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**Ya.M. Vakhrushev, A.P. Lukashevich*, E.V. Suchkova**
Izhevsk State Medical Academy, Department of Internal Diseases, Izhevsk, Russia

# ASSOCIATION OF INTESTINAL BACTERIAL OVERGROWTH AND DISEASES OF HEPATOBIARY TRACT

## Abstract

**The objective:** To find out the nature of the changes of the hepatobiliary system in patients with intestinal bacterial overgrowth syndrome and to study the possible mechanisms of their association. **Materials and methods:** 148 patients with intestinal bacterial overgrowth syndrome and intestinal dysbiosis were examined. The level of total cholesterol, cholestasis and cytolysis markers was determined in the blood using the analyzer of Labsystems (Finland). Intestinal bacterial overgrowth syndrome was assessed using a hydrogen breath test with lactulose on the LactophaH2 apparatus of AMA (St. Petersburg). Intestinal dysbiosis was determined by stool culture on nutrient media. Bile acids in bile were determined on the AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Ultrasound examination of the abdominal cavity was performed via the apparatus SHIMADZU SDN-500 (Japan). Liver elastography was performed using the AIXPLORER apparatus (France). **Results.** The syndrome of intestinal bacterial overgrowth in 67 % of cases was diagnosed in the presence of ileocecal insufficiency, in 33 % of cases — with preserved ileocecal function. The combination of intestinal bacterial overgrowth syndrome and intestinal dysbiosis was detected in 81.8 % of patients. The majority of the examined patients showed clinical symptoms of damage of the hepatobiliary system and intestines, which was confirmed by change of laboratory parameters: the increase in the level of total cholesterol, markers of cholestasis and cytolysis compared with the control group. In the study of bile acids in bile, the decrease of free (mainly cholic) and increase of conjugated (glycodeoxycholic, taurodeoxycholic, glycocholic, taurocholic) bile acids was observed compared with the control group. In general, patients with the syndrome of intestinal bacterial overgrowth revealed the presence of non-calculous cholecystitis — in 11.5 % of cases, I stage of cholelithiasis — in 25.7 %, II stage of cholelithiasis — in 18.9 %, and non-alcoholic fatty liver disease at the stage of steatosis and steatohepatitis — in 43.9 % of cases. **Conclusion:** Intestinal bacterial overgrowth syndrome is the beginning of bacterial translocation, which is the triggering factor for inflammation of the liver and biliary tract. In turn, diseases of the hepatobiliary system contribute to the development of intestinal dysbiosis by reducing the synthesis of bile acids with antibacterial action, as well as violations of their excretion. Thus, strong association of intestinal bacterial overgrowth syndrome with damage to the hepatobiliary system has been established.

***Keywords:*** *bacterial overgrowth syndrome, dysbiosis, cholelithiasis, non-alcoholic fatty liver disease, bile acids*

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ALT — alanine aminotransferase, AST — aspartate aminotransferase, GGTP — gamma-glutamyl transpeptidase, NAFLD — non-alcoholic fatty liver disease, BOS — bacterial overgrowth syndrome, AP — alkaline phosphatase

*Contacts. E-mail: anna.lukashevich.89@mail.ru

In recent years, the development of many digestive diseases has been associated with compositional disorders in the intestinal microbiota [1, 11, 12, 14, 18]. Intestinal dysbiosis, especially the bacterial overgrowth syndrome (BOS), increases the risk of developing metabolic liver disorders and biliary tract diseases [6, 10, 13, 15, 19, 20]. Most researchers associate hepatobiliary system functional disorders in BOS with the close anatomic-physiological relationship between the liver and the intestines [11, 13, 16]. However, there are still few studies on the features of hepatobiliary system damage depending on changes in the intestinal microbiota.

**Our objective** was to find out the nature of hepatobiliary system damages in patients with BOS and to study possible mechanisms of their associations.

## Materials and Methods

One hundred and forty-eight patients with intestinal dysbiosis and BOS were examined, among whom 17 patients had non-calculous cholecystitis, 38 — stage I cholelithiasis, 28 — stage II cholelithiasis, and 65 — non-alcoholic fatty liver disease (NAFLD) at the steatosis and steatohepatitis stage, respectively. There were 128 females and 20 males. The mean age of the female patients was 46.3±3.7 years, and of the male patients — 38.5±2.6 years.

Entry criteria for the study included the age of 18–60 years, diagnosed BOS and/or intestinal dysbiosis, and a signed voluntary informed consent. Exclusion criteria were the age under 18 and over 60 years, pregnancy and lactation, cancer.

General clinical data (medical history, physical examination) and biochemical blood assay including total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGTP), alkaline phosphatase (AP), total bilirubin using Labsystems analyzer (Finland) were used in examining the patients.

BOS was assessed through the lactulose hydrogen breath test using LactophaH2 manufactured by AMA (St. Petersburg). Hydrogen content increase in the air exhaled over 10 ppm from baseline during the first hour of testing was considered a positive result.

Intestinal dysbiosis was examined through stool culturing on aerobic and anaerobic microflora. Intestinal microflora was assessed by the counts of Escherichia, including its hemolytic and lactose negative forms, Lactobacilli and Bifidobacteria, Streptococcus, Enterococcus, Clostridia, Staphylococcus aureus, Klebsiella, yeast-like fungi, Proteus, Pseudomonas aeruginosa and other opportunistic microorganisms per 1 g [4]. The severity of dysbiosis was identified in accordance with the classification of I. B. Kuvaeva and K. S. Ladodo (1991) [9].

Bile acids in gallbladder and liver bile were assessed using AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Calculations were made by registering negative and positive ions in the $m/z$ range from 100 to 2,000. Capillary voltage was 4,500 V. Nitrogen with temperature of 300 °C and flow rate of 8 l·min$^{-1}$ was used as a drying gas. Bile was dissolved in distilled water at a ratio of 1:1. Further, 1 μl of the solution was diluted to 1 ml with water. The resulting figures were interpreted using DataAnalysis 4.0 (Bruker Daltonik GmbH, Bremen, Germany).

Abdominal ultrasound scanning was performed using SHIMADZU SDN-500 (Japan).

Liver elastography was performed to assess the degree of hepatic fibrosis by ultrasound elastography using AIXPLORER (France). The degree of fibrosis in hepatic parenchyma was assessed by the Metavir score system using the SC6-1 convex transducer.

The control group comprised of 40 apparently healthy individuals aged from 18 to 55 years.

The required number of observations was computed based on the sample-size calculation at the statistical power ρ=0.80 with the use of Statistica 6.1 software manufactured by Stat Soft. The patients were assigned to groups through stratified sampling. Parametric statistical methods were applied. Differences between the groups were considered statistically significant with a probability of the valid null hypothesis of no differences between the groups ($p$) < 0.05.

The study was carried out after the patients had signed voluntary informed consent as per Order No. 390n of the Ministry of Health and Social

Development of the Russian Federation dated April 23, 2012 (registered with the Ministry of Justice of the Russian Federation under No. 24082 on May 5, 2012).

## Results and Discussion

Among the patients examined, pain in the right hypochondriac region was observed in 112 (75.7 %) patients, in the paraumbilical area — in 53 (35.8 %) patients, and in the large intestine region — in 76 (51.4 %) patients. Dyspeptic complaints included: abdominal distention — in 126 (85.1 %) patients, bitter taste in the mouth — in 95 (64.2 %) patients, nausea — in 67 (45.3 %) patients, epigastric burning — in 59 (39.9 %) patients, eructation — in 46 (31.1 %) patients, constipation — in 38 (25.7 %) patients, diarrhea — in 33 (22.3 %) patients. According to physical examination, yellow coated tongue was found in 109 (73.6 %) patients, tender abdomen in the right hypochondriac region — in 105 (70.9 %) patients, in the paraumbilical area — in 65 (43.9 %) patients, and in the large intestine region — in 72 (48.6 %) patients. Hepatomegaly was observed in 37 (25 %) patients, positive Ortner's symptoms — in 44 (29.7 %) patients, Lepene's symptoms — in 35 (23.6 %) patients, Murphy's signs — in 29 (19.6 %) patients, Mussy's symptoms — in 22 (14.9 %) patients, Kehr-Gausman's symptoms — in 18 (12.2 %) patients, respectively. Thus, the clinical signs of intestine and hepatobiliary system disorders were identified.

In 67 % of cases, BOS was due to ileocecal failure (the hydrogen breath test results showed significant hydrogen increase in the air exhaled during the first hour of testing, without further decrease). In 33 % of cases, BOS was detected with ileocecal function retained (peaks of hydrogen increase in small and large intestines were found). When examined for BOS, intestinal dysbiosis was identified in 15 % of patients (no hydrogen increase occurred throughout the study).

Results of stool culture for dysbiosis in the patients examined showed a decrease in the number of normal microflora representatives: Bifidobacteria of less than $10^7$ CFU/g — in 72 (48.6 %) patients, Lactobacilli of less than $10^9$ CFU/g — in 55 (37.2 %) patients, Escherichia coli — in 44 (29.7 %) patients, Enterococcus — in 25 (16.9 %) patients, Bacteroides — in 22 (14.9 %) patients, respectively. Increase in lactose-negative E. coli was observed in 52 (35.1 %) patients, and hemolytic E. coli — in 32 (21.6 %) patients. Staphylococcus aureus was cultured in stool of 17 (11.5 %) patients, Klebsiella pneumoniae — of 15 (10.1 %) patients, and Candida — of 10 (6.8 %) patients, respectively. As for the severity of dysbiosis, degree I was identified in 41 (27.7 %) patients, degree II — in 96 (64.9 %) patients, and degree III — in 11 (7.4 %) patients, respectively. Combination of intestinal dysbiosis and BOS was detected in 121 (81.8 %) of the patients examined.

Abdomen ultrasound scanning showed signs of chronic non-calculous cholecystitis (gallbladder wall thickening over 3 mm and/or gallbladder dyskinesia) in 17 (11.5 %) patients, stage I cholelithiasis (sonographic signs by type of microliths and/or inhomogenous thick bile) — in 38 (25.7 %) patients, stage II cholelithiasis (formed gallstones) — in 28 (18.9 %) patients, NAFLD (liver enlargement, hyperechogenicity, liver sound conductivity and density reduction) — in 65 (43.9 %) patients. A number of patients had comorbid pathologies: combination of cholelithiasis and NAFLD — in 25 (16.9 %) patients, non-calculous cholecystitis and NAFLD — in 14 (9.5 %). Mean stiffness of hepatic parenchyma as per Metavir score system according to elastography performed in patients with NAFLD at the stage of hepatic steatosis corresponded to fibrotic changes F0 in 44 (67.7 %) patients, and at the stage of steatohepatitis — to F1 in 18 (27.7 %) patients, and F2 in 3 (4.6 %) patients, respectively.

As shown in Table 1, the majority of patients with BOS showed an increase in total cholesterol, cholestasis (GGTP, AP) and cytolysis (AST, ALT) markers as compared to the control group. The extent of changes in functional indicators of hepatobiliary tract was rather dependent on the type of damage to the liver and biliary tract. So, changes in laboratory parameters were less evident in patients with non-calculous cholecystitis, and maximum abnormalities were observed in patients with NAFLD.

As can be seen from the data provided in Table 2, bile portions B and C from patients with NAFLD and cholelithiasis show a decrease in free (mainly cholic) bile acids and an increase in conjugated (taurodeoxycholic, taurocholic, glycodeoxycholic, glycocholic) bile acids as compared with the control group. In non-calculous cholecystitis, increase in both free and conjugated bile acids was found as compared to the control group. According to literary data [5, 6], free bile acids are produced by the liver only, therefore, their decrease provides evidence of hepatocyte damage in patients with NAFLD and cholelithiasis. This is confirmed by the biochemical blood assays performed, the results of which have revealed increased markers of cytolysis and cholestasis, especially in patients with NAFLD. In cholestasis, bile acids can damage apical membranes of hepatocytes and destroy the epithelium of bile ducts and, consequently, increase the blood concentration of GGTP [7, 8]. In contrast, patients with non-calculous cholecystitis showed minimum biochemical abnormalities.

***Table 1.*** *The results of biochemical blood tests in patients with disorders of the intestinal microflora*

| Parameters | Patients with non-calculous cholecystitis (n=17) | Patients with I stage of cholelithiasis (n=38) | Patients with II stage of cholelithiasis (n=28) | Patients with non-alcoholic fatty liver disease (n=65) | Group of control (n=40) |
|---|---|---|---|---|---|
| Cholesterol, mmol/l | 5.13±0.07* | 5.26±0.05* | 5.15±0.16* | 5.54±0.06* | 4.34±0.08 |
| Alanine aminotransferase, units per liter | 17.12±2.92 | 20.17±1.14 | 23.84±2.85 | 45.0±3.8* | 18.63±0.82 |
| Aspartate aminotransferase, units per liter | 22.7±2.44 | 24.4±2.26 | 23.63±2.08 | 34.2±3.36* | 23.5±2.31 |
| Total bilirubin, umol/l | 13.25±0.63 | 11.85±0.58 | 12.4±1.92 | 14.6±1.2 | 11.61±1.36 |
| Alkaline phosphatase, mmol/l | 105±9.32* | 131±9.91* | 145.63±11.6* | 156.0±5.83* | 73.64±6.53 |
| Gammaglutamyltranspeptidase, units per liter | 27.18±2.3* | 30.09±2.44* | 51.67±4.15* | 49.0±8.6* | 18.5±0.76 |

**Note:** * — reliable changes in relation to group of control ($p < 0.05$); n — number of observations

***Table 2.*** *The bile acids content in bile in patients with disorders of the intestinal microflora (mg/ml)*

| Bile acids | Patients with non-calculous cholecystitis (n=8) | | Patients with non-alcoholic fatty liver disease (n=15) | | Patients with stage I of cholelithiasis (n=12) | | Group of control (n=10) | |
|---|---|---|---|---|---|---|---|---|
| | Portion B | Portion C | Portion B | Portion C | Portion B | Portion C | Portion B | Portion C |
| Cholic | 0.15±0.01* | 0.09±0.01 | 0.01±0.01* | 0.01±0.01* | 0.105±0.03 | 0.048±0.01* | 0.11±0.01 | 0.08±0.01 |
| Chenodeoxycholic | 0.06±0.02 | 0.02±0.01 | 0.03±0.02 | 0.041±0.03 | 0.035±0.03 | 0.01±0.01 | 0.07±0.02 | 0.03±0.01 |
| Glycocholic | 6.9±1.18* | 1.65±0.58* | 17.57±3.33* | 6.7±1.67* | 10.02±2.14* | 2.91±1.16* | 2.7±0.03 | 0.18±0.02 |
| Glycosodeoxycholic | 12.79±3.51* | 2.25±0.86* | 40.05±11.05* | 10.61±2.39* | 19.48±3.91* | 4.12±1.41* | 3.62±0.04 | 0.16±0.02 |
| Taurocholic | 5.37±2.1* | 1.42±0.16* | 6.07±3.5 | 2.45±1.16 | 5.13±2.09 | 1.56±0.95 | 1.15±0.02 | 0.08±0.01 |
| Taurodesoxycholic | 9.35±3.7* | 2.06±0.8* | 10.7±3.9* | 3.5±1.6 | 9.14±3.02* | 2.17±1.04 | 1.49±0.02 | 0.09±0.01 |
| Ursodeoxycholic | 0.07±0.03 | 0.01±0.01 | 0.14±0.05 | 0.03±0.02 | 0.136±0.02 | 0.035±0.03 | 0.1±0.01 | 0.02±0.01 |
| Deoxycholic | 0.06±0.03* | 0.02±0.01 | 0.07±0.06* | 0.08±0.04 | 0.11±0.04* | 0.07±0.04 | 0.22±0.02 | 0.03±0.01 |

**Note:** * — reliable changes in relation to group of control ($p < 0.05$); n — number of observations

The content of glycine conjugates statistically exceeded that in the control group for all the patients examined. Content of glycodeoxycholic and glycocholic acids in patients with non-calculous cholecystitis was lower than in those with NAFLD and cholelithiasis. Concentrations of taurine conjugates in patients with non-calculous cholecystitis had no significant differences from similar figures in patients from other groups, but significantly exceeded those in the control group. As is known from literary data, the degree of bile acid conjugation with glycine or taurine depends on diet and intestinal microflora [1, 8]. Thus, changes in conjugated bile acid concentrations in all the examined patients were unidirectional and were due to intestinal microbiota disorders.

As compared to the control group, concentrations of ursodeoxycholic acid tended to increase in patients with cholelithiasis and NAFLD and to decrease in patients with non-calculous cholecystitis. Deoxycholic acid in bile portion B was significantly lower in all patient groups as compared to the control group. Bile portion C showed no substantive changes in deoxycholic acid concentrations in the patient groups examined.

Changes in bile acid composition are attributable to their increased absorption in ileum in BOS [1]. In addition, BOS is the beginning of bacterial translocation [12, 15]. There are microorganism that are most exposed to translocation due to their adhesiveness to intestinal epithelium (Klebsiella, Enterococcus, Escherichia coli). The examined patients showed a decrease in content of typical Escherichia with an increase in lactose negative and hemolytic forms. These bacteria can even penetrate the histologically unchanged mucous membrane of intestinal walls, then enter the hepatobiliary system and apparently cause inflammatory diseases of the liver and gall bladder. Non-calculous cholecystitis and cholelithiasis are considered successive stages of the same pathologic process, since the pathologic process in the gallbladder wall normally results in the decrease of its contractile function and inspissation of the bile, which can further lead to gallstone formation [3]. Indeed, clinical and laboratory parameters in the examined patients with non-calculous cholecystitis, stage I and II cholelithiasis were unidirectional and merely pronounced to different degrees.

In intestinal dysbiosis, there is an increase in ethanol-producing bacteria. Many bacteria can be qualified as ethanol producers, including Escherichia, the imbalance of quality and number of which was observed in the patients examined. Ethanol production by bacteria results in synthesis of free fatty acids and contributes to oxidative stress which triggers NAFLD [17, 20]. Furthermore, the reduced disintoxication function of the intestinal microflora increases the burden on the liver enzyme systems, leading to metabolic and structural changes in the liver [10, 12]. This is the reason for a sharp reduction in synthesis and excretion of bile acids against the background of cholesterol hypersecretion.

## Conclusion

Our comprehensive studies have shown that BOS is the beginning of bacterial translocation, which is a triggering factor in inflammation of the liver and biliary tract. Through ethanol hyperproduction, pathogenic intestinal bacteria raise the level of free fatty acids and increase endotoxinemia, which contributes to the development of NAFLD. In its turn, synthesis and secretion of bile acids decrease in NAFLD. The decrease in bile acids with an antibacterial effect ensures the activation of opportunistic pathogenic microflora and the development of BOS. Thus, strong association between BOS and hepatobiliary tract diseases, which is characterized by that the development of any one of them acts as an incentive for the development of the other one, has been established.

**Conflict of interests**
The authors declare no conflict of interests.

**References:**

1. Vakhrushev Ya.M., Lukashevich A.P., Gorbunov A. Yu. et al. Intestinal Mechanisms of Enterohepatic Circulation Disturbance of Bile Acids in Cholelithiasis. Vestnik Rossiiskoi akademii meditsinskikh nauk. 2017; 72(2): 105-111 [in Russian]. doi: 10.15690/vramn807

2. Vakhrushev Ya.M., Lukashevich A.P. Specific features of impaired intestinal digestion, absorption, and microbiocenosis in patients with cholelithiasis. Terapevticheskii arkhiv. 2017; 2: 28-32 [In Russian]. doi: 10.17116/terarkh201789228-32
3. Vakhrushev Ya.M., Khokhlacheva N.A., Moseeva M.V. et al. The importance of the morphometric research of bile in early diagnostics of bilious stone formation. Arhiv vnutrennej mediciny. 2018; 6: 458-463 [In Russian]. doi: 10.20514/2226-6704-2018-8-6-458-463
4. Volodin N.N., Kafarskaja L.I., Korshunov V.M. Characteristics of microorganisms colonizing the human intestine. Zhurnal mikrobiologii, jepidemiologii i immunobiologii. 2002; 5: 98-104 [In Russian].
5. Grinevich V.B., Sas E.I. Physiological effects of bile acids. Russkii meditsinskii zhurnal. Meditsinskoe obozrenie. 2017; 1(2): 87-91 [In Russian].
6. Efimenko N.V., Ledovskaya T.I., Fyodorova T.E. et al. Non-alcoholic fatty liver disease: Modern aspects of etiopathogenesis and treatment. Vestnik Avitsenny. 2017; 19(3): 393-398 [In Russian]. doi: 10.25005/2074-0581-2017-19-3-393-398
7. Ilchenko A.A. Diseases of the gallbladder and biliary tract: rukovodstvo dlja vrachej. Moskva: MIA. 2011; 880 p. [In Russian].
8. Ilchenko A.A. Bile acids in normal and pathology. Eksperimentalnaya i klinicheskaya gastroenterologiya. 2010; 4: 3-13 [In Russian].
9. Kuvaeva I.B., Ladodo K.S. Microecological and immune disorders in children. Moskva: Medicina. 1991; 224 p. [In Russian].
10. Ojnotkinova O.Sh., Nikonov E.L., Gioeva I.Z. The role of the intestinal microbiota in the pathogenesis of dyslipidemia and associated metabolic disorders. Dokazatel'naja gastrojenterologija. 2017; 2: 29-34 [In Russian]. doi: 10.17116/dokgastro20176229-34
11. Plotnikova E.Yu. Non-alcoholic fatty liver disease and intestinal microflora. Gastrojenterologija Sankt-Peterburga. 2017; 2: 76-85 [In Russian].
12. Seliverstov P.V., Sitkin S.I., Radchenko V.G. The role of intestinal disbiosis in the development of mitochondrial dysfunction and nonalcoholic fatty liver disease. Meditsinskii sovet. Gastroenterologiya. 2018; 6: 90-95 [In Russian]. doi: 10.21518/2079-701X-2018-6-89-95
13. Uspensky Yu.P., Baryshnikova N.V., Balukova E.V. Microflora of intestine and liver pathology. Medicinskij alfavit. 2016; 3(24): 13-20 [In Russian].
14. Khokhlacheva N.A., Gorbunov A.Yu., Vakhrushev Ya.M. The study of cholelithiasis prevalence basing on prognostic investigation of hepatobiliary diseases. Terapevticheskii arkhiv. 2012; 84(2): 45-49 [In Russian].
15. Jakovenko Je.P., Ivanov A.N., Jakovenko A.V. et al. Metabolic diseases of the liver as systemic manifestations of intestinal dysbiosis. The role of probiotics in the normalization of intestinal microflora. Russkii meditsinskii zhurnal. Gastrojenterologija. 2008; 16(6): 396-401 [In Russian].
16. Miura K., Ohnishi H. Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. World J. Gastroenterol. 2014; 20(23): 7381-7391. doi: 10.3748/wjg.v20.i23.7381
17. Mouzaki M., Comelli E.M., Arendt B.M. et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013; 58: 120-127. doi: 10.1002/hep.26319
18. Nightingale J. Hepatobiliary, renal and bone complications of intestinal failure. Best Practice and Research in Clinical Gastroenterology. 2003; 17(6): 907-929.
19. Wieland A., Frank D.N., Harnke B. et al. Systematic review: microbial dysbiosis and nonalcoholic fatty liver desease. Aliment. Pharmacol. Ther. 2015; 42(9): 1051-1063. doi: 10.1111/apt.13376
20. Zhu l., Baker S.S., Gill C. et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. Hepatology. 2012; 57: 601-609. doi: 10.1002/hep.26093

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