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Small Intestinal Bacterial Overgrowth Is Associated with Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Background: Changes in gut bacteria play a role in type 2 diabetes mellitus (DM) and hepatic steatosis. There is a lack of studies evaluating the frequency and risk factors for non-alcoholic fatty liver disease (NAFLD) in patients tested for small intestinal bacterial overgrowth (SIBO). **Aim**: To evaluate the frequency of NAFLD and associated risk factors in patients tested for SIBO.

Methods: In this case-control study, 372 eligible patients submitted to glucose hydrogen/methane breath test for SIBO who also had an abdominal imaging study were included. Patients were divided into SIBO-positive and SIBO-negative groups. Clinical, demographic and laboratory variables were evaluated in addition to the presence of NAFLD on abdominal imaging.

Results: Of the 372 eligible patients, 141 (37.9%) were tested positive for SIBO (study group) and 231 (62.1%) were negative for it (control group). NAFLD occurred in 45.4% (64/141) of the study group compared to 17.3% (40/231) of the control group ($p<0.001$). Patients in the study group were found to have higher rates of elevated aspartate aminotransferase (AST) (20.6% vs. 11.3%; $p=0.034$) and alanine aminotransferase (ALT) levels (56.0% vs. 40.7%; $p= 0.039$), type 2 diabetes (23.4% vs. 13.9%; $p=0.041$), hypertension (54.6% vs. 40.3%; $p=0.046$) and metabolic syndrome (78.0% vs. 60.2%; $p=0.020$). In the multivariate analysis, SIBO (odds ratio [OR]: 1.95; 95% confidence interval [CI]: 1.14-3.31; $p=0.014$), type 2 DM (OR: 3.04; 95%CI: 1.57-5.90; $p=0.001$) and obesity (OR: 3.58; 95%CI: 1.70-7.54; $p=0.001$) remained associated with NAFLD.

Conclusion: Patients with SIBO have an increased risk for hepatic steatosis and may benefit from aggressive control of the risk factors for NAFLD including metabolic syndrome.

Key words: metabolic syndrome – non-alcoholic fatty liver disease – small intestinal bacterial overgrowth.

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CTE: computed tomography enterography; DM: diabetes mellitus; ETOH: ethanol; IL: interleukin; LPS: lipopolysaccharide; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PPI: proton pump inhibitor; SIBO: small intestinal bacterial overgrowth; TLR-4: toll-like receptor 4; TMAO: trimethylamine-N-oxide (TMAO); TNF-α: tumor necrosis factor alpha.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disorders worldwide, affecting 16-30% of the general population, and its prevalence is rising along with the obesity epidemic [1]. Commonly reported risk factors for NAFLD are obesity, abdominal fat deposition, insulin resistance and type 2 diabetes mellitus (DM) [2]. More recently, a possible role of gut bacteria in NAFLD has been suggested.

There is a growing body of evidence suggesting that gut bacteria influence the hosts' metabolism and predispose them to metabolic syndrome and its consequences. Gut microbiota have been shown to be associated with components of the metabolic syndrome, such as obesity [3, 4], insulin resistance and type 2 DM [5–7], with unclear mechanism. Investigators have speculated the role of an increased inflammatory state in the host, leading to insulin resistance [7–9]. In fact, recent studies point towards a possible role of gut bacteria in the development of NAFLD [10–12].

Small intestinal bacterial overgrowth (SIBO), a condition characterized by excessive gram-negative aerobic and

anaerobic bacteria in the proximal small bowel, has been shown to induce hepatic steatosis in animal models [13, 14]. In humans, the presence of SIBO has been associated with an increased risk and severity of NAFLD in obese individuals [15], who are already at a high risk for hepatic steatosis. Whether patients with SIBO are at an increased risk of developing NAFLD independently of the presence of obesity is unknown.

There is a lack of studies evaluating the frequency and risk factors for NAFLD in patients tested for SIBO. Therefore, the aims of this study were to determine the frequency of NAFLD in patients with SIBO and to evaluate SIBO as an independent risk factor for NAFLD.

PATIENTS AND METHODS

The electronic medical record of 923 consecutive patients from our Gastrointestinal Motility Lab data bank who were submitted to glucose hydrogen/methane (H_2/CH_4) breath test for diagnosis of SIBO at our tertiary care center from the period of January 2004 to January 2014 were reviewed. Detailed demographic and clinical data, including data pertaining to SIBO and liver diseases were obtained. This study was approved by our Institutional Review Board.

Inclusion and exclusion criteria

Included were patients with 1) glucose H_2/CH_4 breath test for evaluation of small intestinal bacterial overgrowth, and 2) abdominal imaging performed at our institution.

Patients who had a prior diagnosis of a liver disease due to causes other than NAFLD were excluded. In addition, patients who had significant alcohol intake defined as greater than 20g of alcohol/day were also excluded.

Study and control groups

Of the initial 923 patients, 372 who had liver imaging before or after the glucose H_2/CH_4 breath test were included in the study. Liver imaging techniques were abdominal ultrasonography (US), abdominal computerized tomography (CT) scan, computerized tomography enterography (CTE) scan and abdominal magnetic resonance imaging (MRI).

Patients were subdivided into the SIBO-positive or study group (n=141) and the SIBO-negative or control group (n=231). All patients were then further subdivided according to the presence or absence of hepatic steatosis on liver imaging.

Diagnosis of SIBO

SIBO was diagnosed using the glucose H_2/CH_4 breath test. The H_2/CH_4 breath concentration was expressed in parts per million (p.p.m.) and measured by gas chromatography after the administration of an oral loading dose of glucose (50 g in 250 ml of sterile water). The test was considered positive for SIBO when one or more of the criteria were present: H_2 and/or CH_4 increase >20 p.p.m. above basal value or H_2 and/or CH_4 increase >12 p.p.m. between minimal and maximal values after glucose ingestion [16].

Diagnosis of NAFLD

The diagnosis of NAFLD was made according to the criteria used in previous studies, based on findings of liver imaging, including abdominal ultrasound, abdominal CT or abdominal MRI. NAFLD was diagnosed when there was: 1) heterogeneous appearance of the liver and echogenicity exceeding that of the renal cortex or spleen on abdominal ultrasound; and/or 2) lower attenuation of the liver parenchyma compared to the spleen and/or intrahepatic blood vessels on abdominal CT; and/or 3) signal intensity loss on opposed-phase images in comparison with in-phase images by MRI. This is in accordance with previous studies [17, 18].

In addition, metabolic syndrome was also studied in patients undergoing the glucose H_2/CH_4 breath test. Metabolic syndrome was defined as the presence of three out of five of the following criteria according to the Adult Treatment Panel III: 1) obesity (body mass index, BMI ≥30), 2) triglycerides ≥150 mg/dL, 3) HDL cholesterol <40 mg/dL in men and <50 mg/dL in women, 4) blood pressure ≥130/85 mmHg, and 5) fasting glucose ≥110 mg/dL [19].

Dyslipidemia was defined as triglycerides ≥150 mg/dL and/or HDL cholesterol <40 mg/dL in men and <50 mg/dL in women.

Study variables

A total of 19 variables were studied. Laboratory variables included were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatinine, platelets and albumin. Clinical variables included were age, sex, BMI, previous abdominal surgery, type of abdominal imaging modality, NAFLD, hypertension, type 2 DM, metabolic syndrome, hypothyroidism and use of proton pump inhibitors (PPI). Patients were further classified according to their BMI into underweight (BMI <18.50 kg/m^2), normal weight (BMI 18.50-24.99 kg/m^2), overweight (BMI 25.00-29.99 kg/m^2) or obese (BMI ≥30 kg/m^2) based on the World Health Organization classification.

Outcome measurements

The primary outcomes of this study were to assess the frequency and associated risk factors for NAFLD in patients undergoing glucose H_2/CH_4 breath test for SIBO.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) or N%. Univariate analysis was performed to identify potential variables associated with SIBO. Student's *t*-tests or the non-parametric Wilcoxon rank sum tests were used for continuous factors and Pearson chi-square test was used for categorical variables. Multivariable logistic regression analysis was performed to assess risk factors associated with NAFLD in SIBO patients. A $p<0.05$ was considered statistically significant. All statistical analysis were performed using SPSS software version 22 (IBM Corp, Armonk, NY).

RESULTS

A total of 923 patients who were tested for SIBO with glucose H_2/CH_4 breath were initially evaluated. Among these patients, 551 who did not have liver imaging or who had liver disease other than NAFLD or who had alcohol consumption > 20g/day were excluded from the study. The remaining 372

patients who fulfilled the inclusion criteria were included for analysis (Fig. 1).

Interval in months between SIBO evaluation and abdominal imaging

A total of 202 patients had SIBO evaluation before abdominal imaging with a median interval of 15.0 months [interquartile range (IQR) of 1.0-35.7months].

A total of 170 patients had SIBO evaluation after abdominal imaging with a median interval of 10.0 months (IQR 4.0-30.0 months).

Frequency of SIBO

The SIBO-positive rate in the whole cohort was 37.9% (141/372). The mean age of the 372 patients included in the study was 59.8±0.8 years, 24.7% (92/372) were males, the mean BMI was 27.9±0.4: 28.0% (104/372) had NAFLD, 17.5% (65/372) had DM, 45.7% (170/372) had hypertension and 66.9 % (249/372) had metabolic syndrome.

Frequency of NAFLD

NAFLD was found in 104 (28.0%) of the 372 patients included in the study. Among the 141 patients with SIBO, 64 (45.4%) had NAFLD compared to 40 (17.3%) of the 231 patients without SIBO (p<0.001).

Univariate comparison of clinical variables and outcomes between patients with or without SIBO

Patients with SIBO were found to have higher rates of elevated AST (20.6% vs. 11.3%; p=0.034) and ALT (56.0% vs. 40.7%; p= 0.039). There was also a higher frequency of NAFLD (45.5% vs. 17.3%; p<0.001), type 2 DM (23.4% vs. 13.9%; p=0.041), hypertension (54.6% vs. 40.3%; p=0.046) and metabolic syndrome (78.0% vs. 60.2%; p=0.020) in SIBO-positive patients when compared to patients without SIBO (Table I).

There was no difference among SIBO-positive and SIBO-negative patients regarding BMI classification, history of abdominal surgery, alcohol use or prior exposure to proton pump inhibitors.

Multivariate analysis of risk factors associated with NAFLD

In the multivariate logistic regression analysis, SIBO, hypertension, type 2 DM, obesity and metabolic syndrome were included in the final model. After adjusting for all other variables in the model, SIBO (odds ratio [OR]: 1.95; 95% confidence interval [CI]: 1.14-3.31; p=0.014), type 2 DM (OR: 3.04; 95%CI: 1.57-5.90; p=0.001) and obesity (OR: 3.58; 95%CI: 1.70-7.54; p=0.001) remained associated with NAFLD (Table II).

DISCUSSION

In this study, the frequency of NAFLD was significantly higher in patients with SIBO when compared to patients without SIBO. This association remained significant even after adjusting for covariables in the multivariate analysis. Our results support the role of the gut-liver axis in the development of NAFLD.

Previous studies have shown a high prevalence of SIBO in patients with NASH, ranging from 50-78% [15, 20, 21]. High rates of SIBO have also been documented in obese patients with NAFLD submitted to bariatric surgery. In such patients, SIBO was associated with severe hepatic steatosis [22]. However, these studies were conducted in patients with known NAFLD/NASH or morbid obesity. To our knowledge, this is the first study to evaluate NAFLD in a population of patients submitted to glucose H_2/CH_4 breath test for diagnosis of SIBO regardless of previously diagnosed obesity, NASH or NAFLD.

The exact mechanism behind the association between fatty liver and SIBO is unknown. It is likely multifactorial and appears to be at least in part due to increased inflammation in patients with SIBO as a result of the production of bacterial byproducts such as endotoxin and LPS. The presence of SIBO in NASH patients has been linked to increased intestinal permeability [19], which predisposes to bacterial translocation and translocation of bacterial byproducts such as LPS. In patients with NASH, SIBO has been associated with increased levels of LPS binding protein and up-regulation of the LPS

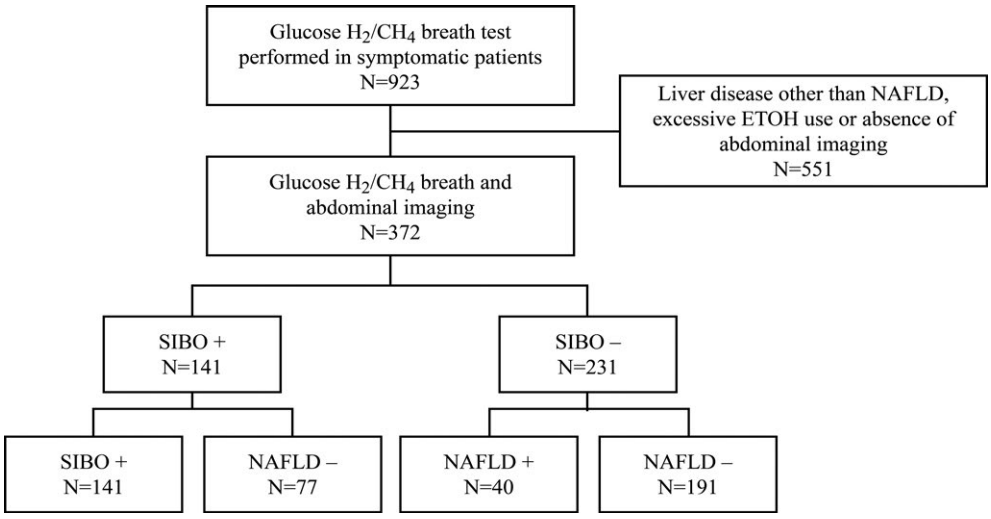

Fig. 1. Distribution of patients according to breath test results and abdominal imaging.

Table I. Univariate analysis of the risk factors associated with SIBO

	All cases (N=372)	SIBO + (N=141)	SIBO - (N=231)	P
Mean age, years	59.76±0.77	60.47±1.24	58.24±0.98	0.159
Male sex	92	44 (31.2%)	48 (20.8%)	0.059
BMI (kg/m^2)	27.85±0.38	28.31±0.61	27.23±0.47	0.158
BMI classification (kg/m^2)				
Underweight	13	7 (5.0%)	6 (2.6%)	0.089
Normal weight	148	46 (32.6%)	102 (44.2%)	
Overweight	98	39 (27.7%)	59 (25.5%)	
Obese	113	49(34.7%)	64 (27.7%)	
Previous abdominal surgery				
Appendectomy	30	15 (10.6%)	15 (6.5%)	0.140
Gastric bypass	3	3 (2.1%)	0 (0%)	
Cholecystectomy	50	23 (16.3%)	27 (11.7%)	
Hernia repair	12	4 (2.8%)	8 (3.5%)	
Small bowel resection	4	1 (0.7%)	3 (1.3%)	
Partial colectomy	27	13 (9.2%)	14(6.1%)	
Liver imaging				
Ultrasound	231	91 (64.5%)	140 (60.6%)	0.550
CT abdomen	123	42 (29.8%)	81 (35.1%)	
CT enterography	14	7 (5.0%)	7 (3.0%)	
MRI abdomen	4	1 (0.7%)	3 (1.3%)	
Use of PPI	153	63 (44.7%)	90 (39.0%)	0.150
Albumin (g/dL)	4.38±0.12	4.12±0.04	4.56±0.21	0.096
AST >35 U/L	55	29 (20.6%)	26 (11.3%)	0.034
ALT >30 U/L if male or >19 U/L if female	173	79 (56.0%)	94 (40.7%)	0.039
Platelets (K/uL)	244.13±3.51	240.19±5.09	246.41±4.58	0.158
Creatinine (mg/dL)	0.94±0.03	1.01±0.05	0.89±0.04	0.120
Dyslipidemia	24	11 (7.8%)	13 (5.6%)	0.325
NAFLD	104	64 (45.4%)	40 (17.3%)	<0.001
Type 2 DM	65	33 (23.4%)	32 (13.9%)	0.041
Hypertension	170	77 (54.6%)	93 (40.3%)	0.046
Metabolic syndrome	249	110 (78.0%)	139 (60.2%)	0.020
Hypothyroidism	47	21 (14.9%)	26 (11.3%)	0.301

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; DM: diabetes mellitus; NAFLD: non-alcoholic fatty liver disease; PPI: proton pump inhibitor; SIBO: small intestinal bacterial overgrowth.

ligand toll-like receptor 4 (TLR-4), which correlated with increased plasma levels of inflammatory markers such as interleukin (IL)-8 and hepatic expression of tumor necrosis factor (TNF)-α [16, 17]. The latter has been shown to induce liver fibrosis and insulin resistance [20, 21]. In a recent study, Kapil et al. reported that patients with NAFLD and SIBO had higher serum endotoxin levels, higher transcript expression of CD14 and nuclear factor (NF)κβ in the liver and higher TLR4 protein expression [23]. These findings suggest that the elevated serum endotoxin levels in patients with SIBO leads to the activation of TLR-4 and CD14, promoting the expression of NFκβ. On the other hand, NFκβ mediates the production of several cytokines including TNF-α, IL-1β, IL-6, and IL-8 [24] leading to inflammation and possibly contributing to the development of NASH in patients with SIBO. Elevated levels of TNF-α induce liver fibrosis and insulin resistance [25, 26]. Some studies in humans and in animals however have failed to document differences between NASH and controls in respect to serum LPS levels [27, 28].

In addition, SIBO may contribute to hepatic steatosis by increased levels of trimethylamine-N-oxide (TMAO). It has been proposed that gut bacteria may also induce fat deposition in the liver through altered choline metabolism. Intestinal bacteria metabolize dietary choline to methylamines which are absorbed in the gut and converted in the liver to TMAO, a component that has been associated with fatty liver in mice [10].

Elevated levels of endogenous alcohol production leading to increased oxidative stress may be an additional mechanism

Table II. Multivariate analysis of the risk factors associated with NAFLD

Variables	Adjusted OR	95%CI	P
SIBO	1.95	1.14-3.31	0.014
Type 2 DM	3.04	1.57-5.90	0.001
Obesity	3.58	1.70-7.54	0.001
Metabolic syndrome	2.15	0.79-5.84	0.131
Hypertension	1.14	0.55-2.39	0.716

DM: diabetes mellitus; NAFLD: non-alcoholic fatty liver disease; OR: odds ratio; PPI: proton pump inhibitor; SIBO: small intestinal bacterial overgrowth.

through which intestinal microbiota contributes to NAFLD. Intestinal bacteria are capable of fermenting carbohydrates from the diet to ethanol (ETOH), contributing to an endogenous alcohol production. Although alcohol is produced in very low levels by this pathway, patients with SIBO have the potential of increased ETOH production, which may further burden the liver in NAFLD. Zhu et al. showed that children with NASH have an increased prevalence of alcohol-producing bacteria in their gut microbiome and increased blood alcohol concentration when compared to healthy controls although the number of patients evaluated in this study was small [11]. In addition, ETOH has been shown to induce increased intestinal permeability, possibly contributing to elevated serum LPS.

Changes in the type of gut bacteria also seem to be associated with fatty liver. In a recent study conducted in humans submitted to a low choline diet, the abundance of *Gammaproteobacteria* and *Erysipelotrichi* bacteria in stool samples correlated with the development of fatty liver [29]. Moreover, obesity is an important risk factor for NAFLD and obese individuals have a different composition of gut bacteria compared with their lean counterparts. Some studies have shown that in obese individuals there is an increase in the *Firmicutes* and a decrease in the *Bacteroidetes* bacterial group in the gut microbiota [30]. In addition, obese individuals appear to be colonized with bacteria that are able to extract energy from food more efficiently [31]. Thus, qualitative changes in gut bacteria may lead to obesity and contribute to NAFLD. It is important to point out that these studies were conducted utilizing stool samples and thus may reflect both small bowel and colonic microbiota, suggesting that colonic bacteria may also contribute to NAFLD.

In this study we also found that the metabolic syndrome occurred more frequently in patients with SIBO. Gut microbiota have been associated with components of the metabolic syndrome such as obesity. Enteric bacteria may contribute to a more efficient energy extraction from the diet by the production of digestive enzymes not produced by humans [32, 33]. Obese mice seem to have a gut microbiome with an increased capacity of energy harvest. In germ-free mice, colonization with the gut microbiota of obese mice resulted in the "transmission" of obesity [31]. Similar results were found when the gut microbiome of human twins discordant for obesity was transferred to germ-free mice [34]. Furthermore, studies in mice and in humans have shown that gut bacteria may contribute to other components of the metabolic syndrome, such as insulin resistance and type 2 DM through elevated levels of LPS [5, 6], leading to an inflammatory state in the host [7, 31, 35].

We also found an association of NAFLD with type 2 DM and obesity, which remained significant after adjusting for confounders. This is in accordance with the literature, as the relation of NAFLD with type 2 DM and obesity has been supported by several studies [36–38].

Our study has several clinical implications. Patients with SIBO may be at an increased risk for metabolic syndrome and NAFLD and they may benefit from routine screening and control of risk factors for NAFLD, including metabolic syndrome. Although routine screening for NAFLD by liver imaging is not recommended, further studies are necessary to evaluate if treatment for SIBO decreases the risk for NAFLD.

There are limitations to our study. Firstly, the sample size was small and this may have compromised the power of the study. Secondly, NAFLD was diagnosed based on imaging. Although ultrasound, CT and MR imaging are known to have a high sensitivity and specificity for moderate to severe steatosis detection, these imaging modalities have far lower sensitivity for the detection of low liver fat contents compared to liver biopsy [39, 40]. Thirdly, as this was a retrospective study, data regarding waist circumference were not available in our electronic medical records. Therefore, we used BMI instead as one of the diagnostic criteria for metabolic syndrome. Fourthly, because this study was conducted in a tertiary center, it is subject to referral bias, as the patients included may have been sicker.

An additional limitation of this study is that SIBO was diagnosed using the glucose H_2/CH_4 breath test. While the gold standard for the diagnosis of SIBO is the culture of duodenal or jejunal aspirate, glucose H_2/CH_4 breath test is commonly used to diagnose SIBO because it is a noninvasive test. In a recent study, Erdogan et al. reported an overall agreement of 65.5% and a sensitivity of 42%, specificity of 84%, positive predictive value of 68% and negative predictive value of 64% when glucose H_2/CH_4 breath test was compared to the duodenal aspirate and culture for the diagnosis of SIBO [41]. Other studies have shown a variable sensitivity of 20% to 93% and a specificity of 30% to 86% of glucose H_2/CH_4 breath test [42].

Further prospective studies are necessary to confirm that patients with SIBO are at an increased risk for NAFLD and metabolic syndrome. It would be interesting to evaluate if treatment for SIBO is associated with an improvement of NAFLD.

CONCLUSION

SIBO-positive patients are at an increased risk for NAFLD and metabolic syndrome. Patients with SIBO may benefit from aggressive control of risk factors for NAFLD. Further studies are necessary to elucidate if treatment for SIBO reduces the risk for NAFLD in these patients.

Conflicts of interest: The authors have no conflicts of interest to disclose.

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that originated the manuscript and participated in the writing of the manuscript; B.S.: mentor for the research project; participated in the writing of the manuscript.

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