Helicobacter pylori infection may increase the severity of nonalcoholic fatty liver disease via promoting liver function damage, glycometabolism, lipid metabolism, inflammatory reaction and metabolic syndrome

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Background and aim Recent clinical trials have confirmed that *Helicobacter pylori* infection is positively associated with nonalcoholic fatty liver disease (NAFLD), although some research has shown a negative association. Therefore, to confirm whether *H. pylori* eradication treatment is feasible for NAFLD patients in our hospital, we aimed to establish the association between *H. pylori* infection and NAFLD.
Methods We enrolled 91 patients with NAFLD diagnosed by abdominal B-mode ultrasonography between January and December 2018. *H. pylori* infection was confirmed by C^{13} urea breath test, and liver function, glycometabolism, insulin sensitivity, lipid metabolism, as well as inflammatory reaction were assessed through blood biochemical analyses.
Results A minority of NAFLD patients had liver dysfunction, increased fasting glucose and insulin levels, a score of insulin-resistance (HOMA-Ir), lipid metabolism, slight inflammatory response, fasting hyperglycemia and hypertension. Most patients were complicated with overweight/visceral obesity and dyslipidemia. Moreover, these abnormal indicators were closely associated with the severity of NAFLD and *H. pylori* infection. Notably, the prevalence of *H. pylori* infection showed a significant difference between mild, moderate and severe NAFLD, and hepatic steatosis with coexistent NAFLD also revealed a striking difference between *H. pylori*-positive and *H. pylori*-negative patients ($P<0.01$).
Conclusion Our results suggest that *H. pylori* infection may be an independent risk factor in NAFLD progress. Eur J Gastroenterol Hepatol 32: 857–866

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common chronic disease worldwide. The disease is characterized by excessive fat deposition in liver cells, and fatty, diffuse and bullous pathological alterations in liver cells, with the absence of alcohol and other liver-damaging factors [1–3]. If NAFLD is not actively prevented or treated, it gradually progresses toward liver fibrosis and cirrhosis [4]. Recently, NAFLD has been reported to affect 20–45% of people worldwide, 60–75% of obese individuals [5], and ~25% of Chinese [6,7]. A meta-analysis published in 2016 reported the prevalence data of NAFLD in different districts, with global prevalence (25%), North America (24%), Europe (24%) and Asia (27%) [8]. A new meta-analysis about massive epidemiological studies from 1999 to 2019 in Asian reported 30% prevalence and 50.9 cases/1000 person/years of incidence of NAFLD [9]. It is worth noting that the pooled nationwide prevalence of NAFLD was 29.2% in China and showing a increased trend from 25.4% in 2008–2010 to 32.3% in 2015–2018, with 14–25% NAFLD sufferers developed advanced fibrosis, which rooted in a updated Chinese NAFLD epidemiology of systematic review and meta-analysis that analyzed 392 studies including more than two million participants from 28 provinces and regions [10]. It is predicted the largest number of liver-associated deaths from NAFLD will appear in China by 2030 [11]. NAFLD is widely recognized as a serious public health issue and has been paid increased attention by researchers because of its high prevalence, unclear etiology and difficult treatment. The risk of NAFLD is also increased in patients with severe chronic diseases such as type 2 diabetes mellitus (T2DM), cardiovascular disease, chronic kidney disease and chronic liver disease [5,12–14]. Additionally, NAFLD is regarded as a hepatic component of metabolic syndromes, based on its high association with overweight/visceral obesity, dyslipidemia, insulin resistance/fasting hyperglycemia, T2DM and hypertension [15]

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Because of the increasing prevalence, and complexity of NAFLD in China, it has been a focus on looking for novel risk factors for prevention and treatment.

Helicobacter pylori was first isolated and cultured from the human gastric mucosal epithelium by researchers Marshall and Warren in 1983. *H. pylori* is a Gram-negative microaerophilic bacterium affecting >50% of humans worldwide, with a higher prevalence in developing countries [16,17]. A JGHF Marshall and Warren Lecture in 2017 about the epidemiology of *H. pylori* infection in Malaysia (three major Asian races living together – Malay, Chinese and Indian) reported that the *H. pylori* prevalence was high in Indians (>50%) and Chinese (40–50%), while a comparatively low prevalence occurred in Malays (10–20%) [18]. Moreover, at the same year, a systematic review and meta-analysis mentioned that the *H. pylori* infection is highly endemic in East Asian countries, with 54% of prevalence in China, Japan, Taiwan and Korea in 2015 [19]. Therefore, *H. pylori* is considered to be the major cause of gastric diseases, including chronic gastritis, gastric ulcer disease, gastric lymphoma such as gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and even gastric cancer such as gastric adenocarcinoma [20–22]. Subsequently, *H. pylori* was also detected in intestinal diseases such as duodenal ulcer [20], and jejunal metaplasia and dysplasia [23], and is reported to be a hazard in several extragastrointestinal diseases including: neurological disorders, such as Parkinson's disease and Alzheimer's disease; cardiovascular diseases such as ischemic heart disease; metabolic diseases or metabolic syndrome such as lipid metabolism disorder, obesity and T2DM; and hematological and immune disorders such as idiopathic thrombocytopenic purpura [24–29]. Recently, *H. pylori* infection has been proved pivotal in liver diseases, including NAFLD, nonalcoholic steatohepatitis, liver fibrosis and cirrhosis, and even liver cancer [30]. Thereby, *H. pylori* may be a novel risk factor for prevention and treatment of NAFLD.

Although several clinical and experimental large sample or cross-sectional studies from different countries have reported that *H. pylori* infection has a positive correlation with NAFLD [12,31,32], some high-quality clinical studies showed a negative correlation [33], or even no association [34–36]. The conclusions about the correlation between *H. pylori* infection and NAFLD are inconsistent, and the pathological mechanisms of *H. pylori* in NAFLD are unclear. Moreover, a case of the *H. pylori* association with NAFLD, the first report on the improvement of metabolic profile after *H. pylori* eradication in a NAFLD patient in 2013, which suggests a possible hypothesis: *H. pylori* treatment may be a promising strategy for liver steatosis [37]. Thus, to establish clinical experiment evidence of our next study about whether *H. pylori* eradication might be a simple and effective therapeutic strategy in NAFLD patients, we primarily and preliminarily investigated the relationship between *H. pylori* infection and NAFLD by analyzing clinical cases of NAFLD in Tianjin Medical University General Hospital, Tianjin, China.

Patients and methods

Patients

We enrolled 91 patients (nonsmokers and nondrinkers, 56 males, 35 females, mean age 52.8 ± 14.7 years, range 18–83 years) who were diagnosed with NAFLD by abdominal B-mode ultrasonography between January and December 2018 at the Tianjin Medical University General Hospital, Tianjin, China. Each patient was immediately required to undergo a face-to-face interview, including symptom description, age, gender, history of becoming overweight, smoking, drinking, structured diet, lifestyle and anamnesis in the preceding 6 months before initiation of the study, as well as a series of detailed medical examinations after hospitalization. NAFLD patients were analyzed for *H. pylori* infection, liver function, carbohydrate metabolism, lipid metabolism and inflammatory response, as well as the presence of metabolic syndrome. This clinical protocol was approved by our Hospital Ethics Committee and conformed to the 2008 Helsinki Declaration and Good Clinical Practice guidelines. Written informed consent was obtained from all patients before study onset.

The diagnosis or inclusion criteria of NAFLD complied with the Clinical Diagnostic Standards of the Chinese Liver Disease Association in 2011 [38] and the guidelines from the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology in 2012 [39]. The required diagnostic criteria for NAFLD were as follows [40,41]: (1) hepatic steatosis detected by abdominal B-mode ultrasonography; (2) echo near the liver was diffuse enhancement, and stronger than that of the kidney and spleen; (3) the liver far-field echo gradually attenuated; (4) the intrahepatic duct structure was not clear; (5) no significant alcohol consumption or <140 g (male) or <70 g (female) alcohol consumption per week; and (6) no coexisting causes of chronic liver disease, such as hepatitis C, medication, parenteral nutrition, Wilson's disease or severe malnutrition.

The exclusion criteria were as follows [40]: (1) ≥140 g (male) or ≥70 g (female) alcoholic consumption per week; (2) any other chronic liver disease; (3) history of gastric surgery; (4) history of cancer or current cancer; (5) use of bismuth, antibiotics, proton pump inhibitors or H2 blockers within the prior 4 weeks; (6) severe infection; (7) significant mental or neurological disorder; (8) pregnancy and lactation; (9) missing data; and (10) steatogenic medication such as methotrexate and corticosteroids.

Classification of nonalcoholic fatty liver disease

NAFLD was detected by an abdominal high-resolution B-mode topographic ultrasound system (ACUSON X300, Siemens, Germany) by two well-trained examiners. The fitness status and laboratory test outcomes of the patients were not revealed to the examiners. NAFLD was divided into three types: mild, moderate and severe based on the Chinese Ultrasonic Grading Criteria of NAFLD published in 2003, as follows [42]. Mild fatty liver: the intrahepatic echo is enhanced, and the boundary of the hepatic vessels and diaphragm muscle can be seen; moderate fatty liver: the intrahepatic echo is moderately increased, and the appearance of intrahepatic ducts or diaphragm muscle is slightly attenuated; severe fatty liver: the intrahepatic echo is significantly increased, and the intrahepatic duct, diaphragm muscle, or posterior right lobe is poorly visible or invisible.

Assessment of H. pylori infection

The C^{13} urea breath test (HCBT-01 tester; Shenzhen Zhonghe Headway Biological Technology Co. Ltd., China) was utilized by experienced examiners for detecting the *H. pylori* infection. The specific steps and diagnostic criteria were as described previously [41].

Blood biochemical detection

Venous blood samples were collected after a 12-hour fast, and centrifuged at 3000 r/min for 10 minutes. The serum supernatant and plasma were collected for lipid metabolism and liver function tests, sugar metabolism, insulin sensitivity and inflammation index detection, using a Beckman Coulter LXi725 automatic biochemical analyzer and other kits. Several representative indexes for each test were randomly selected. The specific indexes were: liver function: alanine aminotransferase (ALT), total bilirubin (TBIL), direct bilirubin (DBIL), total protein, albumin (ALB) and globulin (GLB); glycometabolism: fasting plasma glucose (GLU) and glycosylated hemoglobin (HbAlc); insulin sensitivity: fasting plasma insulin (INS) and a score of insulin-resistance, as a homeostasis model assessment of insulin resistance (HOMA-Ir); lipid metabolism: total cholesterol (CHOL), triglyceride, high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C); inflammation: fasting plasma white blood cell (WBC), lymphocyte (LYMPH), middle cell (MID) and granulocyte (GRAN) counts. The specific check methods were carried out as described previously [43]. HOMA-Ir was calculated as fasting glucose (mmol/L) × fasting insulin level (mIU/L)/22.5 [44].

Diagnosis of metabolic syndrome

Metabolic syndrome was evaluated on the basis of the Japanese criteria published in 2005 [45]. The metabolic syndromes of overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension were most common and co-occurred with NAFLD, and thus, the metabolic syndromes above were chosen for our study. BMI and resting blood pressure were measured by experienced medical nurses, and the diagnosis of these metabolic syndromes was made as previously described [31,46].

Statistical analysis

Statistical analysis was performed using SPSS version 16.0, and all the outcomes were expressed as numbers, percentages and the means ± SD. For significant differences, the quantitative data between two groups were assessed by independent-samples *t* tests. Quantitative data between three groups were evaluated by one-way analysis of variance. For equal variance assumed data, the least significant difference *t* test and Student–Neuman–Keuls *q* test were utilized. For equal variance unassumed data, Dunnett's T3 test was used. For comparison of differences of rate, data between two or more groups, the nonparametric Mann–Whitney *U* test or Pearson's χ^2 test were carried out. $P < 0.05$ was deemed statistically significant.

Results

A small number of nonalcoholic fatty liver disease patients showed liver function damage, increased glucose metabolism, lipid metabolism and inflammatory reactions, while most developed metabolic syndromes

For liver function measurements, 26.37% of NAFLD patients displayed an abnormal increase in ALT in the range of 41–79 U/L, which exceeded the normal reference range of 5–40 U/L. There was an abnormal rise of TBIL in 10.99% of NAFLD patients, and abnormal increase in DBIL in 7.69%. Total protein, ALB and GLB were normal in all NAFLD patients. For carbohydrate metabolism, GLU, HbAlc, INS and HOMA-Ir were all abnormally enhanced in 45.05, 31.87, 36.26 and 45.05% of NAFLD patients, respectively. For lipid metabolism, 42.86% of NAFLD patients showed an abnormal rise in TC compared with the normal reference value, while 5.49% showed an abnormal decrease in TC. A total of 43.96% of NAFLD patients revealed a preternatural increase in triglyceride. Compared with the normal reference ranges, HDL-C showed an abnormal decline in 9.89% of NAFLD patients, while LDL-C showed an exceptional increase in 29.67%. For inflammatory response estimates, WBC count was abnormally increased in 9.89% of NAFLD patients and preternaturally decreased in 2.20%, compared to the normal reference range. A total of 9.89% of NAFLD patients showed an exceptional increase in GRAN count, while 3.30% had an abnormal reduction. MID count was abnormally increased in 8.79% of NAFLD patients, while LYMPH count was within the normal reference ranges (Table 1). As for metabolic syndrome decisions, the incidence rates of overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension were 73.63, 49.45, 73.63 and 30.77%, respectively (Table 2). The above abnormal parameters were analyzed further, as follows.

Severity of nonalcoholic fatty liver disease is associated with liver function, carbohydrate metabolism, lipid metabolism and inflammatory reaction, as well as incidence of metabolic syndromes

From mild to severe NAFLD, excluding a linear decrease in HDL-C, the indexes of liver function, carbohydrate metabolism, lipid metabolism and inflammatory response, as well as the incidence of disparate metabolic syndromes showed a linear upward trend. For liver function, compared with mild NAFLD, moderate NAFLD showed a higher level of ALT ($P = 0.000$), and ALT was higher in severe than in mild NAFLD ($P = 0.000$) and moderate NAFLD ($P = 0.027$). Severe NAFLD patients displayed added TBIL content compared with mild NAFLD ($P = 0.001$) and moderate NAFLD ($P = 0.013$), while DBIL showed no significant difference between each two groups of mild, moderate and severe NAFLD ($P > 0.05$). For carbohydrate metabolism, comparisons of each two groups of mild, moderate and severe NAFLD revealed no significant differences in GLU and HbAlc ($P > 0.05$), and a distinct differences in INS and HOMA-Ir ($P < 0.05$ or 0.01). For lipid metabolism, severe NAFLD patients showed higher LDL-C levels compared with mild NAFLD ($P = 0.000$) and moderate NAFLD ($P = 0.008$) patients. TC, triglyceride and HDL-C showed

Table 1. Estimate of the liver function, carbohydrate metabolism, lipid metabolism and inflammatory response in 91 nonalcoholic fatty liver disease patients

Parameter	NAFLD (range, mean ± SD)	Normal reference value (range)	↑/↓ (*n*, %, range)
Liver function			
ALT (U/L)	9–79, 31.32 ± 17.47	5–40	↑ 24 (26.37), 41–79
TBIL (μmol/L)	6.2–45.2, 13.69 ± 6.16	3.4–20	↑ 10 (10.99), 20.5–45.2
DBIL (μmol/L)	2–16.4, 4.96 ± 2.49	0.1–6.8	↑ 7 (7.69), 7.5–16.4
Total protein (g/L)	64–85, 73.23 ± 4.03	62–85	–
ALB (g/L)	42–55, 46.21 ± 2.45	35–55	–
GLB (g/L)	21–34, 26.88 ± 3.07	20–40	–
Carbohydrate metabolism			
GLU (mmol/L)	4.5–14.2, 6.31 ± 1.88	3.6–5.8	↑ 41 (45.05), 5.9–14.2
HbAlc (%)	4.7–10.7, 6.09 ± 1.18	4.0–6.0	↑ 29 (31.87), 6.1–10.7
INS (mIU/L)	4.3–29.8, 13.42 ± 4.37	3.0–25.0	↑33 (36.26), 26.4–29.8
HOMA-Ir	1.0–1.8, 1.44 ± 0.23	1.0	↑ 41 (45.05), 1.2–1.8
Lipid metabolism			
CHOL (mmol/L)	3.14–6.67, 4.99 ± 0.83	3.59–5.17	↑39 (42.86), 5.27–6.6; ↓ 5 (5.49), 3.14–3.57
TG (mmol/L)	0.64–8.11, 2.24 ± 1.41	0.57–1.71	↑ 40 (43.96), 1.74–8.11
HDL-C (mmol/L)	0.63–1.67, 1.10 ± 0.24	0.8–2.2	↓ 9 (9.89), 0.63–0.76
LDL-C (mmol/L)	1.44–5.42, 3.08 ± 0.85	1.33–3.36	↑ 27 (29.67), 3.42–5.42
Inflammatory response			
WBC# (10^9/L)	2.9–12.4, 6.35 ± 2.03	3.5–9.5	↑ 9 (9.89), 10.7–12.4; ↓ 2 (2.20), 2.9–3.2
LYMPH# (10^9/L)	0.9–3.8, 2.07 ± 0.60	0.8–4	–
MID# (10^9/L)	0.2–1.8, 0.63 ± 0.30	0.2–1	↑8 (8.79), 1.1–1.8
GRAN# (10^9/L)	1.4–9.2, 3.72 ± 1.66	2.0–7.0	↑ 9 (9.89), 7.3–9.2; ↓ 3 (3.30), 1.4–1.9

↑, Above the maximum value of the normal reference value; ↓, Below the lowest value of the normal reference value; –, between the normal reference value; #, count; ALT, alanine aminotransferase; ALB, albumin; CHOL, lipid metabolism: total cholesterol; DBIL, direct bilirubin; GLB, globulin; GLU, fasting plasma glucose; GRAN, granulocyte; HbAlc, glycosylated hemoglobin; HDL-C, high-density lipoprotein; HOMA-Ir, homeostasis model assessment of insulin resistance; INS, fasting plasma insulin; LDL-C, low-density lipoprotein; LYMPH, lymphocyte; MID, middle cell; TBIL, total bilirubin; WBC, white blood cell.

Table 2. Some nonalcoholic fatty liver patients are complicated with metabolic syndromes like overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension in 91 nonalcoholic fatty liver disease patients

Metabolic syndromes	Number	Incidence rate (%)
Overweight/visceral obesity	67	73.63
Fasting hyperglycemia	45	49.45
Dyslipidemia	67	73.63
Hypertension	28	30.77

no significant differences between mild, moderate and severe NAFLD patients ($P > 0.05$). For inflammatory reaction, compared with mild NAFLD patients, both moderate ($P = 0.015$ and 0.023) and severe NAFLD ($P = 0.006$ and 0.007) patients showed higher WBC and GRAN counts. MID count showed no change between each two groups of mild, moderate and severe NAFLD patients ($P > 0.05$) (Table 3). For metabolic syndromes, the severity of NAFLD was worse, and the incidence of overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension was higher ($P < 0.01$) (Table 4).

Severity of nonalcoholic fatty liver disease is related to H. pylori infection

The *H. pylori* infection rate was significantly higher in patients with severe NAFLD ($P < 0.01$) (Table 5). Furthermore, the striograph of abdominal B-mode ultrasonography revealed a consistent outcome with *H. pylori* infection rate comparable between mild, moderate and severe NAFLD patients. Under the premises of the same *H. pylori* infection statuses, the severity degree of NAFLD patients was more fearful, more white background was seen in the image maps, and the intrahepatic ducts were unclear, which suggested that steatosis increased with severity of NAFLD. At the consistent severity degree of NAFLD, patients with positive *H. pylori* infection showed more severe steatosis with white background and unclear intrahepatic ducts in the B-mode ultrasonography image maps, compared with *H. pylori*-negative NAFLD patients (Fig. 1).

Liver function, carbohydrate metabolism, inflammatory response and incidence of metabolic syndromes in nonalcoholic fatty liver disease patients are positively correlated with H. pylori infection

For liver function assessment, in *H. pylori*-positive NAFLD patients, compared with *H. pylori*-negative patients, the DBIL level was increased ($P = 0.027$) and there was no change in ALT and TBIL levels ($P > 0.05$). For carbohydrate metabolism evaluation, in *H. pylori*-positive NAFLD patients, compared with *H. pylori*-negative patients, GLU and HbAlc levels were significantly enhanced ($P < 0.01$), INS and HOMA-Ir were also increased with $P < 0.05$. For lipid metabolism measurement, in *H. pylori*-positive NAFLD patients, compared with *H. pylori*-negative patients, TC, triglyceride, HDL-C and LDL-C levels showed no significant differences ($P > 0.05$). For inflammatory response detection, in *H. pylori*-positive NAFLD patients, compared with *H. pylori*-negative patients, significantly more WBCs ($P = 0.029$) and GRANs ($P = 0.033$) were detected. However, compared with the *H. pylori*-negative NAFLD patients, *H. pylori*-positive NAFLD patients displayed no changed in MID count ($P > 0.05$) (Table 6). As for metabolic syndromes, the occurrence of overweight/visceral obesity, dyslipidemia ($P < 0.05$), fasting hyperglycemia and hypertension ($P < 0.01$) was higher

Table 3. Liver function, carbohydrate metabolism, lipid metabolism and inflammatory response are relative to severity degree in 91 patients with nonalcoholic fatty liver

Parameter (mean ± SD)	Mild-NAFLD	Moderate-NAFLD (*P* value[a])	Severe-NAFLD (*P* value[a,b])
Liver function			
ALT (U/L)	24.6 ± 13.51	40.71 ± 14.46 (0.000[a])	52.75 ± 16.96 (0.000[a], 0.027 [b])
TBIL (μmol/L)	12.75 ± 5.91	13.39 ± 5.53 (0.687[a])	19.03 ± 6.02 (0.001[a], 0.013[b])
DBIL (μmol/L)	4.36 ± 1.43	5.29 ± 2.63 (0.431[a])	7.60 ± 4.44 (0.080[a], 0.322[b])
Carbohydrate metabolism			
GLU (mmol/L)	5.93±1.66	6.76±1.89 (0.298[a])	7.63±2.35 (0.092[a], 0.649[b])
HbAlc (%)	5.83 ± 0.97	6.38 ± 1.25 (0.277[a])	7.05 ± 1.53 (0.056[a], 0.518[b])
INS (mIU/L)	9.43 ± 2.64	13.38 ± 4.22 (0.036[a])	15.89 ± 6.46 (0.001[a], 0.032[b])
HOMA-Ir	1.06 ± 0.08	1.37 ± 0.21 (0.386[a])	1.62 ± 0.48 (0.046[a], 0.265[b])
Lipid metabolism			
CHOL (mmol/L)	4.92 ± 0.79	4.99 ± 0.93 (0.779[a])	5.39 ± 0.85 (0.078[a], 0.202[b])
TG (mmol/L)	1.95 ± 1.01	2.25 ± 1.32 (0.776[a])	3.76 ± 2.28 (0.056[a], 0.152[b])
HDL-C (mmol/L)	1.10 ± 0.22	1.10 ± 0.26 (0.758[a])	1.05 ± 0.30 (0.377[a], 0.606[b])
LDL-C (mmol/L)	2.92 ± 0.73	3.09 ± 0.89 (0.432[a])	3.91 ± 0.96 (0.000[a], 0.008[b])
Inflammatory response			
WBC# (10^9/L)	5.58 ± 1.07	7.41 ± 2.28 (0.015[a])	8.83 ± 2.85 (0.006[a], 0.407[b])
MID# (10^9/L)	0.55 ± 0.20	0.74 ± 0.36 (0.126[a])	0.88 ± 0.48 (0.093[a], 0.766[b])
GRAN# (10^9/L)	3.09 ± 0.76	4.61 ± 2.04 (0.023[a])	5.73 ± 2.31 (0.007[a], 0.461[b])

#, count; ALT, alanine aminotransferase; ALB, albumin; CHOL, lipid metabolism: total cholesterol; DBIL, direct bilirubin; GLB, globulin; GLU, fasting plasma glucose; GRAN, granulocyte; HbAlc, glycosylated hemoglobin; HDL-C, high-density lipoprotein; HOMA-Ir, homeostasis model assessment of insulin resistance; INS, fasting plasma insulin; LDL-C, low-density lipoprotein; LYMPH, lymphocyte; MID, middle cell; NAFLD, nonalcoholic fatty liver; TBIL, total bilirubin; WBC, white blood cell.
[a]Comparison to mild-NAFLD.
[b]Comparison to moderate-NAFLD.

Table 4. The incidence rate of metabolic syndromes such as overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension is related to the severity degree of nonalcoholic fatty liver

Metabolic syndromes (*n*, %)	Mild-NAFLD (*n* = 62)	Moderate-NAFLD (*n* = 17)	Severe-NAFLD (*n* = 12)	*P* value
Overweight/visceral obesity	47 (75.81)	15 (88.24)	11 (91.67)	<0.01
Fasting hyperglycemia	23 (37.10)	12 (70.59)	10 (83.33)	<0.01
Dyslipidemia	42 (67.74)	14 (82.35)	11 (91.67)	<0.01
Hypertension	17 (27.42)	6 (35.29)	5 (41.67)	<0.01

NAFLD, nonalcoholic fatty liver.

Table 5. The severity degree of nonalcoholic fatty liver is associated with *H. pylori* infection

H. pylori infection (*n*, %)	Number	*H. pylori*-positive	*P* value
Mild-NAFLD	62	30 (48.39)	<0.01
Moderate-NAFLD	17	9 (52.94)	
Severe-NAFLD	12	8 (66.67)	
Sum	91	47 (51.65)	

NAFLD, nonalcoholic fatty liver.

in *H. pylori*-positive than in *H. pylori*-negative NAFLD patients (Table 7).

H. pylori infection may be involved in the correlation between severity of NAFLD and liver function, carbohydrate metabolism, lipid metabolism and inflammatory response, as well as the incidence of metabolic syndromes.

In patients with an equivalent severity of NAFLD, liver function (ALT, TBIL and DBIL), carbohydrate metabolism (GLU, HbAlc, INS and HOMA-Ir), lipid metabolism (TC, TG, HDL-C and LDL-C), inflammatory reaction (WBC, MID and GRAN counts), and metabolic syndromes (overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension) all differed significantly between *H. pylori*-positive and *H. pylori*-negative patients ($P < 0.05$ or $P < 0.01$). In patients with similar *H. pylori* infection status, there were marked differences in the above covariates between patients with mild, moderate and severe NAFLD ($P < 0.01$) (Tables 8 and 9).

Discussion

We demonstrated that liver dysfunction, increased glucose and lipid metabolism, slight inflammatory response, fasting hyperglycemia and hypertension occurred in a minority of NAFLD patients, while the majority of patients were complicated with overweight/visceral obesity and dyslipidemia. The abnormalities in the all above indexes can reflect the severity of NAFLD. *H. pylori* infection is positively correlated with the severity or steatosis status of NAFLD patients, and with increased liver dysfunction, abnormal carbohydrate metabolism and inflammatory reaction, as well as incidence of metabolic syndromes such as overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension. However, NAFLD has no effect on lipid metabolism. In order to rule out the influence of NAFLD severity or *H. pylori* infection on the above abnormalities, while independently considering the role of *H. pylori* infection or NAFLD severity, we performed a further analysis. We discovered that *H. pylori* infection had an adverse effect on liver function, glycometabolism, insulin sensitivity, lipid metabolism, inflammatory reaction and occurrence of metabolic syndromes in patients with similar severity of NAFLD. In patients with similar *H. pylori* infection status, NAFLD severity was also positively associated with these parameters. These findings suggest that *H. pylori* infection is an independent risk factor for NAFLD progress through increasing liver dysfunction, abnormal glycometabolism, insulin sensitivity,

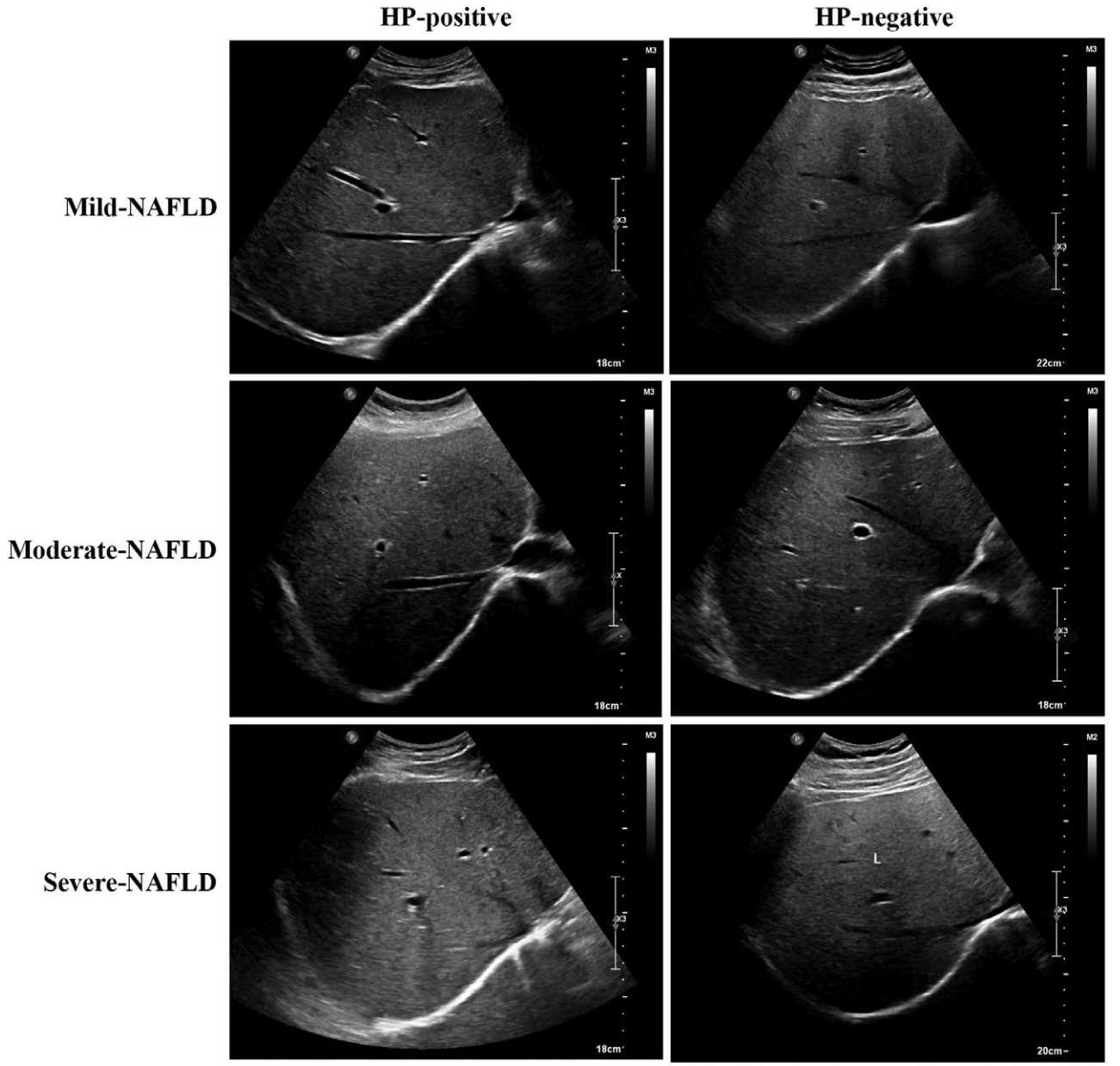

Fig. 1. Representative B-mode ultrasonography image maps of different NAFLD patients. The white background represents steatosis tissues, and the black piping shadow represents intrahepatic ducts. The white standard measurement bar on the top right corner is used for evaluating the brightness of the white background.NAFLD, nonalcoholic fatty liver disease.

lipid metabolism, inflammatory reaction and occurrence of metabolic syndromes.

Previous studies on the relationship between *H. pylori* infection and hepatic diseases have been inconsistent. A systematic review in 2018 showed that *H. pylori* infection has a positive correlation with NAFLD, but a negative association has also been reported in large and small cross-sectional studies, retrospective studies, randomized controlled trials, retrospective studies, and meta-analyses. In addition, except for a negative relationship between *H. pylori* infection and chronic B viral hepatitis identified in a large cross-sectional study in 2011, positive correlations between *H. pylori* infection and other hepatic diseases such as chronic viral hepatitis, cirrhosis, and hepatocellular carcinoma have been reported in cross-sectional studies and meta-analyses [47]. However, the focuses that *H. pylori* infection contributing to NAFLD is disparate.

A cross-sectional study of 4081 individuals found that 52.36% were *H. pylori* positive and 47.82% *H. pylori*-positive individuals were diagnosed with NAFLD. Moreover, in *H. pylori*-positive NAFLD patients, women and patients with dyslipidemia showed a significant increase in the fully adjusted odds ratios and 95% confidence intervals [12]. A prospective cohort multicenter pilot study with 2 years of follow-up in 369 adults without NAFLD at baseline indicated that *H. pylori* infection was positively associated with NAFLD development [increased NAFLD-liver fat score (NAFLD-LFS) and hepatic steatosis index (HSI)]. This was shown by increased markers of insulin resistance, inflammatory mediators such as interleukin-6 and C-reactive protein (CRP), and lipid metabolism such as the leptin/adiponectin ratio, and decreased lipid metabolism marker, HDL. Furthermore, these NAFLD risk factors can be recovered after eradication of *H. pylori*

Table 6. Liver function, carbohydrate metabolism and inflammatory response are relation with *H. pylori* infection in 91 patients with nonalcoholic fatty liver

Parameter (mean ± SD)	*H. pylori*-positive	*H. pylori*-negative	*P* value
Liver function			
ALT (U/L)	34.13 ± 19.88	28.32 ± 14.06	0.11
TBIL (μmol/L)	14.5 ± 7.53	12.83 ± 4.18	0.191
DBIL (μmol/L)	5.51 ± 3.21	4.38 ± 1.15	0.027
Carbohydrate metabolism			
GLU (mmol/L)	7.07 ± 2.28	5.49 ± 0.74	0.000
HbAlc (%)	6.51 ± 1.45	5.64 ± 0.50	0.000
INS (mIU/L)	17.57 ± 6.37	11.44 ± 3.25	0.038
HOMA-Ir	1.86 ± 0.52	1.12 ± 0.13	0.036
Lipid metabolism			
CHOL (mmol/L)	4.91 ± 0.96	5.08 ± 0.66	0.336
Triglyceride (mmol/L)	2.38 ± 1.60	2.10 ± 1.18	0.344
HDL-C (mmol/L)	1.06 ± 0.26	1.15 ± 0.21	0.096
LDL-C (mmol/L)	3.11 ± 1.01	3.05 ± 0.65	0.752
Inflammatory response			
WBC# (10^9/L)	6.80 ± 2.26	5.87 ± 1.65	0.029
MID# (10^9/L)	0.69 ± 0.36	0.57 ± 0.22	0.058
GRAN# (10^9/L)	4.07 ± 1.92	3.34 ± 1.23	0.033

#, count; ALT, alanine aminotransferase; ALB, albumin; CHOL, lipid metabolism: total cholesterol; DBIL, direct bilirubin; GLB, globulin; GLU, fasting plasma glucose; GRAN, granulocyte; HbAlc, glycosylated hemoglobin; HDL-C, high-density lipoprotein; HOMA-Ir, homeostasis model assessment of insulin resistance; INS, fasting plasma insulin; LDL-C, low-density lipoprotein; LYMPH, lymphocyte; MID, middle cell; TBIL, total bilirubin; WBC, white blood cell.

Table 7. The incidence rate of metabolic syndromes such as overweight/visceral obesity, fasting hyperglycemia, dyslipidemia and hypertension is related to the *H. pylori* infection

Metabolic syndromes (*n*, %)	*H. pylori*-positive (*n* = 47)	*H. pylori*-negative (*n* = 44)	*P* value
Overweight/visceral obesity	39 (82.98)	34 (77.27)	<0.05
Fasting hyperglycemia	29 (61.70)	15 (34.09)	<0.01
Dyslipidemia	36 (76.60)	31 (70.45)	<0.05
Hypertension	19 (40.43)	9 (20.45)	<0.01

infection [31]. Additionally, a cohort study carried out in 17 028 adults without NAFLD at baseline showed that *H. pylori* infection was significantly related to NAFLD development, independent of metabolic and inflammatory risk factors [32]. However, the lipid metabolism markers of TC, triglyceride, HDL-C and LDL-C showed no significant difference between *H. pylori*-positive and *H. pylori*-negative NAFLD patients in our study. Notably, a significant difference occurred after removal of the confounding factor of NAFLD status. In a large study of 20 389 Chinese subjects, compared with the control group, the NAFLD group showed a higher prevalence of *H. pylori* infection, and combination of *H. pylori* infection and WBC count was positively correlated with NAFLD by multivariate logistic regression [40]. However, the association between *H. pylori* infection and WBC count was not demonstrated in that study. Moreover, NAFLD patients have a higher incidence of *H. pylori* infection at genetic, small molecular and clinical levels, as analyzed by the Pathway Studio Res Net Mammalian database and the clinical data of 2263 elderly Southern Chinese subjects [41]. A small clinical study indicated that *H. pylori* infection (*H. pylori* IgG seropositivity or C^{13} urea breath test positivity) may be a contributing factor in the pathogenesis of NAFLD, but not in the progression from NAFLD to nonalcoholic steatohepatitis [48]. Based on these previous results, our findings supported that WBC count was positively correlated with NAFLD [40], and *H. pylori* infection was highly associated with occurrence of hypertension [32]. Importantly, we also concluded that *H. pylori* infection was positively related to NAFLD development, but the evidence in our study was distinct. We are the first to demonstrate that *H. pylori* infection is positively involved in development of NAFLD via increasing steatosis and severity of NAFLD. Furthermore, we revealed that *H. pylori* infection may be

Table 8. *H. pylori* infection may be involved in the relations between the severity degree of nonalcoholic fatty liver patients and liver function, carbohydrate metabolism, lipid metabolism and inflammatory response

	Mild-NAFLD (*n* = 62)			Moderate-NAFLD *n* = 17)			Severe-NAFLD (*n* = 12)		
Parameter (*n*, %)	*H. pylori*-positive (*n* = 30)	*H. pylori*-negative (*n* = 32)	*P* value	*H. pylori*-positive (*n* = 9)	*H. pylori*-negative (*n* = 8)	*P* value	*H. pylori*-positive (*n* = 8)	*H. pylori*-negative (*n* = 4)	*P* value
Liver function									
ALT (U/L)	↑4 (13.33)	↑2 (6.25)	<0.01	↑6 (66.67)	↑4 (50)	<0.01	↑6 (75)	↑2 (50)	<0.01
TBIL (μmol/L)	↑2 (6.67)	↑2 (6.25)	>0.05	↑2 (22.22)	–	<0.01	↑3 (37.5)	↑1 (25)	<0.01
DBIL (μmol/L)	↑2 (6.67)	–	<0.01	↑2 (22.22)	–	<0.01	↑3 (37.5)	–	<0.01
Carbohydrate metabolism									
GLU (mmol/L)	↑13 (43.33)	↑2 (6.25)	<0.01	↑7 (77.78)	↑5 (62.5)	<0.01	↑7 (87.5)	↑2 (50)	<0.01
HbAlc (%)	↑12 (40)	↑2 (6.25)	<0.01	↑5 (55.56)	↑3 (37.5)	<0.01	↑5 (62.5)	↑2 (50)	<0.01
INS (mIU/L)	↑11 (36.67)	↑2 (6.25)	<0.01	↑6 (66.67)	↑4 (50)	<0.01	↑6 (75)	↑2 (50)	<0.01
HOMA-Ir	↑13 (43.33)	↑2 (6.25)	<0.01	↑7 (77.78)	↑5 (62.5)	<0.01	↑7 (87.5)	↑2 (50)	<0.01
Lipid metabolism									
CHOL (mmol/L)	↑10 (33.33); ↓3 (10)	↑13 (40.63); –	<0.01	↑5 (55.56);↓ 1 (11.11)	↑4 (50); –	<0.05	↑5 (62.5); ↓ 1 (12.5)	↑2 (50); –	<0.01
Triglyceride (mmol/L)	↑15 (50)	↑13 (40.63)	<0.01	↑6 (66.67)	↑3 (37.5)	<0.01	↑6 (75)	↑2 (50)	<0.01
HDL-C (mmol/L)	↓2 (6.67)	↓1 (3.13)	<0.05	↓3 (33.33)	–	<0.01	↓3 (37.5)	–	<0.01
LDL-C (mmol/L)	↑4 (13.33)	↑7 (21.88)	<0.01	↑5 (55.56)	↑4 (50)	<0.05	↑5 (62.5)	↑2 (50)	<0.01
Inflammatory response									
WBC#(10^9/L)	–	↓2 (6.25)	<0.01	↑3 (33.33)	↑1 (12.5)	<0.01	↑4 (50)	↑1 (25)	<0.01
MID# (10^9/L)	↑ 1 (3.33)	↑1 (3.13)	>0.05	↑3 (33.33)	–	<0.01	↑3 (37.5)	–	<0.01
GRAN# (10^9/L)	↓1 (3.33)	↓2 (6.25)	<0.05	↑3 (33.33)	↑1 (12.5)	<0.01	↑4 (50)	↑1 (25)	<0.01
P value				<0.05					

↑, above the maximum value of the normal reference value; ↓, below the lowest value of the normal reference value; –, between the normal reference value; #, count; ALT, alanine aminotransferase; ALB, albumin; CHOL, lipid metabolism: total cholesterol; DBIL, direct bilirubin; GLB, globulin; GLU, fasting plasma glucose; GRAN, granulocyte; HbAlc, glycosylated hemoglobin; HDL-C, high-density lipoprotein; HOMA-Ir, homeostasis model assessment of insulin resistance; INS, fasting plasma insulin; LDL-C, low-density lipoprotein; LYMPH, lymphocyte; MID, middle cell; NAFLD, nonalcoholic fatty liver; TBIL, total bilirubin; WBC, white blood cell.

Table 9. *H. pylori* infection may be involved in the relations between the severity degree of NAFLD patients and the occurrence rate of metabolic syndromes

NAFLD ($n=91$)	Mild-NAFLD ($n=62$)			Moderate-NAFLD ($n=17$)			Severe-NAFLD ($n=12$)		
Metabolic syndromes (n, %)	*H. pylori*-positive ($n=30$)	*H. pylori*-negative ($n=32$)	*P* value	*H. pylori*-positive ($n=9$)	*H. pylori*-negative ($n=8$)	*P* value	*H. pylori*-positive ($n=8$)	*H. pylori*-negative ($n=4$)	*P* value
Overweight/visceral obesity	23 (76.67)	24 (75)	>0.05	9 (100)	6 (75)	<0.01	7 (87.5)	4 (100)	<0.01
Fasting hyperglycemia	15 (50)	7 (21.88)	<0.01	8 (88.89)	5 (62.5)	<0.01	7 (87.5)	3 (75)	<0.01
Dyslipidemia	20 (66.67)	22 (68.75)	>0.05	9 (100)	5 (62.5)	<0.01	7 (87.5)	4 (100)	<0.01
Hypertension	9 (30)	8 (25)	<0.05	5 (55.56)	1 (12.5)	<0.01	5 (62.5)	0 (0.00)	<0.01
P value					<0.01				

NAFLD, nonalcoholic fatty liver.

an autocephalous risk factor for forecasting NAFLD progress, including liver dysfunction, exceptionally increased glycometabolism and lipid metabolism, inflammatory reaction and occurrence of metabolic syndromes, as well as decresing insulin sensitivity. Weightily, NAFLD is recognized as hepatic manifestation of metabolic syndromes with the characteristics similar to those of metabolic disorders including obesity, inflammation and insulin resistance [49]. A score of insulin-resistance (HOMA-Ir) was increased in NAFLD patients compared with the control people, and insulin-resistance (HOMA-Ir) was used as a diagnostic and predictive factor of NAFLD [50–52]. Additionally, infection of *H. pylori* was related to a higher insulin resistance degree [53]. The eradication of *H. pylori* has been proved beneficial to insulin resistance, atherogenic serum lipids abnormalities and mild inflammation [54]. These previous reports forcefully supported our finds that *H. pylori* infection is positively associated with NAFLD.

Conversely, in a randomized open-label clinical trial in 100 patients, *H. pylori* eradication did not affect liver fat content, liver function tests including serum ALT, AST and ALP, lipid profiles including triglyceride, CHOL, HDL and LDL, and HOMA-IR index in dyspeptic NAFLD patients [55]. *H. pylori* eradication has also been shown to have no long-term effect on hepatic steatosis, but it did improve NAFLD fibrosis score and HSENSI (homocysteine, serum glutamic oxaloacetic transaminase, erythrocyte sedimentation rate, nonalcoholic steatohepatitis index) in an MRI-based pilot open-label study of 13 adults with biopsy-proven nonalcoholic steatohepatitis [56]. A cross-sectional study of 21 456 subjects who underwent a healthy checkup program in China showed that *H. pylori* infection increased BMI, blood pressure and lipid metabolism (higher levels of triglyceride, and lower levels of HDL-C), as well as the prevalence of NAFLD in women but not in men. However, *H. pylori* infection is not associated with NAFLD in the total population after adjusting for the above confounding factors [34]. A large-scale cross-sectional study performed in 13 737 adults in Japan in 2010 revealed that BMI, serum ALT and platelet count were positively correlated with FLD and NAFLD, but not *H. pylori* infection. In addition, in male patients, metabolic syndrome was significantly associated with FLD and NAFLD [35]. A retrospective study of 3663 healthy people who underwent health screening found that *H. pylori* infection was closely associated with older age, male gender, hypertension, higher BMI and dyslipidemic profile, whereas NAFLD (HSI and NAFLD-LFS) was not. Moreover, CRP concentration and smoking were identified as significant risk factors for NAFLD [36]. Despite the opposing conclusions reported in these studies, the study participants and analytical approaches also differed from our study.

Although some novel and valuable discoveries were found in our study, there were still some limitations. Due to the limited clinical data, it was difficult to analyze the effects of sex, age or other sensitive factors on *H. pylori* infection or NAFLD; thus, the outcomes did not rule out these factors having an effect. Additionally, the standards for *H. pylori* detection and classification of NAFLD are one dimensional. For instance, *H. pylori* IgG seropositivity is also a highly sensitive and scientific diagnostic method for *H. pylori* infection [48], and NAFLD-LFS and HSI are frequently used and recognized diagnostic criteria for NAFLD progress or severity [31,36]. Increasing the number of NAFLD patients and further study of therapeutic effects of a follow-up of the patients after *H. pylori* eradication are needed to confirm the relationship between *H. pylori* infection and NAFLD.

In summary, *H. pylori* infection is positively correlated with NAFLD. Considering the limited clinical data in our study, further investigations are necessary to confirm the connection between *H. pylori* infection and NAFLD, which *may be* undoubtedly afford novel insights for effective prevention and treatment of NAFLD.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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