УДК 616.34-008.87:616-056.52:616.36-004:616.36-003.826
DOI http://doi.org/10.30978/MG-2023-1-24

ISSN 1727-5725 (Print)
ISSN 2521-649X (Online)

N. I. Chereliuk, O. E. Gridnyev
GI «L. T. Mala National Therapy Institute of NAMS of Ukraine», Kharkiv

Main phylotypes of gut microbiota and their connection with the degree of obesity and the stage of liver fibrosis in patients with non-alcoholic fatty liver disease

Objective — to analyse the state of the relative composition of gut microbiota (GM) at the level of the main phylotypes in patients with non-alcoholic fatty liver disease (NAFLD) with different body mass index and degree of liver fibrosis.

Materials and methods. The study involved 105 people. The main group consisted of 85 patients with NAFLD with obesity of 36.50 [32.00; 40.60] kg/m^2, who were divided into three subgroups depending on the degree of obesity. Subgroup I included 38 patients who were diagnosed with the first degree of obesity. Subgroup II included 23 patients diagnosed with the second degree. Subgroup III consisted of 24 patients with the third degree of obesity. The control group consisted of 20 practically healthy individuals 23.50 [21.35; 25.78] kg/m^2. Determination of the degree of fibrosis according to METAVIR scale by measuring the average stiffness of the liver parenchyma in the mode of shear wave elastography. The composition of GM at the level of the main phylotypes was studied by identifying total bacterial DNA and DNA of *Bacteroidetes*, *Firmicutes*, as well as *Firmicutes/Bacteroidetes* ratio by quantitative real-time polymerase chain reaction using universal primers for the 16S rRNA gene and taxon-specific primers. Statistical processing was performed using Statistica 13.1.

Results. In the comorbid course of NAFLD and the first degree of obesity, 47.37 % of patients had no signs of fibrosis and the same number were diagnosed with F1 fibrosis, two patients (5.26 %) had F2 fibrosis, and F3 fibrosis was not diagnosed in any patient. In subgroup II, two-thirds actually had liver fibrosis, but F3 fibrosis was not detected in any patient. In patients of subgroup III, only 20.83 % of patients had no signs of liver fibrosis, while 37.50 % were diagnosed with F1 fibrosis, 33.33 % with F2 fibrosis, and 8.33 % with F3 fibrosis. In other words, the most severe F3 fibrosis was observed in patients with the third degree of obesity. In patients of the subgroup I, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* was found compared to the control group: 3.07 times ($p < 0.01$) in the absence of fibrosis and already in the presence of the 1st stage of fibrosis this indicator increased to 3.21 ($p < 0.01$), while in the 2nd stage it increased almost 4 times ($p < 0.01$). However, when comparing this indicator in the first subgroup between the stages of fibrosis, no statistically significant deviations were found ($p > 0.05$), i.e., the changes were only tendency-like. In patients of the subgroup II, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* was also found compared to the control group: 3.25 times ($p < 0.01$) in the absence of fibrosis, 3.81 times ($p < 0.01$) in the 1st stage of fibrosis, 5.08 times ($p < 0.01$) in the 2nd stage of fibrosis, which was the maximum value. Also, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* by 1.57 times ($p < 0.01$) was found at F2 compared to F0, while between other stages of fibrosis, the changes were trending and had no statistically significant differences. In patients of subgroup III in the absence of fibrosis, the relative ratio of *Firmicutes/Bacteroidetes* also exceeded that of the control group and progressively increased with increasing fibrosis. It should be noted that the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* between F2 and F0 by 1.28 times ($p < 0.01$) and between F3 and F0 by 1.35 times ($p < 0.01$) was found.

Conclusions. When analysing the relative composition of GM in the studied subgroups depending on the stage of fibrosis, it was found that the increase in the stage of liver fibrosis is associated with certain disorders of *Firmicutes/Bacteroidetes* ratio, the relative content of *Firmicutes* and *Bacteroidetes*. Thus, in the subgroup I, the relative composition of GM significantly differed only from the control group, while in patients of the subgroup

II with F2 fibrosis there was the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* by more than one and a half times compared to patients without signs of fibrosis. At the same time, in patients of the subgroup III with fibrosis, not only F2, but also F3, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* was found.

Keywords: non-alcoholic fatty liver disease, obesity, gut microbiota, fibrosis.

Non-alcoholic fatty liver disease (NAFLD) remains one of the most common causes of liver disease worldwide, which is closely related to the lifestyle of the population (high energy food intake together with a sedentary lifestyle), which has been further significantly affected by quarantine restrictions due to the COVID-19 pandemic [24].

The analysis of the epidemiological situation gives unfavourable forecasts: according to experts, by 2030 the prevalence of NAFLD will increase by 18.3 %, which will give rise to the exponential increase in the number of patients with non-alcoholic steatohepatitis and cause 43 million new cases of this disease [8, 31].

It should be noted that the prevalence of NAFLD is increasing in parallel with the increase in the prevalence of major diseases associated with metabolic disorders, with obesity taking the leading place. Indeed, studies have shown that more than half of patients with NAFLD are diagnosed with obesity [31], while among obese patients, the prevalence of NAFLD ranges from 60 to 95 % [15, 29]. The above is particularly relevant against the backdrop of the actual obesity epidemic that is sweeping the world. Thus, according to the World Health Organization, in recent decades, there has been a pronounced unfavourable epidemiological trend in the number of obese patients worldwide — from 1975 to 2014, their number doubled [15]. In 2016, the prevalence of obesity for the 20—84 age group ranged from 22.7 % in Portugal to 29.3 % in the UK for men, and from 19.5 % in Switzerland to 31.3 % in the UK for women; and the highest rate was observed in the United States — 37.5 % for men and 39.5 % for women [14]. Mathematical forecasting of the epidemiological situation in the UK suggests a progressive spread of obesity in 2050 to about 50 % of adult women and 60 % of men [14].

It should be noted that the comorbid course of NAFLD and obesity creates a complex etiological basis for the progression of steatosis to steatohepatitis, fibrosis and cirrhosis, which in turn leads to significant socioeconomic consequences: an increase in overall mortality, including that caused by liver disease, and causes a growing burden on public health worldwide [11, 21, 31]. Currently, it has been proven that the presence of fibrosis, rather than proinflammatory changes, is the most important marker associated with the risk of adverse events associated with liver disease or overall mortality [23]. This effect is observed even in the early stages of fibrosis, demonstrating a gradual increase in the frequency of adverse events as the stage of fibrosis progresses [4, 7, 10, 16].

In recent years, the role of the gut microbiota (GM) in the development of liver steatosis and its progression to fibrosis has been increasingly emphasized in the literature. The most convincing evidence of the potential role of the GM in the development and progression of NAFLD is provided by Loomba et al. [19]. In this study, the diagnosis of NAFLD was confirmed by liver biopsy or elastography (n = 86). For the study of the formed cohort of patients, 37 different bacteria were isolated, the composition of which varied depending on the degree of fibrotic changes. The high activity of fibrotic changes in the liver was characterized by the increase in the number of *Proteobacteria* and *E. coli*, the decrease in *Firmicutes*; such features led scientists to believe that it is possible to diagnose progressive fibrosis in NAFLD by changes in the GM. The course of NAFLD-associated cirrhosis is also accompanied by probable changes in GM [5]. In turn, obesity has also been shown to be associated with characteristic changes in GM composition [1, 18].

The above confirms the urgent relevance of studying the role of GM in the progression of NAFLD (including profibrotic processes in the liver) in obese patients.

Objective — to analyse the state of the relative composition of GM at the level of the main phylotypes in patients with NAFLD with different body mass index (BMI) and degree of liver fibrosis.

Materials and methods

The study involved 105 people. The main group consisted of 85 patients with NAFLD with obesity of 36.50 [32.00; 40.60] kg/m^2, who were divided into three subgroups depending on the degree of obesity. Subgroup I included 38 patients (44.71 %) who were diagnosed with the first degree of obesity (BMI from 30.0 to 34.9 kg/m^2). Subgroup II included 23 patients (27.06 %) diagnosed with the second degree (BMI from 35.0 to 39.9 kg/m^2). Subgroup III consisted of 24 (28.24 %) patients with the third degree of obesity (BMI over 40.0 kg/m^2). The control group consisted of 20 practically healthy individuals 23.50 [21.35; 25.78] kg/m^2.

The Bioethics Committee of L.T. Mala Therapy National Institute of the NAMS of Ukraine approved the study. Prior to the study, all patients voluntarily signed the informed consent to participate in the study.

The diagnosis of NAFLD was made in accordance with the Order of the Ministry of Health No. 826 of November 06, 2014 and the European Association for the Diagnosis and Treatment of NAFLD EASL-EASD-EASO Clinical Practice Guidelines for the management of NAFLD, 2016.

WHO criteria [32] were used to diagnose obesity and classify its degree, based on the calculated BMI.

All patients were interviewed to determine the etiological factors of secondary fatty liver and other conditions that affect the composition of GM, as well as a general clinical examination (analysis of complaints, medical history, life history, and objective status) and anthropometry with BMI calculation.

The degree of fibrosis according to the METAVIR scale was determined by measuring the average stiffness of the liver parenchyma during ultrasound examination of the liver in the mode of shear wave elastography [26].

To determine the main GM phylotypes, fecal samples freshly collected in sterile containers were aliquoted, quickly frozen and stored at –20°C until extraction. The DNA was extracted from 400 mg of feces using the Ribo-prep nucleic acid extraction kit (AmpliSens, Russia) according to the manufacturer's instructions.

The DNA concentration in the extracts was measured with Qubit 3 fluorimeter (USA) using Qubit dsDNA HS Assay Kits (Thermo Scientific, USA) and adjusted to ~10 ng/μL. The composition of GM at the level of the main phylotypes was determined by identifying total bacterial DNA and DNA of *Bacteroidetes*, *Firmicutes* and *Actinobacteria* and the ratio of *Firmicutes/Bacteroidetes* by quantitative real-time polymerase chain reaction (qRT-PCR) using universal primers for the 16S rRNA gene and taxon-specific primers [34]. The polymerase chain reaction was performed using CFX96 Touch Real-Time PCR Product Detection System (Bio-Rad, USA). Amplification program: initial denaturation stage for 5 min at 95 °C — 40 cycles: 15 s at 95 °C, 15 s at 61.5 °C, 30 s at 72 °C with fluorescence signal reading; final elongation stage — 5 min at 72 °C.

Statistical processing was performed using Statistica 13.1. According to the Kolmogorov-Smirnov criterion, the distribution of all the studied indicators did not correspond to the normal (Gaussian) distribution, so nonparametric statistics methods were used. The data are presented in the form of Me [LQ; UQ], where Me is the median, LQ and UQ are the lower and upper quartiles, respectively.

Results

The data obtained confirm the frequent comorbidity of obesity and NAFLD (Fig. 1). From the distribution of patients in the main group depending on the degree of obesity (see Fig. 1), we see that patients with the third degree of obesity predominated among them, while patients with the first degree of obesity were actually one and a half times less.

At the next stage, the patients underwent elastometry and the average stiffness of the liver parenchyma in the mode of shear wave elastography to determine the degree of liver fibrosis (Fig. 2) was measured.

As we can see from Fig. 2, in patients with NAFLD and the first degree of obesity (n = 38), almost half of the patients (47.37 %) had no signs of fibrosis and the same number were diagnosed with stage 1 fibrosis, and only two (5.26 %) patients were diagnosed with stage 2 fibrosis, and no patient was diagnosed

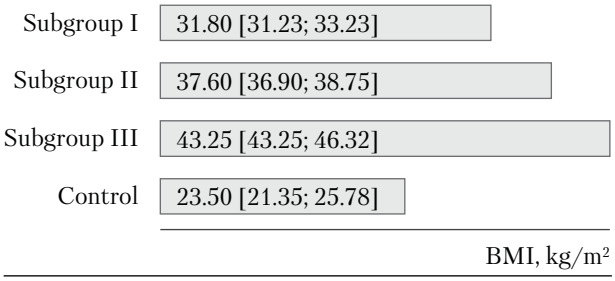

Fig. 1. **Analysis of body mass index in the studied subgroups**

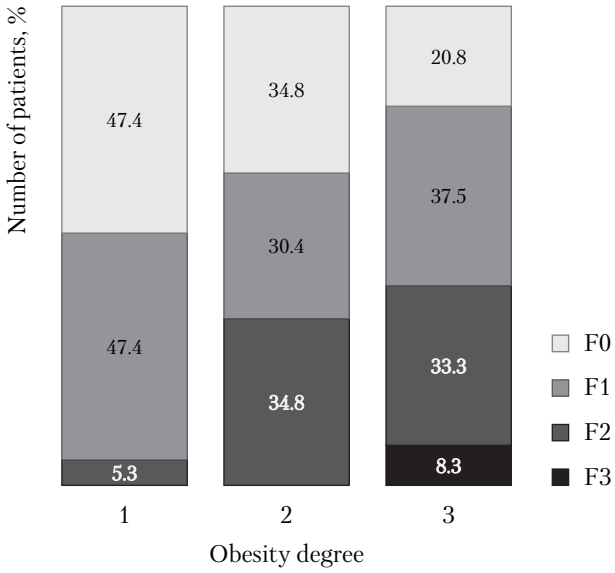

Fig. 2. **Distribution of patients with NAFLD by the degree of liver fibrosis depending on the degree of obesity:** F0 — no liver fibrosis; F1 — stage 1 fibrosis; F2 — stage 2 fibrosis; F3 — stage 3 fibrosis

with stage 3 fibrosis. In contrast, in patients with NAFLD and the second degree of obesity (n = 23), two-thirds had liver fibrosis: 7 (30.43 %) had stage 1, 8 (34.87 %) had stage 2, and no patients had stage 3 fibrosis. And in patients with NAFLD and the third degree of obesity (n = 24), only 20.83 % of patients had no signs of liver fibrosis, while 37.50 % were diagnosed with stage 1 fibrosis, 33.33 % with stage 2, and 8.33 % with stage 3 fibrosis. In other words, the most severe F3 fibrosis was observed in patients with the third degree of obesity, indicating the tendency to increase the number of patients with fibrosis and its severity as the degree of obesity increases.

The study of the ratio of the main GM phylotypes revealed significant differences in the *Firmicutes/Bacteroidetes* ratio depending on the presence of liver fibrosis (Fig. 3).

In patients of the subgroup I, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* was found compared to the control group: 3.07 times ($p < 0.01$) in the absence of fibrosis and already in the presence of the 1st stage of fibrosis this indicator increased to 3.21 ($p < 0.01$), while in the 2nd stage it increased almost 4 times ($p < 0.01$). However, when comparing this indicator in the first subgroup between the stages of fibrosis, no statistically significant deviations were found ($p > 0.05$), i. e., the changes were only tendency-like.

In patients of the subgroup II, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* was also found compared to the control group: 3.25 times ($p < 0.01$) in the absence of fibrosis, 3.81 times ($p < 0.01$) in the 1st stage of fibrosis, 5.08 times ($p < 0.01$) in the 2nd stage of fibrosis, which was the maximum value. Also, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* by 1.57 times ($p < 0.01$) was found at F2 compared to F0, while between other stages of fibrosis, the changes were trending and had no statistically significant differences.

In patients of the subgroup III in the absence of fibrosis, the relative ratio of *Firmicutes/Bacteroidetes* also exceeded that of the control group and progressively increased with increasing fibrosis (Fig. 3). It should be noted that the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* between F2 and F0 by 1.28 times ($p < 0.01$) and between F3 and F0 by 1.35 times ($p < 0.01$) was found.

Further analysis of the results showed that the increase in the relative ratio of *Firmicutes/Bacteroidetes* in the subgroups of the main group was mainly due to the increase in the number of representatives of *Firmicutes* type (Fig. 4).

Thus, in patients of subgroup I, in the absence of signs of fibrosis, the index reflecting the content of *Firmicutes* exceeded the control group by 1.33 times

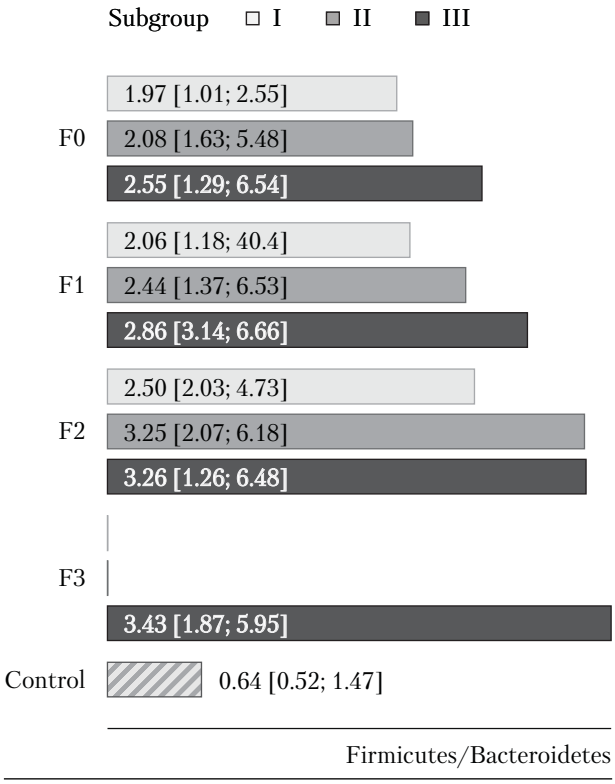

Fig. 3. **Firmicutes/Bacteroidetes ratio in the studied subgroups**

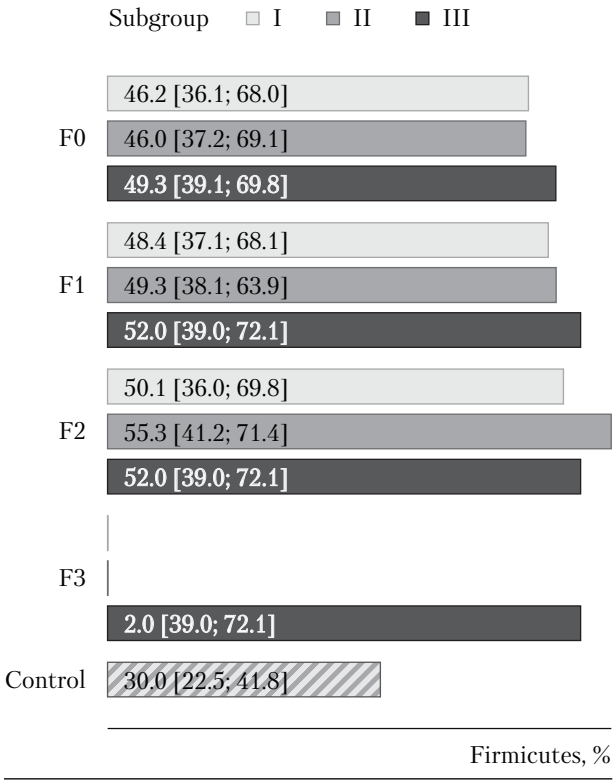

Fig. 4. **Relative content of Firmicutes in the studied subgroups**

($p < 0.01$), in the presence of fibrosis F1 and F2 it gradually increased by 1.40 times. The relative content of *Firmicutes* in the first subgroup significantly exceeded that of the control group, but when compared by fibrosis stage, the differences were trending.

In patients of the subgroup II, in addition to the statistically significant ($p < 0.01$) increase in the relative content of *Firmicutes* depending on the stage of fibrosis, the significant increase in this indicator by 1.2 times ($p < 0.01$) was observed in patients without signs of fibrosis and patients with stage 2 fibrosis.

In the subgroup III, the increase in the relative content of *Firmicutes* had a similar dynamics to that of the subgroup II: their content reached maximum values in patients with fibrosis F2 exceeding the control group by 1.6 times ($p < 0.01$) and decreased in patients with fibrosis F3 by 1.5 times ($p < 0.01$), while in patients with fibrosis F1 and without signs of fibrosis this indicator exceeded the control group by 1.42 times ($p < 0.01$). Also, in patients of the subgroup III, a statistically significant increase in the relative content of *Firmicutes* was found when comparing patients with no fibrosis and patients with fibrosis F2 and F3 by 1.12 times ($p < 0.01$) (see Fig. 4).

Regarding the relative content of *Bacteroidetes*, the data analysis showed that in all subgroups of the main group it was significantly lower than in the control group. Its variability depending on the presence of liver fibrosis was as follows (Fig. 5).

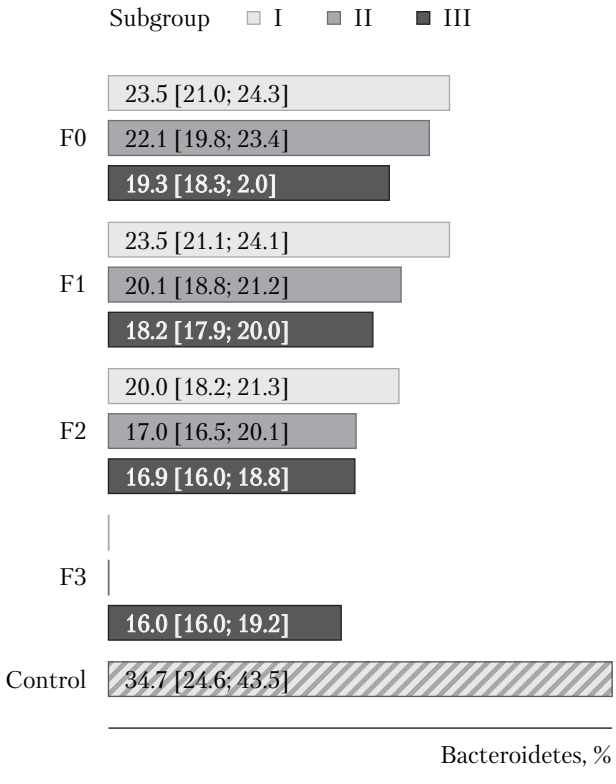

Fig. 5. **Relative content of Bacteroidetes in the studied subgroups**

In patients of the subgroup I, both in the absence of signs of fibrosis and in fibrosis F1, the relative content of *Bacteroidetes* was approximately the same and less than that of the control group ($p < 0.01$), while in the stages of fibrosis F2 it decreased by 1.5 times ($p < 0.01$).

In patients of the subgroup II, the relative content of *Bacteroidetes* in the absence of fibrosis was lower than in patients of the subgroup 1, and the presence of liver fibrosis was associated with the decrease in this indicator. A statistically significant decrease in the relative content of *Bacteroidetes* by 1.18 times ($p < 0.01$) was found in patients with F2 fibrosis compared with patients without fibrosis, while changes in this indicator did not differ significantly between other stages of fibrosis (see Fig. 4).

In patients of the subgroup III, the relative content of *Bacteroidetes* was the lowest. At the same time, in patients of the subgroup III with no signs of fibrosis, this indicator was significantly lower ($p < 0.01$) compared with the control group and gradually decreased with increasing fibrosis: in F1, it was reduced by 1.65 times ($p < 0. 01$), in F3 by 1.87 times ($p < 0.01$).

Similarly to the previous results regarding the relative content of *Firmicutes*, there was an inverse statistically significant ($p < 0.01$) decrease in the relative content of *Bacteroidetes* from patients in the subgroup III with no fibrosis to patients with fibrosis F2—F3 (see Fig. 5).

Discussion

Over the past decades, the study of GM, including its main phylotypes: *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*, in NAFLD and obesity has been studied extensively [25, 28], but often the results are heterogeneous or even contradictory. The data obtained in our study show that the presence of comorbidity of NAFLD and obesity significantly affects the above indicators, and this effect increases with increasing BMI. This is consistent with the data of other authors [2, 13], but A. Schwiertz et al. [27] do not confirm the presence of changes in the main GM phylotypes in this category of patients.

Changes in the *Firmicutes/Bacteroidetes* ratio do not support the results obtained by California researchers who refuted the existence of the relationship between *Firmicutes/Bacteroidetes* ratio [29] and the formation of overweight, and confirm the findings of other studies [15, 24].

It is also known that liver fibrogenesis is often accompanied by changes in GM, primarily by the

decrease in its diversity due to the increase in the number of *Firmicutes* and the decrease in the number of *Bacteroidetes* [3, 17]. In our study, the increase in obesity severity influenced the increase in the number of patients with stage 1 and stage 2 fibrosis ($\chi^2 = 37.23985$, df = 9; $p < 0.002$). Our findings also confirm the role of obesity as a risk factor that potentiates the development of liver fibrosis and, accordingly, the progression of NAFLD. This is consistent with a recent meta-analysis by Feng-Bin Lu [13, 20, 22]. We found that stage 3 fibrosis was associated with the most severe degree of obesity and more severe abnormalities in BM filotypes, which is also consistent with current literature [30, 33]. For example, the study by Dong et al [6] found that patients with advanced fibrosis have marked changes in the composition of GM, characterized by the increase in the genus *Prevotella* and decrease in *Bacteroides*, compared with patients with lower stages of fibrosis [22].

It should be noted that in our study, patients without signs of fibrosis and stage 1 fibrosis predominated among the examined patients, and a small number of patients with NAFLD with stage 3 fibrosis was due to the small number of patients with the third stage of obesity included in our study, which in turn imposes certain limitations on our results.

Conclusions

The results of the study indicate that the composition of GM in terms of the content of the main phylotypes in obese patients with NAFLD has statistically significant differences depending on BMI and the stage of fibrosis compared with the control group. Thus, there was a statistically significant increase in the *Firmicutes/Bacteroidetes* ratio, the relative content of *Firmicutes*, against the background of the reverse decrease in the relative content of *Bacteroidetes* at all degrees of obesity compared to the control group.

When analysing the relative composition of GM in the studied subgroups depending on the stage of fibrosis, it was found that the increase in the stage of liver fibrosis is associated with certain disorders of *Firmicutes/Bacteroidetes* ratio, the relative content of *Firmicutes* and *Bacteroidetes*. Thus, in the first subgroup, the relative composition of GM significantly differed only from the control group, while in patients of the second subgroup with F2 fibrosis there was a statistically significant increase in the relative ratio of *Firmicutes / Bacteroidetes* by more than one and a half times, the relative content of *Firmicutes* by 1.2 times, against the decrease in the relative content of *Bacteroidetes* by 1.18 times compared with patients without signs of fibrosis. At the same time, in patients of the subgroup III with fibrosis, not only F2, but also F3, the statistically significant increase in the relative ratio of *Firmicutes/Bacteroidetes* and the increase in the relative content of *Firmicutes* were found against the background of the decrease in the relative content of *Bacteroidetes* of the subgroup compared with patients without fibrosis.

Our results have certain limitations, which may explain their heterogeneity — first of all, a rather small number of patients studied, which especially affects the results due to their division into subgroups, which only emphasizes the high relevance of further large-scale studies in this area.

Conflicts of interest: none.
Authorship contributions: conception and design — O. Y. H.; acquisition of data, analysis and interpretation of data — N. I. C.; drafting the article, critical revision of the article — N. I. C., O. Y. H.

References

1. Черелюк НІ. Співвідношення основних філотипів кишкової мікробіоти у хворих на неалкогольну жирову хворобу печінки та ожиріння. Сучасна Гастроентерологія. 2019;(5):26-33. doi: 10.30978/MG-2019-5-26.
2. Arumugam M, Raes J, Pelletier E et al. Enterotypes of the human gut microbiome. Nature. 2011;473:174-180. doi: 10.1038/nature09944.
3. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol. 2014 May;60(5):940-7. doi: 10.1016/j.jhep.2013.12.019. Epub 2013 Dec 25. PMID: 24374295; PMCID: PMC3995845.
4. Campos-Murguía A, Ruiz-Margáin A, González-Regueiro JA, Macías-Rodríguez RU. Clinical assessment and management of liver fibrosis in non-alcoholic fatty liver disease. World J Gastroenterol. 2020; 26 (39):5919-43. doi: 10.3748/wjg.v26.i39.5919.
5. Caussy C, Tripathi A, Humphrey G, Bassirian S, Singh S, Faulkner C et al. A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. Nat Commun. 2019;10:1406. doi: 10.1038/s41467-019-09455-9.
6. Dong TS, Katzka W, Lagishetty V, Luu K, Hauer M, Pisegna J, Jacobs JP. A Microbial signature identifies advanced fibrosis in patients with chronic liver disease mainly due to NAFLD. Sci Rep. 2020 Feb 17;10(1):2771. doi: 10.1038/s41598-020-59535-w. PMID: 32066758; PMCID: PMC7026172.
7. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology (Baltimore, Md). 2017;65(5):1557-65.
8. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J et al. Modeling NA.FLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol. 2018;69(4):896-904. doi: 10.1016/j.jhep.2018.05.036.

9. Godoy-Matos AF, Silva Júnior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. Diabetol Metab Syndr. 2020;12:60. doi: 10.1186/s13098-020-00570-y.
10. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67(6):1265-73. doi: 10.1016/j.jhep.2017.07.027.
11. Heyens LJ.M., Busschots D, Koek GH, Robaeys G, Francque S. Liver fibrosis in non-alcoholic fatty liver disease: from liver biopsy to non-invasive biomarkers in diagnosis and treatment. Front Med (Lausanne). 2021;8:615978.
12. Huh Y, Cho YJ, Nam GE. Recent epidemiology and risk factors of nonalcoholic fatty liver disease. J Obes Metab Syndr. 2022;31(1):17-27. doi: 10.7570/jomes22021.
13. Huttenhower C, Gevers D et al. Structure, function and diversity of the healthy human microbiome: The Human Microbiome Project. Nature. 2012;486:2.
14. Janssen F, Bardoutsos A, Vidra N. Obesity Prevalence in the long-term future in 18 European countries and in the USA. Obes Facts. 2020;13:514-27. doi: 10.1159/000511023.
15. Ko JS. New perspectives in pediatric nonalcoholic fatty liver disease: epidemiology, genetics, diagnosis, and natural history. Pediatr Gastroenterol Hepatol Nutr. 2019;22 (6):501-510. doi: 10.5223/pghn.2019.22.6.501.
16. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC et al. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PloS one. 2017;12(3):e0173499.
17. Liang Q, Zhang M, Hu Y, Zhang W, Zhu P, Chen Y, Xue P, Li Q, Wang K. Gut microbiome contributes to liver fibrosis impact on T cell receptor immune repertoire. Front Microbiol. 2020 Nov 27;11:571847. doi: 10.3389/fmicb.2020.571847. PMID: 33329430; PMCID: PMC7729130.
18. Liu BN, Liu XT, Liang ZH, Wang JH. Gut microbiota in obesity. World J Gastroenterol. 2021 Jul 7;27 (25):3837-3850. doi: 10.3748/wjg.v27.i25.3837. PMID: 34321848; PMCID: PMC8291023.
19. Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. CellMetab. 2017;25:1054-1062.e5. doi: 10.1016/j.cmet.2017.04.001.
20. Lu FB, Hu ED, Xu LM, Chen L, Wu JL, Li H et al. The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol. 2018;12(5):491-502. doi: 10.1080/17474124.2018.1460202.
21. Mitra S, De A, Chowdhury A. Epidemiology of non-alcoholic and alcoholic fatty liver diseases. Transl Gastroenterol Hepatol. 2020;5:16. doi: 10.21037/tgh.2019.09.08.
22. Moran-Lev H, Cohen S, Webb M, Yerushalmy-Feler A, Amir A, Gal DL, Lubetzky R. Higher BM. I. predicts liver fibrosis among obese children and adolescents with NAFLD — an interventional pilot study. BMC Pediatr. 2021;21(1):385.
23. Ng CH, Lim WH, Hui Lim GE, Hao Tan DJ, Syn N, Muthiah MD, Huang DQ, Loomba R. Mortality outcomes by fibrosis stage in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2022 May 2:S1542-3565 (22)00439-6. doi: 10.1016/j.cgh.2022.04.014. Epub ahead of print. PMID: 35513235.
24. O'Gorman P, Norris S Exercising in the COVID-19 era: implications in non-alcoholic fatty liver disease (NAFLD). BMJ Open Gastroenterology. 2021;8:e000568.
25. Porras D, Nistal E, Martínez-Flórez S, González-Gallego J, García-Mediavilla MV, Sánchez-Campos S. Intestinal microbiota modulation in obesity-related non-alcoholic fatty liver disease. Front Physiol. 2018;9:1813.
26. Samir AE, Dhyani M, Vij A, Bhan AK, Halpern EF, Méndez-Navarro J, Corey KE, Chung RT. Shear-wave elastography for the estimation of liver fibrosis in chronic liver disease: determining accuracy and ideal site for measurement. Radiology. 2015 Mar;274(3):888-96. doi: 10.1148/radiol.14140839. Epub 2014 Nov 13. PMID: 25393946; PMCID: PMC4455676.
27. Schwiertz A, Taras D, Schafer K et al. Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring). 2010;18:190-195.
28. Suk KT, Kim DJ. Gut microbiota: novel therapeutic target for nonalcoholic fatty liver disease. Expert Rev Gastroenterol Hepatol. 2019;13(3):193-204.
29. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology. 2020;158(6):1611-125.e12. doi: 10.1053/j.gastro.2020.01.043.
30. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med. 2012;18(7):1028-40. .
31. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. doi: 10.1002/hep.28431.
32. Yumuk V, Tsigos C, Fried M, Schindler K, Busetto L, Micic D, Toplak H. Obesity management task force of the european association for the study of obesity. European guidelines for obesity management in adults. Obes Facts. 2015;8(6):402-24.
33. Zhan S, Li N, Liu C, Mao R, Wu D, Li T et al. Intestinal fibrosis and gut microbiota: clues from other organs. Front Microbiol. 2021;12:694967. doi: 10.3389/fmicb.2021.694967.
34. Zhu L, Baker SS, Gill C et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology. 2013;57,2:601-609. doi: 10.1002/hep.26093.

Н. І. Черелюк, О. Є. Гріднєв
ДУ «Національний інститут терапії імені Л. Т. Малої НАМН України», Харків

Основні філотипи кишкової мікробіоти та їхній зв'язок зі ступенем ожиріння та стадією фіброзу печінки у хворих на неалкогольну жирову хворобу печінки

Мета — проаналізувати стан відносного складу кишкової мікробіоти (КМ) на рівень основних філотипів у хворих на неалкогольну жирову хворобу печінки (НАЖХП) з різним індексом маси тіла та ступенем фіброзу печінки.

Матеріали та методи. У дослідження було залучено 105 осіб. Основну групу утворили 85 хворих на НАЖХП на тлі ожиріння 36,50 [32,00; 40,60] кг/м^2, яких розподілили на три підгрупи залежно від ступеня ожиріння: I підгрупа — 38 хворих з I ступенем ожиріння, II підгрупа — 23 хворих з II ступенем, III підгрупа — 24 хворих з III ступенем. Контрольну групу утворили 20 практично здорових особи, середній індекс

маси тіла — 23,50 [21,35; 25,78] кг/м^2. Ступінь фіброзу визначали за шкалою METAVIR з використанням показника середньої жорсткості паренхіми печінки в режимі зсувнохвильової еластографії. Вивчення складу КМ на рівні основних філотипів проводили шляхом ідентифікації загальної бактеріальної ДНК і ДНК *Bacteroidetes*, *Firmicutes*, а також співвідношення *Firmicutes*/*Bacteroidetes* методом кількісної полімеразної ланцюгової реакції в режимі реального часу з використанням універсальних праймерів для гена 16S рРНК і таксон-специфічних праймерів. Статистичну обробку здійснювали за допомогою пакета програм Statistica 13.1.

Результати. При коморбідному перебігу НАЖХП та ожиріння I ступеня у 47,37 % пацієнтів не виявлено ознак фіброзу, ще у 47,37 % — діагностовано фіброз 1 стадії (F1), у 2 (5,26 %) — F2, випадків F3 не було. У II підгрупі дві третини пацієнтів мали фіброз печінки, але випадків F3 не було, у III підгрупі лише 20,83 % пацієнтів не мали ознак фіброзу печінки, тоді як у 37,50 % діагностовано F1, у 33,33 % — F2, у 8,33 % — F3. У хворих I підгрупи виявлено статистично значуще збільшення величини співвідношення *Firmicutes*/*Bacteroidetes* порівняно з контрольною групою: за відсутності фіброзу — у 3,07 разу ($p < 0,01$), за наявності F1 — у 3,21 разу ($p < 0,01$), за наявності F2 — майже у 4 рази ($p < 0,01$). При порівнянні цього показника у пацієнтів з різними стадіями фіброзу статистично значущої різниці не виявлено ($p > 0,05$), зміни мали лише характер тенденції. У хворих II підгрупи також зафіксовано статистично значуще збільшення величини співвідношення *Firmicutes*/*Bacteroidetes* з контрольною групою: за відсутності фіброзу — у 3,25 разу ($p < 0,01$), за наявності F1 — у 3,81 разу ($p < 0,01$), за наявності F2 — у 5,08 разу ($p < 0,01$). Виявлено статистично значуще підвищення величини співвідношення *Firmicutes*/*Bacteroidetes* у 1,57 разу ($p < 0,01$) при F2 порівняно з F0, відмінності між іншими стадіями фіброзу не були статистично значущими і мали характер тенденції. У хворих III підгрупи за відсутності фіброзу величина співвідношення *Firmicutes*/*Bacteroidetes* перевищувала показник контрольної групи та прогресивно збільшувалася зі зростанням ступеня фіброзу. Установлено статистично значущу відмінність за величиною співвідношення *Firmicutes*/*Bacteroidetes* між F2 та F0 у 1,28 разу ($p < 0,01$), між F3 та F0 — у 1,35 разу ($p < 0,01$).

Висновки. Виявлено, що підвищення стадії фіброзу печінки асоціюється з певними порушеннями співвідношення *Firmicutes*/*Bacteroidetes*, відносного вмісту *Firmicutes* і *Bacteroidetes*. У I підгрупі відносний склад КМ статистично значущо відрізнявся лише від показника контрольної групи, тоді як у хворих II підгрупи з фіброзом F2 зафіксовано статистично значуще підвищення більш ніж у 1,5 разу величини співвідношення *Firmicutes*/*Bacteroidetes* порівняно з хворими без ознак фіброзу. У хворих III підгрупи з фіброзом не лише F2, а й F3 зафіксовано статистично значуще збільшення величини співвідношення *Firmicutes*/*Bacteroidetes*.

Ключові слова: неалкогольна жирова хвороба печінки, ожиріння, кишкова мікробіота, фіброз.

Контактна інформація

Черелюк Наталія Ігорівна, доктор філософії, мол. наук. співр. відділу вивчення захворювань органів травлення та їхньої коморбідності з неінфекційними захворюваннями
E-mail: nat.chereliyk@gmail.com
https://orcid.org/0000-0002-4227-6529

Стаття надійшла до редакції 02.12.2022 р.; рекомендована до опублікування 13.01.2023 р.

ДЛЯ ЦИТУВАННЯ

Chereliuk NI, Gridnyev OE. Main phylotypes of gut microbiota and their connection with the degree of obesity and the stage of liver fibrosis in patients with non-alcoholic fatty liver disease. Modern Gastroenterology (Ukraine). 2023;1:24-31. http://doi.org/10.30978/MG-2023-1-24.