ORIGINAL ARTICLE

ASSESSMENT OF COLON MICROBIOCENOSIS DISORDERS IN PATIENTS WITH CHRONIC HEPATITIS C

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ABSTRACT

The aim: To investigate the peculiarities of colon microbiocenosis disorders in patients with chronic hepatitis C.

Materials and methods: 142 patients with CHC were under observation, determination of the degree of liver fibrosis (FibroMax), bacteriological examination of stools and pancreatic elastase was performed.

Results: It was found that 59.2% of patients with CHC had gut dysbiosis (DB), of which 61.9% had increased body weight. Intestinal microbiocenosis disorders were manifested by constipation in 57.1% of patients, diarrhea in 31% of patients, and alternating constipation and diarrhea in 11.9% of patients. Bacteriologically, gut dysbiosis was characterized by suppression of the growth of normal microflora: *Escherichia coli* in 47.6%, *bifidobacteria* in 61.9%, *lactobacilli* in 53.6%, complete absence of *bifidobacteria* in 20.2% of cases. In patients with CHC combined with DB deep stages of liver fibrosis (F2-3 and F3-4) are registered 3.6 times more often compared to patients without intestinal dysbiosis (53.6% versus 24.1% and 11.9% versus 3.4%). The degree of gut DB increased in proportion to the stage of liver fibrosis ($p<0.05$). 32.1% of patients with CHC with dysbiosis were diagnosed with exocrine insufficiency of the pancreas.

Conclusions: Gut dysbiosis occurs more often in CHC patients with increased body weight and is characterized by constipation in 59.2% of patients. Intestinal microbiocenosis is characterized by suppression of the growth of normal microflora. In 32.1% of CHC patients with intestinal dysbiosis, according to the results of the pancreatic elastase-1 test, pancreatic exocrine insufficiency of various degrees was found.

KEY WORDS: chronic hepatitis C, colon microbiocenosis, liver fibrosis, pancreas

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INTRODUCTION

Identifying clinical signs typical for early intestinal dysbiosis in patients with CHC is a difficult task, because against the background of polymorbidity characteristic to this contingent of patients, various clinical symptoms are layered and masked, which does not allow clearly differentiating the clinical picture of one specific pathology [1,2].

For today the factors of progression of chronic hepatitis C (CHC), which have not been finally clarified, continue to be studied [3]. The activation and dominance of opportunistic microorganisms and their associations (*staphylococci, Proteus, Escherichia, Klebsiella, Pseudomonas aeruginosa and fungi*) worsen the functional state of the biliary tract and liver [4]. With a long course of intestinal dysbiosis, the risk of developing metabolic liver diseases, such as non-alcoholic fatty liver disease (NAFLD), cholestasis, hepatocellular dysfunction and dyskinetic disorders of the biliary tract increases [5-7]. The presence of NAFLD in patients with CHC contributes to the emergence and further progression of fibrosis from the initial stages to liver cirrhosis within a short time. On the one hand, the hepatitis C virus itself has a direct cytotoxic effect on the liver and, in a genotype-specific way, causes its fatty degeneration. On the other hand, insulin resistance, increased body weight, obesity, intestinal dysbiosis lead to the independent formation of NAFLD, which affects the development of the necrobiotic process in the liver tissue and leads to the progression of fibrosis [8-10].

There are several reasons for the development of microbiocenosis disorders in the large intestine. These are primarily immunodeficiency states that occur with severe infections, AIDS, tumors. The second common cause of dysbiosis is long-term treatment with antibiotics, which destroy not only pathogenic, but also beneficial microorganisms. The third cause of dysbiosis is the lack or absence of some digestive enzymes, especially in patients with impaired exocrine function of the pancreas. Normal human microbiota is a natural biosorbent of the gastrointestinal tract. According to modern ideas, the basis of the normal microbiota of the human colon is autochthonous obligate anaerobic bacteria (genera *Bifidobacterium, Lactobacillus, Propionibacterium*). Bacterial translocation from the intestine into the systemic circulation plays an important role in the progression of liver diseases, in particular CHC, but the literature data are contradictory [11-13].

Therefore, the study of the intestinal microbiome in patients with CHC and assessment of its relationship with the stages of liver fibrosis is relevant.

THE AIM

To investigate the peculiarities of colon microbiocenosis disorders in patients with chronic hepatitis C.

MATERIALS AND METHODS

142 patients with CHC were under observation, of whom 47.8% (65) were men, 54.2% (77) were women. The control group consisted of 20 healthy people. The diagnosis of HCV was made according to the International Classification of Diseases of the 10th revision and confirmed by the detection of total IgG class antibodies to HCV by enzyme immunoassay, as well as by the detection of RNA-HCV in the blood by polymerase chain reaction. General clinical, biochemical, serological, molecular genetic studies were conducted in certified laboratories. The functional state of the liver was assessed by the activity level of alanine and aspartic aminotransferases (ALT, AST), alkaline phosphatase (ALP), bilirubin, and gamma-glutamyl transpeptidase (GGT). The lipid spectrum of blood was determined: total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. All patients underwent USO of the abdominal organs and determination of the degree of steatosis and liver fibrosis using a non-invasive diagnostic method – FibroMax. Patients were evaluated for their trophic status according to generally accepted anthropometric indicators. Anthropometric criteria for obesity were considered to be the Quetelet index, or the body mass index (BMI), with the help of which 89 out of 142 (62.7%) people were found to have an increased body weight.

The state of microbiocinosis of the intestine was determined by microbiological examination of feces. To detect dysbiosis of the large intestine, a quantitative count of microorganisms that grew on agar, Saburo, Endo and 5% blood agar based on 1 g of feces was carried out, taking into account the dose of the inoculated material and the degree of its dilution. Identification of cultures was carried out on the basis of biochemical tests and the "Enterotest" system. At the time of material collection, all patients did not have acute infectious diseases, did not receive antibacterial, pre- or probiotic therapy. According to the unified working classification of dysbacteriosis (I.B. Kuvayeva, K.S. Ladodo, 1991), 4 degrees of intestinal dysbiotic disorders were distinguished:

I degree was characterized by a decrease in bifido- and *lactobacteria* 10^{5}-10^{6} CFU (with a norm of 10^{7}) and other microflora indicators within the norm.

II degree – reduction of *bifidobacteria* (10^{4}), *lactobacilli* (10^{4}), *Escherichia coli* with normal enzymatic activity (10^{5}) and growth of conditionally pathogenic microflora (proteus 10^{6}) of CFU.

III degree – a decrease in the number of bifido- and *lactobacteria* several times below the norm (10^{3} and 10^{3}) CFU and their complete absence, the appearance of proteus + hemolytic enterococci, proteus 10^{7}, replacement of full-fledged escherichia with bacteria of the genera *Klebsiella*, *Enterobacter*, *Citrobacter*, opportunistic flora acquires aggressive properties.

IV degree – is characterized by the complete absence of bifido- and *lactobacteria*, a significant decrease in the number of *Escherichia coli* and its qualitative changes, the subsequent increase and dominance of opportunistic and pathogenic microorganisms, fungi of the genera *Candida*.

Simultaneously with the bacteriological examination of feces, all patients underwent fecal coproscopy and determination of pancreatic elastase-1 and fecal calprotectin by ELISA method.

According to the obtained results, all patients were divided into two groups: 1st group (n=84) patients with CHC+DB – and 2nd group (n= 58) patients with CHC without intestinal DB; group 1, in its turn, was divided into 1a (n=56) – patients with CHC + DB and 1b (n = 28) – patients with CHC + DB + chronic pancreatitis (CP).

The analysis and processing of the results of the examination of patients was carried out using the Statistics for Windows v.7.0 computer program (StatSoft Inc, USA) using parametric and non-parametric assessment methods. The difference was considered to be significant at $p<0.05$.

RESULTS

It was established that 59.2% (84/142) of CHC patients had intestinal DB, of which 52 people had increased body weight, which was 61.9%.

Clinical manifestations of intestinal microbiocenosis disorders in 57.1% of patients consisted in the predominance of constipation (48/84), in 31% of people (26/84) ($p<0.001$) – the appearance of a typical diarrheal syndrome and in 11.9% of people (10/84) – alternating constipation and diarrhea. The predominance of constipation was contributed not only by intestinal DB, but also by other related factors, such as lifestyle. Thus, 35.4% of patients were engaged in mental work and led a sedentary lifestyle, 6.1% performed work that was associated with business trips in uncomfortable conditions, irregular nutrition was noted by 38.1% (32/84) of patients, insufficient use of vegetable fiber and liquid was noted by 16.7% (14/84) of patients, and only 4.8% (4/84) indicated self-administration of antibiotics 3 months before inclusion in the study. Intestinal dysfunction was observed in 53.6% (45/84) of patients in the form of a decrease in the number of defecation, changes in the consistency of fecal masses, the need for additional straining and the feeling of incomplete bowel emptying. These complaints were not found in patients with CHC without DB.

Also, 82.1% (69/84) of patients complained of reduced work capacity, headache, mood depression, and sleep disturbances, which confirmed the presence of asthenovegetative syndrome. In patients with CHC without intestinal DB asthenovegetative syndrome was noted in a smaller number of patients (32/58), namely in 55.2%.

The study of clinical manifestations of DB in patients with CHC revealed their connection with the degree of DB. It was established that 15 patients had DB of the I degree, DB of the II degree – 39, 30 patients of the III degree, and DB of the IV degree – was not detected in any patient.

Table I. Severity of liver fibrosis in the examined patients

Stage of fibrosis	Group, (abs./%)					
	1 (n=84) CHC+ DB			2 (n=58) CHC		
	1 genotype (n=75)	not 1 genotype (n=9)	total (n= 84)	1 genotype (n=52)	not 1 genotype (n=6)	total (n= 58)
F0-1	7/9,3	1/11,1	8/9,5	19 / 36,5	3 /50,0	22/37,9
F1-2	16/21,3	5/55,5*	21/25,0	18 /34,6	2 /33,3	20/34,5
F2-3	42/56*	3/33,3*	45/53,6*	13 /25,0	1 /16,7	14/24,1
F3-4	10/13,3*	0	10/11,9*	2 /3,8	0	2/3,4

Note. Significance of the difference:* – in comparison with the indicator of group 2 (the indicator is calculated according to the Mann-Whitney test, $p<0.05$).

Table II. Degree of dysbiosis and stages of liver fibrosis

Stage of fibrosis	Degree of dysbiosis (n=84) abs/%			
	1 (n=15)	2 (n=39)	3 (n=30)	4 ((n=0)
F0-1 (n=8)	5/62,5	3/37,5	0	0
F1-2 (n=21)	7/33,3	13/61,9*	1/4,8	0
F2-3 (n=45)	2/4,4	20/44,4	23/51,1*	0
F3-4 (n=10)	1/10,0	3/30	6/60*	0

Note. Significance of the difference:* – in comparison between degrees of dysbiosis ($p<0.05$)

In 9 patients with CHG with DB of the III degree periodical discomfort with localization in the lower abdomen was significantly more often registered (30.0%), compared to patients with I and II degrees of DB (13.3% and 20.5%), respectively. The presence of this localization of discomfort in patients with CHC is obviously explained by an increase in dysbiotic processes and a violation of intestinal function. This was confirmed by the presence of a reliable connection of discomfort in the lower abdomen with dyspeptic manifestations, such as: nausea (p=0.007), flatulence (p=0.02), decreased appetite (p=0.003).

The next most frequent complaint was flatulence, which was observed in 38.1% (32/84) of patients with DB. The frequency of flatulence increased against the background of the progression of DB and was one of the persistent dyspeptic complaints that are difficult to correct with medication in patients with CHC. Flatulence was constantly present in 100% of patients with the III degree of DB, in persons with the II degree it bothered much less often (69.4%), and was not registered at all in the I degree of DB.

Analysis of the results of microbiological research in patients with CHC showed that the intestinal microbiocenosis disturbance is characterized by inhibition of the growth of normal microflora: *Escherichia* coli in 47.6% (40/84 patients), *bifidobacteria* 61.9% (52/84), *lactobacilli* 53.6 % (45/84); complete absence of growth of *bifidobacteria* was found in 20.2% (17/84) of cases. In 22.6% (19/84) of patients, an increase in *Escherichia coli* with reduced enzymatic properties was noted. In general, quantitative and qualitative changes in *Escherichia coli* were found in 69.0% (58/84) of the examined persons. Along with deviations of the normal intestinal microflora, 54.8% (46/84) of 54.8% (46/84) of patients with CHC had opportunistic microflora, represented mainly by gram-negative bacteria. It was hemolytic *Escherichia coli* 43.4% (20/46), *Klebsiella* 30.4% (14/46), *Enterobacter* 15.2% (7/46). In addition, *Staphylococcus aureus* was isolated in 14.3% (12/84) of people and *Proteus* in significant amounts in 33.3% (28/84) of patients.

As the degree of CHC activity increased, there were statistically significant changes in the normal intestinal microflora, which mainly related to the number of *Escherichia coli, bifidobacteria, and lactobacilli.*

It should be noted that the expressiveness of the degree of liver fibrosis also depended on the presence of intestinal DB, and not only on the HCV genotype. It was established that in patients with CHC combined with intestinal DB, deep stages of liver fibrosis (F2-3 and F3-4) are registered 3.6 times more often compared to patients without DB (53.6% vs. 24.1% and 11. 9% against 3.4% $p<0.05$) (Table I).

The degree of intestinal microbiocenosis disorders increased in proportion to the stage of fibrotic changes in the liver ($p<0.05$) (Table II).

Simultaneously with the bacteriological examination of feces, all patients underwent fecal coproscopy, where the appearance of a small amount of neutral fat, altered muscle fibers, and extracellular starch in the stool of these patients made it possible to suspect a violation of the exocrine function of the pancreas and the formation of chronic pancreatitis (CP). Next, the exocrine function of the PZ was evaluated based on the results of pancreatic elastase-1 in feces, which gave the reason to distinguish a group of patients with CHC combined with CP. The obtained data showed that 32.1% (27/84) of patients with CHC under the

condition of DB have lower than normal levels of the fecal elastase test and correspond to: exocrine insufficiency of mild degree in 25.9% (7 out of 27) of patients, moderate in 55.5% (15) and severe – in 18.5% (5) people.

DISCUSSION

We have established various kinds of violations of the normal flora of the colon cavity in 59.2% of patients with CHC. We found similar studies in the literature, however, without dividing patients with CHC with different body weights [14]. Analyzing the degree of dysbiosis, we found a difference in the violations of the normal flora of the colon in patients with CHC with constipation and patients with CHC with exocrine pancreatic insufficiency. In the majority of CHC patients with constipation, the microbiocenosis of the colon was characterized by the disappearance or decrease in the number of obligate representatives (*Bifidobacterium spp. and Lactobacillus spp.*), an increase in the population level of conditionally pathogenic microflora (*Bacteroides spp, bacteria of the genera Clostridium*) and corresponded to the 2nd degree of DB. These patients had increased body weight, which, in our opinion, played a significant role in the formation of colon dysbiosis. Data on the participation of microbiota in the development of obesity have been proven by many studies [15-17]. There are a number of reports that the composition of the gut microbiota differs between overweight and lean or normal weight individuals, suggesting that microbiota imbalances may contribute to weight change. Changes in the intestinal microbiotic landscape lead to increased intestinal permeability, endotoxemia, which is a link in the pathogenesis of chronic systemic inflammation in CHC, contribute to the development of obesity and other metabolic-associated diseases [18].

In CHC patients with insufficiency of the exocrine function of the pancreas, the quantitative changes of the microflora were more pronounced, and they showed a significant, statistically reliable deficiency of bifidobacteria and lactobacilli compared to patients with normal exocrine function (p=0.02), and they more often had the 3rd degree DB. Violations of the intestinal microbiocenosis in patients with CHC of this group, in our opinion, are due to the long persistence of the virus and its direct cytotoxic effect on the pancreatocyte with the formation of exocrine insufficiency of the pancreas. Researches of recent decades prove the extrahepatic replication of the hepatitis C virus, which allows HCV infection to be considered not as a liver disease, but as a systemic (generalized) infection, which explains one of the mechanisms of the development of insufficiency of the exocrine function of the pancreas [19-21]. The data on the damage to the pancreas caused by the hepatitis C virus are consistent with the data of other scientists who report that concomitant CHC worsens the course of CP, as evidenced by reliable negative changes in the indicators of the exocrine activity of the pancreas [22]. Enzymatic insufficiency of the digestive tract leads to disturbances in digestion processes and entry of incompletely digested food ingredients into the intestinal cavity, which is a nutrient medium for microbial flora [23], as a result of which the formation of intestinal dysbiosis in patients with CHC is possible.

CONCLUSIONS

1. Intestinal dysbiosis occurs more often in CHC patients with increased body weight and is characterized by constipation in 59.2% of patients.
2. In CHC patients, the intestinal microbiocenosis is characterized by inhibition of the growth of normal microflora: *Escherichia coli* in 47.6% of patients, *bifidobacteria* in 61.9%, *lactobacilli* in 53.6%, and the complete absence of bifidobacteria growth in 20.2% of people.
3. In 32.1% of CHC patients with intestinal dysbiosis, according to the results of the pancreatic elastase-1 test, pancreatic exocrine insufficiency of various degrees was found.

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The Authors declare no conflict of interest.

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