ASSOCIATION OF NONALCOHOLIC FATTY LIVER DISEASE WITH SMALL INTESTINE BACTERIAL OVERGROWTH IN OBESE CHILDREN

ZWIĄZEK NIEALKOHOLOWEJ STŁUSZCZENIOWEJ CHOROBY WĄTROBY Z ZESPOŁEM ROZROSTU BAKTERYJNEGO JELITA CIENKIEGO U DZIECI Z OTYŁOŚCIĄ

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ABSTRACT

Introduction: In recent years, NAFLD is considered as the key of the so-called metabolic inflammation, in which the intestinal microbiota plays an important role.

The aim: To determine the effect of small intestine bacterial overgrowth on the liver structural and functional parameters in children with obesity and overweight.

Materials and methods: The object of the study was 89 children with obesity/overweight. Depending on the presence of SIBO based on the results of the hydrogen breath test with glucose, the patients were divided into 2 groups: first (I) consisted of 31 children with SIBO, the second (II) included 58 children without SIBO. All the patients under study performed a general blood analysis and a biochemical blood test, immuno-enzyme test method with insulin level determination HOMA index calculation. For diagnostics of the liver steatosis, transient elastography with the CAP (controlled attenuation parameter) function was carried out using FibroScan® 502 touch (Echosens, Paris, France).

Results: According to fibroscan data, the presence of SIBO in obese children can lead to raise of CAP level; liver steatosis was diagnosed in 22 patients (70.9%) of the 1st group and 24 patients (41.4%) of the 2 group (p<0.05). We found significant differences in the the ratio of neutrophils and lymphocytes (NLR) (p <0.05). The average glucose level and HOMA index were significantly higher in SIBO group (p<0,05). The analysis of risk factors of SIBO showed that metabolic syndrome and NAFLD as the risk factors for SIBO development (p<0,05).

Conclusions: SIBO has an effect on the structural and functional characteristics of the liver resulting in higher insulin and glucose level, higher NLR level and greater prevalence of NAFLD.

KEY WORDS: nonalcoholic fatty liver disease, small intestine bacterial overgrowth, children, obesity

INTRODUCTION

Childhood obesity has become a worldwide epidemic nowadays. According to World Health Organization (WHO), overweight and obesity affect more than 42 million children under the age of 5 years. At the same time, the prevalence of the consequences of this problem is growing: every second child with severe obesity has manifestations of the metabolic syndrome (MS) [1, 2, 3]. The prevalence of non-alcoholic fatty liver disease (NAFLD), which is the hepatic manifestation of MS, reaches 5-18% in countries in Asia, 30% in Europe, and its incidence in the child population is about 8-10% [4, 5]. It is known that in most cases the accumulation of fat in hepatocytes is a fairly benign condition, but in a certain number of patients (about 3-5%) the pathological process leads to the development of non-alcoholic steatohepatitis (NASH) with the further formation of fibrosis and cirrhosis. The latter consequence is the formation of portal hypertension and hepatic-cell insufficiency. The cases of liver transplantation in children, the need for which was caused by the terminal stage of the NAFLD, have already been described [6].

In recent years, NAFLD is considered as the key component of the so-called metabolic inflammation, in which the intestinal microbiota plays an important role [7, 8]. Microbiota is involved in regulation of both inflammatory and anti-inflammatory homeostasis, and in the accumulation of triglycerides in the liver (Fig. 1).

Patients with obesity are characterized by a certain composition of microflora with a relatively low proportion of Bacteroidetes and a predominance of Firmicutes. The microbiota composition of people with steatohepatitis even without obesity also has a low proportion of Bacteroidetes, which can lead to the multiplication of other bacteria that are more effective in extracting and storing energy [9].

Firstly, the metabolites produced by microbiota-short-chain fatty acids-can act as energy storage form and modulate the signaling pathways of the host organism metabolism. Participation in the accumulation of lipids in
the liver occurs by enhancing the intestinal absorption of monosaccharides, thereby increasing hepatic lipogenesis and being suppressed by the fasting-induced adipocyte factor, resulting in the accumulation of triglycerides in adipocytes [10].

Secondly - the intestinal epithelium is a natural barrier to prevent the translocation of harmful bacteria and their elements into circulation. NASH patients are usually characterized by small intestine bacterial overgrowth (SIBO) which can damage intestinal joints and increase its permeability. SIBO also induces hepatic expression of the TLR-4 receptors and the release of IL-8, which stimulates the inflammatory response. Consequently, intestinal dysbiosis is the cause of increased secretion of lipopolysaccharides (LPS), the constituent components of the intestinal bacterial membranes, and inflammation caused by it during the development of NAFLD [11]. In support of the hypothesis on the influence of microflora in the NAFLD development, numerous experimental studies are available; it is showing that normalization of the microbiota helps to prevent the development of fatty liver disease [12, 13]. It should be noted that, despite of numerous studies, the molecular mechanisms involved in this process in children and adolescents have not been fully determined yet.

**THE AIM**

To determine the effect of small intestine bacterial overgrowth on the liver structural and functional parameters in children with obesity and overweight.

**MATERIALS AND METHODS**

The object of the study was 89 children with obesity/overweight, who were on treatment at the Children’s Gastroenterology Department of the State Institution “Institute of Gastroenterology of NAMS of Ukraine”. Depending on the presence of SIBO based on the results of the hydrogen breath test with glucose, the patients were divided into 2 groups: first (I) consisted of 31 children with SIBO, the second (II) included 58 children without SIBO. The objective study, anthropometry with BMI calculation were conducted.
The presence of obesity / overweight was determined by the standard sigma deviations of BMI values for age and sex according to WHO recommendations [14]. The presence of abdominal obesity was diagnosed with a waist circumference (WC) of a patient that exceeded 90 percentiles for the corresponding age and sex according to the recommendations of the International Diabetic Federation (IDF) [15].

All the patients performed a general blood analysis and a biochemical blood test with the determination of the total protein, total bilirubin, ALT, AST, GGTP levels using the biochemical analyzer Stat Fax 1904 Plus, Awareness Technology (USA). The content of insulin in serum was determined by the immuno-enzyme test method with HOMA (Homeostasis model estimation) index calculation. A quantitative determination of the concentration of IL-6, IL-10, TNF-α in serum was carried out using ELISA.

Diagnosis of the small intestine bacterial overgrowth was performed according to the breath test with glucose loading with gas analyzer “Gastro” Gastrolyzer of Bedfont Scientific Ltd (UK). For diagnostics of the liver steatosis, transient elastography with the controlled attenuation parameter (CAP) measurement was carried out using FibroScan® 502 touch (Echosens, Paris, France).

Diagnosis of NAFLD was established in case of liver steatosis being detected according to CAP level and exclusion of the secondary etiological factors of steatosis in children with overweight / obesity.

The statistical analysis was carried out using the Statistica program. Under normal distribution conditions, the data were presented as the mean (M) and standard deviation (m). The significance of differences between the groups was assessed using Student’s t-test. The difference was considered reliable at a value of p less than 0.05. In the absence of a normal distribution, the data was displayed using the median and quartiles, and the difference between the groups was estimated using the Mann-Whitney test. Analysis of risk factors was performed with calculation of odds ratio (OR).

RESULTS

ANTHROPOMETRIC DATA

Analyzing the anthropometric data, it was found that the abdominal type of obesity prevailed in children with SIBO - the waist circumference was higher in 1 group than in the second group (p = 0.04) (Table I). Abdominal obesity was diagnosed in 16 (51.6%) of the 1 group and in 25 (43.1%) of the 2 group.

It should be noted that metabolic syndrome (according to IDF) was diagnosed in 6 (19.3%) patients of SIBO group and 5 (15.6%) patients of 2 group.

BLOOD ANALYSIS

The data of the general clinical analysis of the blood are presented in Table II.

Children of SIBO+ group had higher level of ESR in comparison with SIBO- group, but difference was not significant. As can be seen from the table, there were significant differences in the ratio of neutrophils to lymphocytes - in the group with bacterial overgrowth it was significantly higher (p <0.05).
Interesting data were obtained by analyzing the carbohydrate metabolism parameters of patients (Table III). So, although the average glucose level did not go beyond the normal level, in the group without SIBO it was significantly higher, and conversely, insulin resistance (IR) was greater in the group with bacterial overgrowth.

Indices of the content of various cytokines (IL-6, IL-10 and TNF-alpha) are presented in Table IV. It was found that the median level of IL-10 in patients in the 2 group was 1.8 times higher than in the 1 group (Tab. IV).

CARBOHYDRATE METABOLISM

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FIBROSCAN DATA

Significant differences were observed in the analysis of CAP level (Fig. 2).

According to fibroscan data, the presence of SIBO in obese children led to raise in CAP level (tab. V); liver steatosis was diagnosed in 22 patients (70.9%) of the 1st group and 24 patients (41.4%) of the 2nd group. In addition, different degrees of steatosis were observed. In SIBO group S1 degree was observed in 7 patients (22.6%), S2 - in 9 (29.0%) and S3 - in 6 patients (19.3%), while in children without SIBO the maximum degree of steatosis was ob-
Table V. Basic statistical indicators characterizing the structure of the liver

<table>
<thead>
<tr>
<th>Parameters, units</th>
<th>M ± m</th>
<th>Median</th>
<th>IQR (25%-75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver stiffness (kPa)</td>
<td>4,6 ± 1,3</td>
<td>4,2</td>
<td>3,6 – 5,1</td>
</tr>
<tr>
<td>CAP (dB/m²)</td>
<td>262,7 ± 20,1</td>
<td>265,0</td>
<td>246,5–274,5</td>
</tr>
</tbody>
</table>

Table VI. Risk factors for SIBO

<table>
<thead>
<tr>
<th>Factors, n (%)</th>
<th>I group (n=31)</th>
<th>II group (n=58)</th>
<th>OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>18 (58,1)</td>
<td>30 (51,7)</td>
<td>1,29</td>
<td>0,54-3,11</td>
</tr>
<tr>
<td>Overweight</td>
<td>12 (38,7)</td>
<td>19 (32,7)</td>
<td>1,49</td>
<td>0,57-3,92</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>16 (51,6)</td>
<td>25 (43,1)</td>
<td>1,40</td>
<td>0,58-3,37</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>18 (58,1)</td>
<td>23 (53,4)</td>
<td>2,10</td>
<td>0,87-5,11</td>
</tr>
<tr>
<td>NAFLD</td>
<td>22 (19,3)</td>
<td>24 (41,4)</td>
<td>3,46</td>
<td>1,36-8,82</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>6 (32,3)</td>
<td>9 (15,5)</td>
<td>3,54</td>
<td>1,12-11,64</td>
</tr>
</tbody>
</table>

served in only 4 (6.9%) patients, the 1 degree of steatosis in 9 patients (15.6%); S2 - 11 patients (18.9%).

Also we observed that liver stiffness showed a trend to be higher in SIBO+ children.

RISK FACTORS

The analysis of risk factors of SIBO was carried out, according to them it is possible to carry out metabolic syndrome and NAFLD as the risk factors for SIBO development (tab. VI).

DISCUSSION

Previous studies have already confirmed the relationship between the intestinal microflora and the development of fatty liver disease. It is known that metabolic profiles differ between children with and without obesity, as well as with and without NAFLD, and a significant contribution to these differences is made by the state of the entero-hepatic axis [15]. Intestinal microflora is involved in the development of obesity and MS by enhancing the interaction between the intestinal bacterial products and Toll-like receptors 4 (TLR4).

According to the studies in patients with nonalcoholic steatohepatitis (NASH) there are specific changes in the microflora and the amount of alcohol produced by Escherichia is increased. This results in intestinal wall intracellular connections disruption, the concentration in the portal blood of ethanol and other metabolic products of bacteria and their constituents - amino acids and lipopolysaccharides elevation [16].

Excessive reproduction and growth of microflora promote a cascade of reactions of oxidative stress, parenchymal inflammation and fibrogenesis. Thus, studies by Italian, American, Finnish scientists have shown that a high content of aromatic amino acids and branched chain amino acids in the plasma, as products of the vital activity of the intestinal microflora, is associated with the development of obesity-related MS. Metabolism is also disturbed by changes in the carnitine cycle, which leads to damage to the nucleotide, lysolipids, and activation of proinflammatory markers [17, 18, 19, 20].

The increase in lipopolysaccharide levels is associated with an increase in tumor necrosis factor-alpha (TNF-α) gene expression in the hepatic tissue, which confirms the role of endotoxemia in the development of steatohepatitis [16]. It has been shown that SIBO with steatohepatitis is associated with an increase in hepatic expression of TLR4 and the release of interleukin-6 and 8 (IL) -8 [9]. In our study, we did not find differences between the levels of the proinflammatory cytokines TNF, IL-6 and IL-10 in obese patients, depending on whether or not they had SIBO. Perhaps the fact is that previous studies were conducted in adults, and among our patients, the inadequate exposure of SIBO has not yet led to significant changes in these indicators.

Since the early 1970s, adipose tissue has been recognized as the link between inflammation and metabolism, demonstrating that endotoxin enhances the release of free fatty acids from visceral adipose tissue [8]. The identification of chronic release of cytokines such as TNF-alpha from adipose tissue has triggered a wave of studies showing that obesity presents a complex and integrated immune system of adipose tissue that includes both congenital and acquired components [9, 10]. Within this network, multiple innate pathways of inflammatory signals such as inflammasomes, lysosomes, NF-kb, and JNK contribute to metabolic destruction [11-14]. These acute inflammatory signals cannot always counteract the metabolic adaptation to excessive nutrition and in fact may be required for an adequate response in adipose tissue. Proinflammatory cascades within adipocytes are required for adipogenesis and hypertrophy of adipocytes with a change in the high-fat diet (HFD) [15]. Within a short time, the HFD in mice induces IR regardless of inflammation and is characterized by NKT-mediated alternative activation of resident tissue...
macrophages of adipose tissue [16, 17]. Thus, inflammatory responses in this context probably represent an adaptive response that allows the expansion of healthy adipose tissue and effective fat storage [18].

On the one hand, SIBO can induce the activation of blood leukocytes with the help of circulating LPS, on the other hand the metabolic inflammation in obesity stimulates the bone marrow for leukopoiesis [19]. In our study children with SIBO had higher level of ESR but the significance of differences wasn’t sufficient, also we observed the higher neutrophil-to-lymphocyte ratio (NLR) level in SIBO group that indicates signs of more intensive inflammation in obese children with SIBO.

Several studies have demonstrated the importance of NLR as an indicator of subclinical inflammation in coronary artery disease. It has been shown that high levels of NLR could predict a risk of recurrence in patients with various malignancies [20]. The study of Mehmet Asil et al. showed that the neutrophil-to-lymphocyte ratio was higher in patients with steatohepatitis than in patients with simple steatosis and healthy controls. Taking into account that the difference between patients with simple steatosis and healthy controls was not statistically significant, the increased neutrophil-to-lymphocyte ratio in the patients with steatohepatitis can be attributed to a low level of systemic inflammation accompanying the hepatic inflammation [21]. Also it has been shown that neutrophil-to-lymphocyte ratio increases with increasing grade of non-alcoholic fatty liver disease in patients with type 2 diabetes, and may be a convenient marker to follow progression of non-alcoholic fatty liver disease [22]. We’ve calculated ratio of relative count and it was significantly higher in patients with SIBO, we suppose that this ratio also can be considered as inflammation index that can be useful in NAFLD patients.

We also found an increase in glucose and insulin levels in patients with SIBO that can be explained by the anabolic properties of microflora. Also it is known that the small intestine plays a key role in glucose regulation and diabetes pathogenesis. The central role of small intestinal motility on the development of SIBO was highlighted in a recent study; the authors concluded the primacy of altered small intestinal motility on the development of SIBO. SIBO is present in up to 40% of patients with diabetes with diarrhea [23].

One potential explanation for this effect might be the fact that the microbiotas of obese individuals have an increased capacity to breakdown non-digestible carbohydrates and produce short-chain fatty acids, which would be subsequently absorbed, metabolized to more complex lipids in the liver, and then stored in adipose tissue [8, 24, 25].

Indeed, as we see association between NAFLD and SIBO can have bilateral character. Children with SIBO have inverse metabolic and functional liver profile and also NAFLD seem to be a risk factor for SIBO development in this group of patients.

The limitations of the study are due to the fact that the diagnosis of hepatic steatosis was not established on the basis of the “gold standard” - the morphological study of liver biopsy, but on the basis of the method of noninvasive diagnostics - transient elastography of the liver with determination of the CAP function. However, it should be noted that in most studies on the effect of SIBO on the course of NAFLD, the routine ultrasound was used as the basis for the diagnosis of steatosis, which is significantly inferior to fibroscan in sensitivity and specificity.

CONCLUSIONS
Thus, SIBO has an effect on the structural and functional characteristics of the liver resulting in higher insulin and glucose level, higher NLR level and greater prevalence of NAFLD. We can assume that NAFLD as far as MS are the risk factors for SIBO development in obese children.

REFERENCES

**Authors’ contributions:**
According to the order of the Authorship.

**Conflict of interest:**
The Authors declare no conflict of interest.

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