

Small Intestinal Bacterial Overgrowth and Orocecal Transit Time in Patients of Nonalcoholic Fatty Liver Disease

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Abstract

Background: To explore the frequency of small intestinal bacterial overgrowth (SIBO) and orocecal transit time (OCTT) in patients with nonalcoholic fatty liver disease (NAFLD). **Methods:** 103 patients with NAFLD and 49 healthy controls were enrolled. Clinical indicators such as body mass index (BMI), liver function, blood lipids, HOMA-IR, serum endotoxin of NAFLD patients were collected and examined. Fibro Touch was used to detect the (controlled attenuation parameter) CAP and liver stiffness measurement (LSM). SIBO and OCTT were measured by lactulose hydrogen breath test. **Results:** The incidence of SIBO in NAFLD patients (58.3%) was significantly higher than that in healthy controls (26.5%). The level of serum endotoxin in NAFLD patients was higher than that in healthy controls. The levels of CAP, LSM, serum endotoxin, ALT, AST and HOMA-IR in SIBO-positive NAFLD patients were higher than those in SIBO-negative patients. There was no significant difference in GGT, TG, LDL and BMI between the two groups. OCTT in NAFLD patients was longer than that in healthy controls. It was also observed that OCTT in SIBO-positive NAFLD patients was significantly delayed compared with SIBO-negative NAFLD patients. **Conclusions:** Patients with NAFLD exhibit the increased incidence rate of SIBO and prolonged OCTT; SIBO in NAFLD patients is a contributing factor to the elevated transaminase, hepatic steatosis, progression of liver fibrosis and prolonged OCTT.

Background

Nonalcoholic fatty liver disease (NAFLD) is a group of metabolic liver diseases closely related to genetic factors and insulin resistance, the pathology of which is mainly manifested by diffuse hepatic macrovesicular steatosis. The pathogenesis of NAFLD is widely recognized by “two hit hypothesis”[1] proposed by Day, namely, the “first hit” caused by excessive accumulation of triglycerides in the liver resulting from insulin resistance, and the “second hit” caused by hepatocellular injury resulting from oxidative stress, inflammatory factors, and lipid peroxidation, etc. In recent years, it has been found that intestinal bacteria also play an important role in the occurrence and progression of NAFLD with unclear mechanism. Investigators have speculated the role of endotoxemia induced increased inflammatory in liver and insulin resistance. 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. However, there are still few studies on the effects of SIBO on various clinical indicators of patients with NAFLD. SIBO can possibly lead to delayed orocecal transit time (OCTT) due to mucosal injury, bacterial translocation, and worsening of small bowel motility[2]. Lactulose hydrogen breath test is a sensitive, convenient and non-invasive test for the diagnosis of small intestinal bacterial overgrowth (SIBO) and for the evaluation of orocecal transit time (OCTT). This study is intended to clarify the effects of SIBO on various clinical indicators of patients with NAFLD, providing new clues to reveal the pathogenesis of NAFLD and offering evidence for its prevention and treatment.

Methods

Study population

103 NAFLD patients and 49 healthy controls were enrolled in the Second Affiliated Hospital of Xi'an Jiaotong University from March 2015 to December 2017. The NAFLD was diagnosed based on the criteria recommended by Guidelines for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Diseases formulated by Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association: imaging examination consistent with the performance of diffuse fatty liver; no alcoholic history or alcohol intake less than 140 g/week of ethanol (less than 70 g/week for females); exclusion of specific hepatic diseases that can cause hepatic steatosis, such as viral hepatitis, drug-induced liver damage, autoimmune liver disease, and hepatolenticular degeneration, etc. Exclusion: patients with severe organ damage; patients with intestinal organic lesions and a past medical history of abdominal surgery; patients with diseases that clearly affect gastrointestinal motility and intestinal microecology, such as infection, inflammatory bowel diseases, and hyperthyroidism; patients who have recently taken drugs affecting the results, like antibiotics, active microbial agents, and prokinetics; pregnant and lactating patients. This study has been approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University, and all the research subjects have been informed and agreed to participate in this trial.

General Information

Basic information of all subjects was collected and recorded, such as age, gender, alcoholic history, body mass index (BMI) = body mass (Kg) /height (m)², past medical history and recent medication history.

Blood Biochemical Index

Fasting venous blood of NAFLD patients were collected. Alanine transaminase (ALT), aspartate aminotransferase (AST), glutamyl transpeptidase (GGT), triglyceride (TG), low density lipoprotein (LDL), fasting plasma glucose (FPG) were detected using a biochemical analyzer. Fasting insulin and endotoxin were detected by radioimmunoassay. Homeostasis model assessment-insulin resistance (HOMA-IR) = fasting blood glucose level × fasting insulin/22.5.

Non-invasive Examination Of Liver Fibrosis

The liver fibrosis diagnostic apparatus FibroTouch (FibroTouch-B-004-004; Wuxi Hisky Medical Co., Ltd.) was used to detect the controlled attenuation parameter (CAP) of liver fat and liver stiffness measurement (LSM) in subjects. Patients were classified according to CAP as mild (240 to 264 db/m), moderate (265 to 294 db/m) and severe (> 295 db/m).

Lactulose Hydrogen Breath Test (lhbt)

An EC60 Gastrolyzer gastrointestinal hydrogen detector and lactulose oral liquid (66.7 g/100 ml) manufactured by Bedfont Science Ltd (UK) were used. The background value of hydrogen breath concentration in the fasting state was measured. Subjects were suggested to drink 250 ml of warm water with 10 g of lactulose dissolved in. In the next 3 h, the hydrogen breath value was measured every 15 min. During the test, subjects were kept quiet, and banned from eating, smoking and sleeping. Among the following three items, patients who conformed to one of them were diagnosed as SIBO positive: (1) in the fasting state, the baseline hydrogen breath concentration is ≥ 20 ppm, and it is still ≥ 20 ppm measured after 30 min interval; (2) a typical double-peak pattern appears, namely, the small intestine peak above the baseline value 12 ppm occurs within 60 min, and the second colon peak with a higher peak value appears after 60 min; (3) fusion waveform: the hydrogen breath value above the baseline value 12 ppm occurs within 60 min, and the subsequent hydrogen breath values are higher or not lower than this value. Each hydrogen breath value is connected to present a rising platform. The latent and higher hydrogen breath value occurring after 60 min is the colon peak. The time when the colon peak appears is the OCTT.

Statistical analysis

Data statistical analysis was performed using SPSS20.0 software. Serum ALT, AST, GGT, TG, LDL levels and CAP, LSM, OCTT, HOMA-IR were in accordance with normal distribution, expressed as $\bar{x} \pm s$ and with the t test. Serum endotoxin level did not conform to normal distribution, expressed as M (P25, P75) and with the nonparametric rank sum test. The comparison of the grouped count data was performed using the chi-square test. $P < 0.05$ was considered to be the difference with statistical significance.

Results

No significant differences in gender or age distribution were present among the controls, NAFLD and subgroups (Table 1).

Table 1
Characteristics of the subjects

	Control	NAFLD			
		total	mild	moderate	sever
N	49	103	46	32	25
Gender(Male/female)	26/23	54/49	24/22	17/15	13/12
Age(years)	46.39 \pm 8.89	48.52 \pm 12.34	49.25 \pm 10.18	47.14 \pm 9.28	47.75 \pm 9.36

Sibo And Serum Endotoxin In Subjects

The incidence of SIBO in NAFLD group (58.3%) was significantly higher than that in healthy control group (26.5%) ($P < 0.05$). The level of serum endotoxin in NAFLD group was higher than that in healthy control group ($P < 0.05$). With the increase of the severity of NAFLD graded by CAP, the incidence of SIBO and serum endotoxin increased ($P < 0.05$) (Table 2).

Table 2
Small intestinal bacterial overgrowth and serum endotoxin in patients
with NAFLD and control

Group	N	Incidence rate of SIBO (%)	Endotoxin (pg/ml)
Control	49	26.5	5.0(3.0-6)
NAFLD	103	58.3*	6.0(5.00-45)*
mild	46	32.6	5.0(3-6.2)
moderate	32	71.8*	7.76(5.0-55.86)*
severe	25	88.0*	47.14(6.62-325.5)*

* $P < 0.05$ compared with the control group.

Comparison of clinical biochemical parameters between SIBO positive NAFLD and negative NAFLD

No significant differences in gender or age distribution were present between the SIBO positive and negative group. The levels of CAP, LSM, serum endotoxin, ALT, AST and HOMA-IR in SIBO-positive NAFLD patients were higher than those in SIBO-negative patients ($P < 0.05$). There was no significant difference in GGT, TG, LDL and BMI between the two groups (Table 3).

Table 3
Comparison of clinical biochemical parameters between SIBO positive and negative NAFLD

	SIBO positive (N = 60)	SIBO negative (N = 43)	p value
Gender(Male/female)	32/28	22/21	0.828
Age(years)	48.25 ± 9.98	48.79 ± 11.36	0.799
CAP(db/m)	277.45 ± 18.45	268.70 ± 19.25	0.02
LSM(kPa)	8.88 ± 3.16	7.66 ± 2.60	0.042
endotoxin(pg/ml)	12.00(5.00-64.75)	6.00(3.00-6.72)	< 0.001
ALT (IU/L)	46.13 ± 20.54	35.18 ± 9.14	0.002
AST (IU/L)	34.18 ± 13.02	27.95 ± 7.52	0.006
GGT(U/L)	41.30 ± 17.02	37.16 ± 17.45	0.231
TG (mmol/L)	2.16 ± 0.59	2.33 ± 0.72	0.181
LDL(mmol/L)	3.51 ± 0.96	3.17 ± 0.69	0.055
BMI (Kg/m ²)	26.52 ± 2.27	26.13 ± 2.45	0.413
HOMA-IR	2.57 ± 0.68	2.24 ± 0.79	0.031

Octt In Subjects

OCTT in NAFLD group was longer than that in healthy control group ($P < 0.05$). With the increase of the severity of NAFLD graded by CAP, OCTT prolonged significantly ($P < 0.05$) (Table 4). OCTT in the positive SIBO group was significantly longer than the negative group (Table 5).

Table 4
Oro-cecal transit time (OCTT) in patients with
NAFLD and control

Group	N	OCTT (min)
Control	49	(107.55 ± 22.96)
NAFLD	103	(142.18 ± 32.36)*
mild	46	(125.21 ± 27.06)*
moderate	32	(145.16 ± 27.11)*
severe	25	(169.60 ± 27.99)*
*P < 0.05 compared with the control group.		

Table 5
Comparison of Oro-cecal transit time (OCTT) between SIBO-
positive and negative NAFLD

	SIBO positive (N = 60)	SIBO negative (N = 43)	p value
OCTT (min)	154.08 ± 30.27	125.58 ± 27.78	< 0.001

Discussion

In recent years, the incidence rate of NAFLD has increased year by year, becoming the second largest liver disease in China after viral hepatitis. As the study on intestinal bacteria increases, the association between them and NAFLD is revealed. Intestinal bacteria have also become the key to exploring the pathogenesis of NAFLD. Under normal circumstances, only a small number of bacteria survive in the duodenum and proximal jejunum, while the terminal ileum is the transitional site of a small number of aerobic bacteria in the small intestine and a large number of anaerobic bacteria in the colon. SIBO is a syndrome in which the number of bacteria in the small intestine is excessive or the type of bacteria is abnormal. It can cause various symptoms such as abdominal pain, abdominal distension, diarrhea, and abdominal discomfort, etc. In patients with NAFLD, the secretion of gastric acid, bile, pancreatic secretions and immunoglobulin is reduced, and the regulation of intestinal pH and the inhibition of bacteria are weakened[3], resulting in the occurrence of SIBO. This study showed that the incidence rate of SIBO in patients with NAFLD was significantly higher than that in healthy people, consistent with the findings at home and abroad[4]; and the incidence rate of SIBO in NAFLD patients with severe CAP was obviously increased compared with patients with mild CAP, suggesting that the occurrence of SIBO may be related to the severity of hepatic steatosis. At the same time, this study showed that the OCTT of patients with NAFLD was obviously prolonged compared with the controls, suggesting that the intestinal

peristalsis slowed down in patients with NAFLD, consistent with the findings of Soza et al [5], which might be related to the decrease in the number of interstitial cells of Cajal at the end of the jejunum. Interstitial cells of Cajal are mainly distributed in the muscular and intermuscular plexus in the gastrointestinal tract and play an important role in the gastrointestinal movement. The decrease in the number of interstitial cells of Cajal or abnormal morphology can cause changes in gastrointestinal motility. The slowing of the intestinal peristalsis will reduce the discharge of bacteria in the intestine and further promote the occurrence of SIBO. Meanwhile, this study also founded that OCTT in SIBO-positive NAFLD patients was significantly delayed compared with SIBO-negative NAFLD patients. As reported by Cuoco et al [6] SIBO could slow human intestinal transit, prolong OCTT and affect intestinal motility.

The excessive growth and reproduction of bacteria in the intestine leads to an increase in the number of metabolites, wherein endotoxin is one of the metabolites closely related to NAFLD. Endotoxin is an important component of the cell wall of Gram-negative bacteria, which will be released when bacteria in the intestine grow rapidly or are dissolved after death [7]. Under normal circumstances, the barrier function of the intestinal mucosa can effectively prevent intestinal bacterial translocation and endotoxin invasion [8]. When NAFLD occurs, the endotoxin released by intestinal bacteria increases, the permeability of intestinal mucosa increases, and the barrier function is impaired, which cannot effectively prevent endotoxin from entering the portal system [9]. According to Abdul-Hai A et al [10], it was found that the intestinal mucosal permeability of patients with NAFLD was increased compared with the healthy people, and the degree of increase was associated with the severity of hepatic steatosis. This phenomenon is related to the destruction of the tight junction integrity among intestinal mucosal epithelial cells. In addition, the immune function of the liver in the pathological state is disordered, and thus its clearance effect on endotoxin is weakened. The combination of the above factors leads to an increase in endotoxin levels in patients with NAFLD. This study showed that patients with SIBO-positive NAFLD had higher endotoxin levels than the negative patients, consistent with the past research results [11].

After the endotoxin reaches the liver through the portal system, it recognizes and activates Toll-like receptor 4 (TLR4) [12]. Once TLR4 is activated, the region within TLR4 cells binds to the adaptive molecule of myeloid differentiation factor 88, activating the downstream interleukin (IL)-1 receptor associated kinase/tumor necrosis factor receptor association factor 6 complex, further activating nuclear transcription factor- κ B (NF- κ B) [13], thereby inducing the gene expression of pro-inflammatory cytokines in the nucleus and releasing various cytokines such as tumor necrosis factor- α (TNF- α), IL-1, IL-6 and IL-8 [14] [15] [16]. The released inflammatory factors can also activate other Kupffer cells, expanding the inflammatory response and causing more cell injury. In this study, the levels of serum ALT and GGT in SIBO-positive NAFLD patients were higher than those in SIBO-negative patients, suggesting that SIBO might promote the inflammatory injury response in the liver.

The inflammatory factors that are released by Kupffer cells activated by endotoxins can also induce the formation of insulin resistance by interfering with the insulin signaling [17]. After insulin reaches the target organ through blood circulation, it binds to the insulin receptor on the cell surface, which phosphorylates tyrosine protein kinase and changes the conformation of insulin receptor. Phosphokinase can

phosphorylate the insulin receptor substrate (IRS) to make it activated. Inflammatory factors such as TNF- α can inhibit the phosphorylation of IRS, thereby interfering with the insulin signaling and leading to insulin resistance[18]. Cani et al.[19] injected subcutaneously endotoxin into mice to form endotoxemia. After 4 weeks, it was found that the fasting blood glucose and insulin levels of the mice increased compared with the previous. Mehta et al.[20] injected endotoxin into healthy volunteers, causing an increase in levels of serum IL-6 and TNF- α , which induced insulin resistance. Insulin resistance is the key link in the development of NAFLD, which promotes dyslipidemia and triglyceride accumulation in the liver. In this study, SIBO-positive NAFLD patients had higher CAP and serum TG levels than SIBO-negative patients, and patients with moderate and severe CAP had significantly increased serum endotoxin levels compared with CAP mild patients, suggesting that the occurrence of SIBO aggravated the severity of liver steatosis.

In addition, inflammatory factors such as TNF- α can stimulate the activation, proliferation and transformation of hepatic stellate cells into myofibroblasts, and on the other hand promote the synthesis of transforming growth factor β 1, resulting in the deposition of extracellular matrix in the liver because of its synthesis beyond the degradation capacity of the liver, which leads to the formation of liver fibrosis. This study showed that SIBO-positive patients had higher LSM than the negative patients, indicating that SIBO also promoted liver fibrosis in patients with NAFLD. Bacterial overgrowth increased intestinal permeability and serum endotoxin and all likely to play a role in the liver fibrosis and cirrhosis. Regulation of gut microbiota represents a therapeutic method to NAFLD and other chronic liver disease[21] [22] .

Conclusion

In summary, this study found that patients with NAFLD had an increased incidence rate of SIBO and prolonged OCTT. SIBO had a promoting effect on elevated transaminase, dyslipidemia, hepatic steatosis, and progression of liver fibrosis in patients with NAFLD. Regulating the micro-ecological environment of the intestine and improving the small intestinal bacterial overgrowth are expected to be the key to the treatment of NAFLD.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Second Affiliated Hospital of Xi'an Jiaotong University. The written informed consent was obtained from each participant.

Consent for publication

Not Applicable.

Availability of data and materials

The relevant raw data from this study can be readily available on request for non-commercial purpose per request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FD and HS participated in conception, design and constructive review of the study. HS, LM, LW and XX participated in data collection and processing. HS and LM participated in manuscript writing. XQ, YC and SZ participated in statistical analysis. All authors read and approved the final version of the manuscript.

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Abbreviations

ALT: Alanine transaminase; AST: asparate aminotransferase; BMI: body mass index; CAP: controlled attenuation parameter; FPG: fasting plasma glucose; GGT: glutamyl transpeptidase; HOMA-IR: homeostasis model assessment-insulin resistance; IL: interleukin; IRS: insulin receptor substrate; LDL: low density lipoprotein; LSM: liver stiffness measurement; MyD88: myeloid differentiation factor 88; NAFLD: nonalcoholic fatty liver disease; NF- κ B: nuclear transcription factor- κ B; OCTT: orocecal transit time; SBIO: small intestinal bacterial overgrowth; TG: triglyceride; TLR4: Toll-like receptor 4; TNF- α : tumor necrosis factor- α

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