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Liver disease symptoms in non-alcoholic fatty liver disease and small intestinal bacterial overgrowth

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Introduction. It seems that there is a relationship between small intestinal bacterial overgrowth (SIBO) and non-alcoholic fatty liver disease (NAFLD). The main objective of this study was to evaluate the prevalence of SIBO among NAFLD patients.

Methods. In this descriptive-analytical cross-sectional study, 98 eligible NAFLD patients were evaluated for SIBO using hydrogen breath test (HBT). They were divided into SIBO-positive and SIBO-negative groups. Demographic, clinical, and laboratory data were obtained.

Results. Based on the HBT, 38 patients (39%) had bacteria overgrowth. There were no significant differences between SIBO-positive and SIBO-negative regarding demographic data and BMI classification ($P > 0.05$). Biochemical variables, the results of abdominal ultrasound, and liver elastography did not show any significant difference between SIBO-positive and SIBO-negative patients ($P > 0.05$). Patients with SIBO were found to have higher rates of bloating, while abdominal pain was more prevalent in SIBO-negative patients ($P < 0.001$).

Conclusions. SIBO is prevalent in NAFLD and associated with bloating in these patients. Further studies are necessary to elucidate if therapeutic manipulation of gut microbiota reduces the risk of NAFLD, fibrosis, and liver cirrhosis.

Key words: Non-alcoholic fatty liver disease, Small intestinal bacterial overgrowth, Breath tests, Abdominal pain.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of ≥ 5% of hepatic steatosis without any competing liver disease etiologies including a variety of liver damages, which may increase the risk of mortality [1]. A recent meta-analysis reported that worldwide prevalence of NAFLD was 25.24%, mostly observed in the Middle East and South America [2]. Unfortunately, the prevalence of this disease has increased at an alarming rate in recent decades due to lifestyle changes (physical activities, diet) and rise of obesity [3].

Although NAFLD occurs most frequently during the 4^{th}-6^{th} decade, it is almost seen in all age, gender, and racial groups [4]. The pathogenesis of NAFLD is not yet fully understood. Several theories address the role of insulin resistance in steato-hepatitis, resulting in the resistance of the adipose tissue to the anti-lipolytic effect of insulin with an increase of free fatty acids [5]. There are also other hypotheses proposing "second hit", or additional oxidative injury, as the reason of necro-inflammation in steatohepatitis [6]. Factors have been suggested as potential oxidative stressors, including hepatic iron [7], leptin, antioxidant deficiencies [8], and intestinal bacteria.

Some studies reinforced the concept that small intestinal bacterial overgrowth (SIBO) plays an important role in the pathogenesis of NAFLD through endotoxin of bacteria and tumor necrosis factor (TNF) as effective mediators [9, 10]. SIBO is defined as presence of more than 10^5 colony forming unit (CFU)/mL in duodenal aspirate cultures [11]. Clinical manifestations of SIBO include bloating, abdominal discomfort, watery diarrhea, dyspepsia, weight loss, hepatic steatosis and macrocytic anemia resulting from accumulation of bacteria such as *E. coli* and *Bacteroides* in the small intestine. Bacterial overgrowth has been associated with severe hepatic steatosis in morbidly obese patients [12].

As SIBO is common in NAFLD patients and plays a role in its pathogenesis, it is likely that correct treatment of SIBO (using antibiotics and probiotics) can reduce the risk of liver fibrosis,

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cirrhosis, and quality of life of patients. Therefore, we aimed to determine the prevalence of SIBO in NAFLD patients regarding liver disease symptoms.

MATERIALS AND METHODS

We conducted a descriptive-analytical cross-sectional study in NAFLD patients recruited from Shariati Hospital (Isfahan, Iran) during the period from March 2012 to March 2013. The sample size was determined to provide 0.8 power to detect the frequency of SIBO in patients with NAFLD. It was supposed that the prevalence of SIBO in NAFLD patients was 0.5. Using sample size calculation formula in cross-sectional studies,

$$n = \frac{Z_{1-\frac{a}{2}}^{2} \times \rho(1-p)}{d^2}$$

with the absolute error of 1% and a type 1 error of 5%, a sample of 96 patients was required (p = 0.5); however, we included 98 patients. The study was approved by the Ethics Committee of Islamic Azad University of Najafabad. Demographic and clinical data were obtained.

Patients were included if abdominal ultrasound (heterogeneous appearance of the liver and echogenicity exceeding that of the spleen or renal cortex) and liver elastography confirmed NAFLD. Abdominal ultrasonography was performed by a single expert physician. Liver dimensions, borders, and parenchymal texture were evaluated in order to classify liver steatosis as follows: 1 – increased liver echogenicity; 2 – echogenic liver obscuring; 3 – diaphragmatic outline obscuring. Individuals having a prior diagnosis of liver diseases due to viral, metabolic and genetic causes, chronic pulmonary disease, alcohol drinking, and antibiotic use within recent two weeks were excluded. Liver elastography was performed by FibroScan 402 (Echosens, France).

SIBO was diagnosed using glucose hydrogen breath test (HBT), (Micro Medical Company, England). The H_2 breath levels were measured after an overnight fasting and the patients were then given 1 g/kg glucose dissolved in 150 mL sterile water and readings were conducted every 30 minutes for two hours. The test was considered positive for SIBO when fasting breath hydrogen concentrations were > 20 parts per million (ppm) or the hydrogen measured in the exhaled breath had a rise of > 20 ppm above the baseline value [13].

Patients were questioned about bloating and abdominal pain and their medical history was assessed to confirm the symptoms. Using ATP III criteria [14], metabolic syndrome was evaluated in patients to rule out the negative effects of poor glucose control on abdominal symptoms.

Laboratory variables, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, serum albumin, and prothrombin time (PT) were also measured.

Continuous variables were demonstrated as n (%), mean ± standard deviation (SD), or median interquartile range (IQR) whenever the distribution of data was not normal. The data were analyzed using chi square and student t-tests and $P < 0.05$ was considered statistically significant. SPSS software version 21.0 was used.

RESULTS

Ninety-eight patients with NAFLD were recruited with the mean age of 48.5 ± 12.1 years and a total of 38 (39%) were males. Based on HBT, 38 patients (39%) had bacteria overgrowth. Comparing the groups, there were no difference in gender, age, and BMI categories between the groups ($P > 0.05$). There were no significant differences between SIBO-positive and SIBO-negative in terms of demographic data and BMI classification ($P > 0.05$). The number of patients with metabolic syndrome was similar between the groups ($P > 0.05$) (Table 1).

Table 1
Baseline characteristics of NAFLD patients with and without SIBO[†]

Variable		SIBO positive (n = 38)	SIBO negative (n = 60)	P-value
Sex, n (%)	Male	11 (30)	27 (45)	0.11
	Female	27 (71)	33 (55)	
BMI[‡], kg/m^2, n (%)	Normal	3 (8)	6 (10)	0.13
	Overweight	8 (21)	25 (42)	
	Obese	27 (71)	29 (48)	
Age, years, mean ± SD		48.8±11.6	48.4±12.5	0.87
Metabolic syndrome, n (%)		21 (55)	29 (48)	0.229

[*]NAFLD: non-alcoholic fatty liver disease, [†]SIBO: small intestinal bacterial overgrowth; [‡]BMI: body mass index

Biochemical variables, the results of abdominal ultrasound, and liver elastography did not show any significant difference between SIBO-positive and SIBO-negative patients (P > 0.05). Patients with SIBO were found to have higher rates of bloating (P < 0.001), while abdominal pain was more prevalent in SIBO-negative patients (P < 0.001) (Table 2). We performed a multinomial logistic regression to find odds ratio of bloating in patients with SIBO, after adjusting for metabolic syndrome. It was more likely that NAFLD patients had SIBO if they experienced bloating (OR: 38.35, 95% CI: 9.44-155.73, P < 0.001).

Table 2
Biochemical variables in the groups

Variable		SIBO* positive (n = 38)	SIBO negative (n = 60)	P-value
AST†(U/T), n (%)	< 35	33 (87)	50 (83)	0.64
	≥ 35	5 (13)	10 (17)	
ALT‡(U/L), n (%)	< 40	33 (87)	55 (92)	0.50
	≥ 40	5 (13)	5 (8)	
ALP§(IU/L), n (%)	< 306	36 (95)	58 (97)	0.64
	≥ 360	2 (5)	2 (3)	
PT□(Sec), n (%)	< 15	38 (100)	56 (93)	0.16
	≥ 15	0	4 (7)	
Total Bilirubin (mg/dL), n(%)	< 1.2	35 (92)	52 (87)	0.52
	≥ 1.2	3 (8)	8 (13)	
Serum albumin(g/dL), mean ± SD		4.7 ± 0.41	4.58 ± 0.49	0.23
Clinical symptoms, n (%)	Bloating	34 (90)	13 (22)	**< 0.001**
	Abdominal pain	4 (11)	47 (78)	**< 0.001**
Grade of fatty liver, n (%)	1	15 (40)	32 (53)	0.19
	2	19 (50)	19 (38)	
	3	4 (11)	9 (3)	
Elasticity of Liver (kpa), median (IQR[d])		7.9 (6.9-10.2)	7.8 (5.8-10.5)	0.56

[a]SIBO: small intestinal bacterial overgrowth; [b]AST: aspartate aminotransferase; [c]ALT: alanine aminotransferase; [d]ALP: alkaline phosphatase; [e]PT: prothrombin time; [d]IQR: interquartile range

DISCUSSION

In this study, the frequency of SIBO was quietly higher in patients with NAFLD. These features are compared in terms of presence or absence of SIBO. It was found that bloating was associated with SIBO.

High prevalence of SIBO in patients with NASH (non-alcoholic steatohepatitis) has been reported previously [15-17]. A number of studies have shown that SIBO is seen more in patients with cirrhosis [18-20]. Miele *et al*. found that SIBO had a high prevalence in NAFLD patients compared with normal people [21]. Proinflammatory metabolic by-products of gut microbiota, ethanol production by intestinal bacteria, and volatile organic compounds delivery to the liver *via* portal circulation may have toxic effects on the liver [22]. In one study by Wigg *et al*., a role for SIBO in development of NAFLD through intestine permeability and increased endotoxins absorption was suggested [15]. There are also interventional studies revealing beneficial effects of supplementation with probiotics on reduction of hepatic fat accumulation in rat model of NAFLD [23].

According to the previous studies, bacteria overgrowth might be more common in elderly people compared to young people (5.9% in adults aged 24-59 years *vs*. 15.6% in the older group) [24]. The reason can be related to a reduction in physical activity, slower metabolism, and low gastrointestinal (GI) motility. On the other hand, some metabolic and GI disorders may lead to movement of undigested food residues to the large intestine, where no food absorption but bacterial overgrowth occurs. However, we could not find any age difference between SIBO-positive and SIBO-negative patients.

Obesity is an important risk factor for presence of NAFLD. It has been shown that composition of gut microbiota of obese and lean people is different. Here, there was no significant difference in BMI between the SIBO-positive and SIBO-negative patients. SIBO has been associated with BMI, and obese patients with SIBO have been diagnosed with severe hepatic steatosis [12]. In this study, such a relationship was not observed; it seems that the most important reason of lack of similarity between our study and other studies is that our study population has consisted of patients with

known fatty liver. Comparing the results with a control group could shed light on the relationship between SIBO and BMI.

In the present study, it was shown that serum levels of liver enzymes had not significant differences between the patients with and without bacteria overgrowth. SIBO per se is not a risk factor of elevated liver enzymes and other conditions such as insulin resistance, diabetes mellitus, obesity, dyslipidemia, and oxidative stress may be also involved.

The following symptoms can be experienced by patients with SIBO: excessive bloating, gas, abdominal pain, flatulence, and steatorrhea. Bloating, in this study, was more common than abdominal pain in those with bacteria overgrowth. It has been demonstrated that the number of alcohol producing bacteria can increase in NASH microbiomes; as a result, blood ethanol levels are higher in these patients and they might face more oxidative stress owing to alcohol metabolism. Consequently, observation of more bloating in NAFLD patients can be explained. In this regard, Alkhouri *et al.* used exhaled breath analysis (*via* measuring isoprene, acetone, trimethylamine, acetaldehyde, and pentane) as a promising noninvasive method to detect fatty liver in children [25]. To the best of our knowledge, this is the first study to evaluate frequency of SIBO symptoms in NAFLD patients. Although bacteria overgrowth symptoms are non-specific, further studies should be conducted for a better diagnosis of SIBO based on clinical signs and preventing its progression to fatty liver.

There are some limitations to our study. Firstly, SIBO was diagnosed using HBT, and we could not measure methane concentration in the breath. Secondly, we did not compare our results with a healthy control group (without NAFLD). Future well-designed studies are needed to confirm the relationship between SIBO and NAFLD. We also recommend comparing results in NASH patients with non-NASH NAFLD ones.

In summary, SIBO is prevalent in NAFLD and associated with bloating in these patients. Further studies are necessary to elucidate if therapeutic manipulation of gut microbiota reduces the risk of NAFLD, fibrosis, and liver cirrhosis.

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Introducere. *Se pare că există o legătură între creşterea bacteriilor la nivelul intestinului subțire (SIBO) şi steatoza hepatică non-alcoolică (NAFLD). Obiectivul studiului a fost de a evalua prevalența SIBO în cadrul NAFLD.*

Metode. *În acest studiu descriptiv transversal, 98 de pacienți eligibili cu NAFLD au fost evaluați pentru SIBO folosind testul respirator cu hidrogen (HBT). După rezultatele acestui test, aceştia au fost împărțiți în SIBO pozitivi şi SIBO negativi. Au fost obținute date demografice, clinice şi de laborator.*

Rezultate. *Bazat pe datele obținute cu HBT, 38 de pacienți (39%) aveau SIBO. Nu au fost diferențe semnificative între pacienții SIBO pozitivi şi cei negativi din punctul de vedere al datelor demografice sau din punctul de vedere al BMI. Nu au fost găsite diferențe nici între variabilele biochimice, modificările ecografice sau ale elastografiei. Pacienții cu SIBO însă au avut mai prevalent senzație de balonare, pe când pacienții SIBO negativi au avut mai frecvent discomfort abdominal ($p < 0.001$).*

Concluzii. *SIBO este relativ frecventă la pacienții cu NAFLD şi se asociază cu balonarea. Mai multe studii sunt necesare pentru a determina dacă intervenția asupra microbiomului intestinal scade riscul de dezvoltare a NAFLD, a fibrozei şi cirozei hepatice.*

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