted to determine the peculiarities of the quantitative and qualitative composition of microorganisms in the colon lumen in the examined patients with NAFLD in combination with IBS or UC – Table 2.

In both groups of examined patients, a decrease of *Bifidobacteria* and *Lactobacilli*, as well as *Enterococcus* and *E. coli* with normal enzymatic properties was found compared with the control group. This was accompanied by an increase in the number and percentage of pathogenic and opportunistic microflora in both study groups of patients with NAFLD. However, in patients of group 1, more pronounced changes in the microbial composition of faeces in the colon were found.

A detailed analysis of the microbial composition of faeces made it possible to establish a difference in the data obtained, namely, in patients of group 1 with NAFLD and UC, an increased number of haemolytic form of *E.coli, Enterobacter, Citrobacter, Staphylococcus, Klebsiella, Clostridium, Candida* were significantly more often detected in the faeces. Therefore, the data obtained indicate pronounced dysbiotic changes in the colon in patients with NAFLD and UC, which is manifested by a decrease in normal microflora (*Bifidobacterium, Lactobacillus*) and an increase and activation of opportunistic microflora (mainly gram-negative forms of facultative anaerobes that acquired aggressive properties).

The level of faecal calprotectin and a1-AT in the examined patients with NAFLD was determined to study the severity of inflammation and intestinal barrier permeability in these patients (Table 3).

In patients with NAFLD and UC, an increase in the level of FC was found in 23.8 times compared with the control group, while in patients with NAFLD and IBS, the level of FC did not actually differ from the norm.

Interesting results were obtained regarding changes in the level of a1-AT. As expected, there was an increase in the level of a1-AT in the blood serum, faeces and its clearance in patients of group 1. However, patients

Table 2. Quantitative and qualitative composition of the colon microflora in the examined subjects

Indicator	Examined patients	
	Group 1 (n=40)	Group 2 (n=40)
Bifidobacterium:	Control group 100.0 % (8.55±0.07)	
frequency (%)	65.0 % **	75.0 %*+
lg colony forming units (CFU)/gr	5.71±0.06**	6.95±0.07**+
Lactobacillus:	Control group 100.0 % (6.74±0.09)	
frequency (%)	70.0 % **	80.0 % *+
lg CFU/gr	4.96±0.05**	5.58±0.07*+
E.coli (with normal enzymatic properties):	Control group 93.3 % (7.99±0.10)	
frequency (%)	75.0 % **	82.5 % *
lg CFU/gr	4.87±0.08**	6.76±0.07*+
E.coli (haemolytic form):	Control group 0 % (0.75±0.04)	
frequency (%)	22.5 %+	10.0 %
lg CFU/gr	4.74±0.08***++	2.93±0.06**
Enterococcus:	Control group 90.0 % (7.52±0.04)	
frequency (%)	55.0 %**	70.0 %*++
lg CFU/gr	5.12±0.09**	6.76±0.11*+
Enterobacter:	Control group 23.3 % (1.18±0.05)	
frequency (%)	50.0 %**+	40.0 %*
lg CFU/gr	3.04±0.05**+	2.12±0.04*
Citrobacter:	Control group 26.7 % (1.54±0.03)	
frequency (%)	60.0 %**+	35.0 %
lg CFU/gr	3.06±0.12*+	1.81±0.11
Staphylococcus:	Control group 26.7 % (3.31±0.07)	
frequency (%)	65.0 % **++	30.0 %
lg CFU/gr	4.99±0.14*+	4.08±0.12*
Klebsiella:	Control group 16.7 % (1.44±0.06)	
frequency (%)	45.0 %**+	30.0 %*
lg CFU/gr	3.86±0.10**+	2.75±0.14*
Clostridium:	Control group 16.7 % (4.56±0.16)	
frequency (%)	45.0 %**	27.0 %*
lg CFU/gr	5.82±0.12*+	4.58±0.14
Proteus:	Control group 10.0 % (0.55±0.03)	
frequency (%)	32.5 %**+	22.5 %*
lg CFU/gr	2.24±0.08***+	1.71±0.05**
Candida:	Control group 3.3 % (2.97±0.11)	
frequency (%)	20.0 %**	15.0 %*
lg CFU/gr	4.77±0.07**+	3.65±0.05*

Note: differences between the indicators of the control group and patients of groups I and II are significant: * - $p<0.05$; ** - $p<0.01$; ** - $p<0.001$; differences between the indicators of patients of groups I and II are significant: + - $p<0.05$; ++ - $p<0.01$.

with NAFLD and IBS also had a slight increase in the level of a1-AT. This indicates a violation of intestinal permeability and barrier function of the colon not only in patients with NAFLD and UC, but also in patients with a combination of NAFLD and IBS.

DISCUSSION

It is known that the disruption of the connection between the intestine and the liver is characterised by a number of pathogenic mechanisms, including a weakening of the intestinal barrier and increased intestinal permeability,

Table 3. Indicators of biomarkers of intestinal lesions in the examined patients

Indicator	Examined patients		
	Контрольна група (n=30)	Group 1 (n=40)	Group 2 (n=40)
FC, mkg/l	26.17±0.95	623.44±3.74***+++	35.71±0.42
α1-AT:			
in blood serum, mg/dl	124.15±1.85	397.50±3.81**++	171.23±1.56*
in faeces, mg/dl	14.22±0.16	40.06±0,50**+	23.88±0.31*
clearance α1-AT, ml/day	17.41±0.42	83.45±2.12**++	29.45±0.23*

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 groups I and II, the difference is statistically significant: + - p<0.05; ++ - p<0.01; +++ - p<0.001.

which leads to endotoxemia and inflammation, as well as changes in the profile of bile acids and levels of metabolites produced by the intestinal microbiome [11, 12]. It is particularly relevant and important to study changes in the functional activity of the intestine in patients with NAFLD and such common conditions as IBS and UC.

Calprotectin or the S100A8/S100A9 complex is located in the cytosol of neutrophilic granulocytes and is defined as an acute phase protein. Calprotectin is associated with a number of inflammatory conditions, including rheumatoid arthritis, psoriasis, and cardiovascular diseases. During inflammatory reactions, calprotectin is produced in increased amounts by neutrophils as a body's response to stress, performing its pro-inflammatory functions. Faecal calprotectin is used in clinical practice as a biomarker of active intestinal inflammation in patients with inflammatory bowel disease. Given the pathogenetic role of hepatic neutrophil infiltration and systemic inflammation in NAFLD, circulating calprotectin may reflect the involvement of neutrophilic inflammation in the pathogenesis of NAFLD. However, only a limited number of experimental studies have been conducted to investigate the role of calprotectin as a biomarker in patients with NAFLD, and the results have been controversial [13, 14].

Calprotectin levels are elevated as a sign of intestinal inflammation in obese patients with NAFLD. This is directly proportional to body weight, waist circumference and waist-to-height ratio. It is believed that FC, which is an easy-to-use and inexpensive biomarker, can be safely used to demonstrate the presence of intestinal inflammation in obesity [15].

Thus, the group of patients with NAFLD and symptoms of bowel lesions requires a detailed analysis of not only clinical manifestations, but also a mandatory element at the stage of research is to determine the level of FC, which makes it possible to diagnose IBD and differentiate it from functional bowel lesions.

CONCLUSIONS

In patients with NAFLD, both UC and IBS have similar clinical symptoms. An effective biomarker for differentiating and choosing treatment tactics in patients with NAFLD and UC is the determination of the level of FC.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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