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Study of fatty acid-binding protein and Bacteroidetes and Firmicutes levels in patients with metabolic-associated fatty liver disease in combination with type 2 diabetes mellitus and small intestinal bacterial overgrowth syndrome

V. V. Cherniavskyi[A,B,E,F], **O. K. Didyk***[A,B,C,D]

Bogomolets National Medical University, Kyiv, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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***E-mail:** olgadidyk06@gmail.com

The aim of the study was to examine serum levels of liver and intestinal fatty acid-binding proteins (L-FABP and I-FABP), fecal numbers of *Bacteroidetes* (B) and *Firmicutes* (F) in patients with metabolic-associated fatty liver disease (MAFLD) in combination with type 2 diabetes mellitus (T2DM) and small intestinal bacterial overgrowth (SIBO) syndrome.

Materials and methods. The prospective, interventional, randomized study included 51 patients with MAFLD in combination with T2DM, who were examined and divided into 2 groups. Group 1 consisted of 24 patients with MAFLD and T2DM without SIBO. Group 2 was comprised of 27 patients with MAFLD in combination with T2DM and SIBO. The control group included 20 apparently healthy individuals. Serum levels of L-FABP and I-FABP were measured by ELISA method using the Human L-FABP and I-FABP ELISA Kit test systems, respectively (Elabscience, USA). Fecal numbers of B and F were determined by real-time PCR. Bacterial DNA was detected in a thermal cycler Rotor-Gene 6000 (QIAGEN, Germany) using DNA 16S rRNA primers and NanoDrop ND-8000 reagents (Thermo Scientific, USA).

Results. To assess the state of intestinal permeability, serum levels of L-FABP and I-FABP were examined and numbers of phylum F and B as well as their ratio were calculated. Patients of both groups have been found to have increased serum levels of L-FABP, I-FABP, numbers of B in fecal samples and decreased numbers of F and F/B ratio.

Conclusions. The study results obtained have revealed increased intestinal permeability and demonstrated an important diagnostic value of serum L-FABP and I-FABP as a biomarker of intestinal permeability in diabetic MAFLD patients with or without SIBO. Increased fecal numbers of *Bacteroidetes*, decreased numbers of *Firmicutes* and F/B ratio have been detected in diabetic MAFLD patients with or without SIBO.

Дослідження рівня білків, що зв'язують жирні кислоти, та вмісту Bacteroidetes, Firmicutes у пацієнтів із метаболічно асоційованою жировою хворобою печінки в поєднанні з цукровим діабетом 2 типу та синдромом надмірного бактеріального росту

В. В. Чернявський, О. К. Дідик

Ключові слова: кишкова проникність, білки, що зв'язують жирні кислоти, Bacteroidetes, Firmicutes, синдром надмірного бактеріального росту, цукровий діабет 2 типу, метаболічно асоційована жирова хвороба печінки.

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Мета роботи – дослідження рівня печінкової та кишкової фракцій білків, що зв'язують жирні кислоти (L-FABP та I-FABP), у сироватці крові, вмісту *Bacteroidetes*, *Firmicutes* у калі у пацієнтів з метаболічно асоційованою жировою хворобою печінки (МАЖХП) у поєднанні з цукровим діабетом (ЦД) 2 типу та синдромом надмірного бактеріального росту (СНБР).

Матеріали і методи. У проспективне інтервенційне рандомізоване дослідження залучили 51 пацієнта з МАЖХП у поєднанні з ЦД 2 типу. Хворих обстежили та поділили на 2 групи. До першої групи залучили 24 пацієнтів із МАЖХП та ЦД 2 типу без СНБР; до другої – 27 хворих на МАЖХП у поєднанні з ЦД 2 типу та СНБР. Контрольна група – 20 практично здорових осіб. Рівень печінкової та кишкової фракцій білків, що зв'язують жирні кислоти, у сироватці крові визначали методом ELISA з використанням тест-систем Human I-FABP та L-FABP ELISA Kit (Elabscience, США). Вміст *Bacteroidetes*, *Firmicutes* у калі визначали методом ПЛР у реальному часі. Бактеріальну ДНК досліджували на термоциклері Rotor-Gene 6000 (QIAGEN, Німеччина) з використанням праймерів ДНК 16S рРНК та реагентів NanoDrop ND-8000 (Thermo Scientific, США).

Результати. Для оцінювання кишкової проникності досліджували рівень L-FABP, I-FABP у сироватці крові, вміст типів *Firmicutes*, *Bacteroidetes* та їх співвідношення. У хворих першої та другої груп встановлено підвищення рівня L-FABP, I-FABP у сироватці крові, вмісту *Bacteroidetes* (B) у пробах калу та зниження вмісту *Firmicutes* (F), а також співвідношення F/B.

Висновки. У результаті дослідження виявили підвищення рівня L-FABP та I-FABP у сироватці крові, що свідчить про підвищення кишкової проникності у пацієнтів із МАЖХП у поєднанні з ЦД 2 типу та СНБР та хворих без СНБР. У пацієнтів із МАЖХП у поєднанні з ЦД 2 типу та СНБР і хворих без СНБР визначили підвищення вмісту *Bacteroidetes*, зниження вмісту *Firmicutes* та співвідношення F/B у калі.

Alterations in gut microbiota increase intestinal permeability favoring the absorption of pathogen-associated molecular patterns such as lipopolysaccharides (LPSs) [1].

This phenomenon activates the TRL4 receptors that increase the NF-κB-related gene transcription in the Kupffer cells triggering inflammatory pathways by the activation

of proinflammatory genes (TNF-α, IL-6, IL-8, and IL-12) and generating reactive oxygen species (ROS) [2]. The consequent inflammatory response induces production of profibrotic factors by the hepatic stellate cells, impairs insulin signaling with a subsequent increase in free fatty acids (FFAs) afflux and alters mitochondrial beta-oxidation, which results in hepatic steatosis [3].

Intestinal microbiota can alter bile acid metabolism, contributing to the pathogenesis of metabolic-associated fatty liver disease (MAFLD) by modulating farnesoid X receptor (FXR) stimulation and thus affecting fat and glucose homeostasis [4]. Hyperglycemia has been reported to induce an increased intestinal permeability through GLUT2-dependent mechanisms and the alteration of tight junction integrity, thus creating a leaky gut state [5].

Fatty acid-binding proteins (FABPs) are small (14–15 kDa) cytosolic water-soluble proteins, present in mature enterocytes of the small and large intestine. Their function is the transport of fatty acids from the enterocyte apical membrane to the endoplasmic reticulum where complex lipid biosynthesis occurs.

Thus, this shows that diagnostic methods for assessing the intestinal barrier function are of great scientific interest, and in several studies, FABPs were considered as markers of the intestinal barrier function [5], which requires further research.

Aim

The aim of the study was to examine serum levels of liver and intestinal FABPs (L-FABP and I-FABP), fecal numbers of *Bacteroidetes* and *Firmicutes* in patients with MAFLD in combination with type 2 diabetes mellitus and small intestinal bacterial overgrowth syndrome.

Materials and methods

The study was approved by the commission on biotic expertise and ethics of scientific research (protocol No. 150 dated October 18, 2021) at Bogomolets National Medical University and performed at the clinical base of the Department of Internal Medicine No. 1 from 2021 to 2023. All patients gave their informed consent for participation in the study.

The prospective, interventional, randomized study included 51 patients with MAFLD in combination with type 2 diabetes mellitus (T2DM), who were examined and divided into the 2 groups. Group 1 comprised 24 patients with MAFLD and T2DM without small intestinal bacterial overgrowth (SIBO). Group 2 consisted of 27 patients with MAFLD in combination with T2DM and SIBO. The control group was composed of 20 apparently healthy subjects.

The inclusion criteria were male or female, 25–78 years of age, MAFLD patients with T2DM diagnosed by estimating the steatosis degree based on the results of ultrasound steatometry (Ultrasign soneus P7 device with a 1–6 MHz convex sensor) performed on the scale of ultrasound attenuation (ultrasound attenuation coefficient ≥2.2 dB/cm) proposed by M. Sasso et al. and diagnostic criteria of carbohydrate metabolism disorders according to the 2023 American Diabetes Association guidelines [6].

The exclusion criteria were the following: viral hepatitis, alcoholic liver disease, autoimmune hepatitis, drug-induced liver damage, Wilson–Konovalov disease, type 1 diabetes mellitus, decompensated T2DM, cancer, pregnancy, refusal to participate in the study.

The serum levels of L-FABP and I-FABP were measured by ELISA method using the Human L-FABP and I-FABP ELISA Kit test systems, respectively (Elabscience, USA). The fecal numbers of B and F were determined by real-time PCR. Bacterial DNA was detected in a thermal cycler Rotor-Gene 6000 (QIAGEN, Germany) using DNA 16S rRNA primers and NanoDrop ND-8000 reagents (Thermo Scientific, USA).

The Lactulose Hydrogen Breath Test was used to diagnose SIBO. The test was performed using the Advanced Hydrogen Breath Testing (Micro H2 Meter) device.

The program GraphPad Prism Version 9.5.1.733, Microsoft Office 2016 software package, MedStat version 5.2. and EZR version 3.4.1. (R Foundation Statistical Computing) were used for statistical processing of the obtained results. Quantitative and qualitative variables were evaluated through a statistical analysis. Qualitative data were presented as absolute values and percentages. The Shapiro–Wilk test was used to check the distribution of the obtained data for normality. In the case of a normal distribution, quantitative variables were described by arithmetic mean values with a standard deviation (Mean ± SD) and by medians with the first and third quartiles (Median [Q1; Q3]) in non-normal distribution. The method of multiple comparisons ANOVA was used to check differences between the 3 groups when the data were normally distributed, non-normally distributed variables were compared by the Kruskal–Wallis test. Differences between groups were considered significant at a value of $p < 0.05$.

Results

The clinical and diagnostic characteristics patient groups are shown in *Table 1*. To assess the state of intestinal permeability, serum levels of L-FABP and I-FABP were examined and numbers of phylum *Firmicutes* (F) and *Bacteroidetes* (B) as well as their ratio were calculated.

Patients of both groups have been found to have increased serum levels of L-FABP, I-FABP, numbers of B in fecal samples and decreased numbers of F and F/B ratio (*Table 1*).

Quantitative studies have revealed the L-FABP levels to be significantly increased by 6.1 times in Group 1 patients and by 6.8 times in Group 2 patients compared to the control group ($p < 0.001$), and the L-FABP levels were 1.1 times increased in Group 2 patients compared to patients of Group 1 ($p < 0.001$).

The I-FABP levels have been found to be significantly increased by 6.0 and 9.2 times in Group 1 and Group 2 patients, respectively, compared to the control group individuals ($p < 0.001$). The study has shown 1.5 times increased I-FABP levels in patients of Group 2 as compared to Group 1 patients ($p < 0.05$).

A significant increase in the number of B has been observed by 4.6 and 5.1 times in Group 1 and Group 2 patients, respectively, compared to the control group individuals ($p < 0.001$). The number of B was 1.1 times increased in Group 2 patients as compared to Group 1 patients ($p < 0.05$). The number of F was reduced by 8.2 and

Table 1. Clinical and diagnostic characteristics of patient groups

Parameter, units of measurement	Group 1, n = 24	Group 2, n = 27	Control group, n = 20
Sex (female / male), n (%)	14 (58 %) / 10 (42 %)	18 (67 %) / 9 (33 %)	12 (60 %) / 8 (40 %)
Age, years	57.4 ± 2.3	58.8 ± 3.1	55.2 ± 3.6
BMI, kg/m^2	30.4 [29.8; 32.0]	31.1 [29.3; 33.2]	30.2 [29.6; 32.8]
L-FABP, ng/ml	31.2 ± 2.4*,#	34.7 ± 3.2*	5.1 ± 2.9*
I-FABP, ng/ml	8.4 ± 1.1*,#	12.9 ± 0.9*	1.4 ± 0.1*
Firmicutes, %	5.6 [3.1; 7.6]*,#	3.4 [3.2; 6.5]*	45.8 [41.1; 52.6]*
Bacteroidetes, %	79.4 [84.3; 91.1]*,#	87.6 [84.1; 90.9]*	17.2 [15.5; 26.3]*
F/B ratio	0.07 [0.04; 0.09]*,#	0.04 [0.03; 0.06]*	2.7 [2.3; 3.7]*

BMI: body mass index; ***:** $p < 0.001$ compared to the control group; **#:** $p < 0.05$ – Group 1 compared to Group 2.

13.5 times in patients of Group 1 and Group 2 ($p < 0.001$), respectively, compared to the control group, but it was 1.5 times increased in Group 2 patients as compared to Group 1 patients ($p < 0.05$).

The F/B ratio was decreased by 38.6 and 67.5 times in Group 1 and Group 2 patients, respectively, compared to the control group ($p < 0.001$), and it was 1.8 times increased in Group 2 patients as compared to Group 1 patients ($p < 0.05$).

Discussion

The data presented indicate a possible association between the intestinal microbiota composition, intestinal barrier disruption and higher grades of endotoxemia, which leads to chronic delayed inflammation, that underlies insulin resistance [7,8]. Thus, the intestinal microbiome can be considered as a factor that determines tissue insulin sensitivity, and violations in this sensitive system result in the development and progression of MAFLD [9].

Translocation of bacteria or bacterial products such as LPSs from the intestine to the liver has been proposed as a triggering factor of liver inflammation and fatty liver disease [10]. It has been found that LPS translocation induced hepatic steatosis in mice suggesting that increased intestinal permeability was associated with fatty liver disease [11].

Basal FABP levels have been reported to reflect the physiological enterocyte turnover rate, whereas elevated levels have been shown to indicate altered intestinal permeability and epithelial cell damage [12].

Patients with MAFLD, particularly those with nonalcoholic steatohepatitis and T2DM, have been revealed with greater numbers of B and differences in the presence of F, resulting in a decreased F/B ratio in most analyses [13].

The study has demonstrated increased serum levels of L-FABP and I-FABP in patients with MAFLD combined with T2DM and with or without SIBO, indicating increased intestinal permeability and epithelial cell damage.

According to the study results, diabetic MAFLD patients with or without SIBO have been shown to have increased fecal numbers of B, decreased numbers of F and F/B ratio.

The study findings completely coincide with currently known scientific data and are complementary to them, however, more research still needs to be done.

Conclusions

1. The study results obtained have revealed elevated serum levels of L-FABP and I-FABP, indicating increased intestinal permeability in diabetic MAFLD patients with or without SIBO.

2. Increased fecal numbers of *Bacteroidetes*, decreased numbers of *Firmicutes* and F/B ratio have been detected in diabetic MAFLD patients with or without SIBO.

Prospects for further research. Further studies on the state of intestinal permeability in patients with MAFLD combined with T2DM and SIBO will help to establish the involvement of intestinal microbiota and FABPs in the pathogenetic mechanisms of MAFLD development and progression and to improve diagnostics and complex treatment measures compared to standard therapy.

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Information about the authors:
Cherniavskyi V. V., MD, PhD, DSc, Professor, Department of Internal Medicine No. 1, Bogomolets National Medical University, Kyiv, Ukraine.
ORCID ID: 0000-0001-5831-8810
Didyk O. K., MD, PhD student, Department of Internal Medicine No. 1, Bogomolets National Medical University, Kyiv, Ukraine.
ORCID ID: 0000-0003-4536-3377

Відомості про авторів:
Чернявський В. В., д-р мед. наук, професор каф. внутрішньої медицини № 1, Національний медичний університет імені О. О. Богомольця, м. Київ, Україна.
Дідик О. К., аспірант каф. внутрішньої медицини № 1, Національний медичний університет імені О. О. Богомольця, м. Київ, Україна.

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