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Helicobacter pylori infection is not an independent risk factor of non-alcoholic fatty liver disease in China

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Abstract

Background: The role of *Helicobacter pylori* (*H. pylori*) infection in the development of non-alcoholic fatty liver disease (NAFLD) remains controversial. The exact relationship requires further investigation. This study aimed to determine the association between them in China.

Methods: A retrospective study was conducted on 71,633 participants who underwent physical examinations. ¹³C urea breath test (¹³C-UBT) was conducted to detect *H. pylori* infection, and ultrasonography was used to detect NAFLD.

Results: Body mass index (BMI), blood pressure (BP), and triglyceride (TG) levels were higher in participants with *H. pylori* infection than in those without *H. pylori* infection. While the levels of high-density lipoprotein cholesterol (HDL-C) for participants with *H. pylori* infection was lower than without *H. pylori* infection ($P < 0.001$). After adjusting for confounding factors (age, sex, BMI, BP, Scr, BUN, LDL-C, HDL-C, triglycerides, FBG and HbA1c), multivariate logistic regression analysis indicated that there was no independent relationship between them ($P = 0.574$). Subgroup analysis (stratified by sex, age, BMI, hypertension, diabetes and dyslipidemia) showed that *H. pylori* infection was not included as an independent risk factor for NAFLD. Moreover, the different grades of NAFLD were not related to *H. pylori* infection.

Conclusions: These results indicate that *H. pylori* infection is not an independent risk factor for NAFLD in China.

Keywords: *H. pylori*, NAFLD, Risk factor, Infection, Association

Background

Non-alcoholic fatty liver disease (NAFLD), manifested by hepatic steatosis without alcohol consumption or other causes of liver disease, is an important form of chronic liver disease [1]. It is increasingly recognized that NAFLD can cause both liver disease and extrahepatic

manifestations, including obesity, dyslipidemia, diabetes, insulin resistance, chronic kidney disease and extrahepatic malignancies [2–5]. Currently, the prevalence of NAFLD is rapidly increasing, contributing to enormous clinical and financial burden [1]. Therefore, it is of great importance to identify risk factors that may have therapeutic implications for the prevention and treatment of NAFLD, as well as the associated burden.

Various mechanisms related to the intestinal microbiome and NAFLD have been proposed, such as its impact on the innate immune system, intestinal endothelial barrier function, intestinal production of metabolites, and fermentation of indigestible carbohydrates [6, 7]. Animal

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experiments also suggest that intestinal dysbiosis or *Helicobacter pylori* (*H. pylori*) infection may lead to the occurrence and development of NAFLD [8]. *H. pylori* is a gram-negative bacterium that colonizes the gastric epithelium [9]. It is a key element of the human microbiome. Although current evidence is not definitive, chronic *H. pylori* infection has been demonstrated to be related to inflammatory bowel diseases (IBD), gastrointestinal cancers, and extra-digestive tract diseases, including cardiovascular, pulmonary, hematological, ophthalmic, skin, neurological, and metabolic diseases [10, 11]. A previous study reported that *H. pylori* infection may lead to gastrointestinal flora dysbiosis, increase the levels of inflammatory cytokines, promote insulin resistance, and accelerate fatty deposits in the liver tissue, which contribute to NAFLD [12].

There have been increasing numbers of studies investigating the association of *H. pylori* and NAFLD [13, 14]. Several researches have shown that *H. pylori* infection is related to the development of NAFLD, regardless of inflammatory and metabolic risk factors [13]. On the contrary, other retrospective studies indicated that in apparently healthy subjects, *H. pylori* infection is not an independent risk factor for patients suffering from NAFLD [14]. To clarify this inconsistency, it is necessary to further investigate the association between *H. pylori* and NAFLD.

A broad population-based study may help clarify the relationship between these factors. This cross-sectional study of 71,633 participants recruited from the Wuhan Union Hospital aimed to determine the association between *H. pylori* infection and different grades of NAFLD (total/mild/moderate/severe). Meanwhile, the role of *H. pylori* in NAFLD was further studied in subgroups, characterized by sex, age, body mass index (BMI), hypertension, diabetes, and dyslipidemia.

Methods

Study population

The cross-sectional study included healthy adults who underwent comprehensive medical checkups at the Wuhan Union Hospital, from January 2015 to December 2019. Participants with both abdominal ultrasound (US) for the detection of liver steatosis and ^{13}C urea breath test (^{13}C -UBT) for the detection of *H. pylori* infection were included ($n = 122,764$). Participants with a self-reported history of other chronic liver diseases (viral hepatitis, autoimmune hepatitis, etc.), daily alcohol consumption (male: > 30 g; female: > 20 g); positive markers for hepatitis A, B, or C virus; self-reported history of malignancy; and missing data on basic information were excluded from the study. In total, 71,633 healthy participants were included in the analysis. The chart of this study is

presented in Additional file 1: Fig. S1. Table 1 shows the baseline characteristics of the participants. This study was approved by the Wuhan Union Hospital Ethics Committee and the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (IORG No: IORG0003571). Informed consent was waived by the ethics committees.

Data collection

Clinical examination data comprised demographic features, anthropometry, laboratory examination, image examination, and a self-administered health questionnaire. The weight, height, and blood pressure were measured by three experienced physicians. Blood samples were collected from the elbow vein after an overnight fast by three experienced nurses. Fasting total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transpeptidase (GGT), serum creatinine, and urea nitrogen were measured using an automatic biochemical analyzer. Plasma glucose was analyzed using the glucose oxidase method. Glycosylated hemoglobin (HbA1c) levels were measured using high-performance liquid chromatography [15].

Diagnosis of *Helicobacter pylori* infection

H. pylori infection status was diagnosed using ^{13}C -UBT. After fasting overnight, each participant ingested an oral pill labeled with the radiocarbon-13 isotope. Respiratory samples were stored at room temperature and evaluated using $^{13}\text{CO}_2$. *H. pylori* infection was determined by comparing the $^{13}\text{CO}_2$ content of the baseline and 30-min samples, and a ratio > 4.0 was considered positive. This was based on the Fourth Chinese National Consensus Report on the management of *H. pylori* infection [16].

Diagnosis of NAFLD

NAFLD was diagnosed by hepatic ultrasonography in the absence of excessive alcohol intake and viral or autoimmune hepatitis. Ultrasonographic examinations were carried out by five experienced specially trained doctors. NAFLD was diagnosed according to the standard USS criteria. Liver steatosis was defined as having at least two of the following three abnormal manifestations: echogenicity enhancement of the liver compared with spleen or kidney; ultrasound beam attenuation; poor visualization of intrahepatic architectural details [17, 18]. In addition, NAFLD was also divided into three different grades (mild, moderate, and severe) according to the Chinese Ultrasonic Grading Criteria of NAFLD published in 2003.

Table 1 Characteristics of study participants at baseline

| Variables | Full sample (n = 71,633) | | | Matched sample (n = 29,974) ^a | | |
|----------------------------|---------------------------------|---------------------------------|---------|--|---------------------------------|---------|
| | <i>H. pylori</i> – (n = 46,888) | <i>H. pylori</i> + (n = 24,745) | P value | <i>H. pylori</i> – (n = 14,987) | <i>H. pylori</i> + (n = 14,987) | P value |
| Age | 44.8 ± 13.3 | 46.5 ± 12.5 | < 0.001 | 47.3 ± 12.9 | 46.6 ± 12.2 | |
| Male (%) | 57.6 | 58.6 | 0.010 | 58.3 | 57.5 | 0.134 |
| BMI (kg/m ²) | 23.8 ± 3.4 | 24.1 ± 3.4 | < 0.001 | 24.3 ± 3.3 | 24.2 ± 3.4 | 0.043 |
| Year of screening exam (%) | | | < 0.001 | | | |
| 2015 | 20.8 | 21.7 | | 21.6 | 12.6 | |
| 2016 | 24.4 | 24.2 | | 48.1 | 27.1 | |
| 2017 | 19.3 | 18.7 | | 25.1 | 23.0 | |
| 2018 | 19.8 | 21.3 | | 4.8 | 24.5 | |
| 2019 | 18.8 | 17.3 | | 0.4 | 12.7 | |
| Systolic BP (mmHg) | 124.3 ± 19.5 | 125.9 ± 20.0 | < 0.001 | 127.1 ± 19.8 | 126.4 ± 20.0 | |
| Diastolic BP (mmHg) | 77.8 ± 11.4 | 78.9 ± 11.8 | < 0.001 | 79.7 ± 11.5 | 79.1 ± 11.8 | |
| Hypertension (%) | 13.7 | 15.1 | < 0.001 | 13.5 | 13.9 | 0.129 |
| FBG (mM) | 5.05 ± 1.27 | 5.18 ± 1.47 | < 0.001 | 5.11 ± 1.29 | 5.12 ± 1.29 | |
| HbA1C (%) | 5.48 ± 0.75 | 5.55 ± 0.83 | < 0.001 | 5.50 ± 0.77 | 5.50 ± 0.77 | |
| Diabetes (%) | 4.74 | 6.14 | < 0.001 | 5.49 | 5.60 | 0.565 |
| Total cholesterol (mM) | 4.80 ± 0.92 | 4.86 ± 0.92 | < 0.001 | 4.89 ± 0.91 | 4.86 ± 0.92 | |
| LDL-C (mM) | 2.76 ± 0.77 | 2.82 ± 0.78 | < 0.001 | 2.22 ± 0.77 | 2.82 ± 0.77 | |
| HDL-C (mM) | 1.43 ± 0.34 | 1.41 ± 0.34 | < 0.001 | 1.46 ± 0.35 | 1.41 ± 0.35 | |
| Triglycerides (mM) | 1.58 ± 1.39 | 1.65 ± 1.50 | < 0.001 | 1.65 ± 1.41 | 1.64 ± 1.44 | |
| Dyslipidemia (%) | 24.9 | 28.0 | < 0.001 | 26.8 | 26.9 | 0.887 |
| AST (IU/L) | 23.3 (18–25) | 23.4 (18–25) | 0.011 | 22.7 (17–25) | 23.3 (18–26) | |
| ALT (IU/L) | 26.5 (15–31) | 27.0 (15–31) | < 0.001 | 27.4 (15–32) | 27.0 (15–31) | |
| GGT (IU/L) | 30.2 (14–33) | 31.0 (15–35) | < 0.001 | 32.6 (15–36) | 30.5 (14–35) | |
| Scr (μM) | 70.5 ± 17.2 | 71.1 ± 20.1 | 0.001 | 69.6 ± 18.6 | 70.2 ± 17.6 | |
| BUN (mM) | 4.88 ± 1.30 | 4.95 ± 1.37 | < 0.001 | 4.99 ± 1.32 | 4.94 ± 1.32 | |
| NAFLD (%) | 31.3 | 34.8 | < 0.001 | 34.8 | 34.6 | 0.716 |

Continuous variables were shown as mean ± SD or median (interquartile range), and categorical variables were expressed as counts (percentage)

^a Matched sample were selected with propensity score matching analysis (PSM) (Covariate: Age, sex, BMI, Systolic BP, Diastolic BP, FBG, HbA1C, Total cholesterol, LDL-C, HDL-C, Triglycerides, Scr, BUN; Matching tolerance: 0.001)

H. pylori, *Helicobacter pylori*; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; HbA1C, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; Scr, serum creatinine; BUN, blood urea nitrogen; NAFLD, nonalcoholic fatty liver disease

Diagnosis of hypertension, diabetes, and dyslipidemia

Hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg [19]. Patients with fasting blood glucose (FBG) ≥ 7.0 mmol/L were defined as having diabetes [20]. Dyslipidemia was confirmed by increases in total cholesterol (≥ 240 mg/dL [6.20 mmol/L]), LDL-C (> 160 mg/dL [4.13 mmol/L]), and triglyceride levels (> 200 mg/dL [2.25 mmol/L]), or a decrease in HDL-C (< 40 mg/dL [1.03 mmol/L]) [21].

Statistical analysis

Statistical analyses were performed using SPSS, version 26.0 (Chicago, Illinois, USA). Continuous variables were shown as mean ± SD, and categorical variables were

expressed as counts (percentage). The Mann–Whitney *U* test and χ^2 test were used to compare the differences between the two groups. Propensity score matching (PSM) analysis was also conducted. Logistic regression analysis was used to assess the association between the factors of interest. And multicollinearity test was performed before multivariate regression analysis. *P* values < 0.05 (two-tailed) were considered statistically significant.

Results

Clinical and demographic characteristics

Among the 71,633 involved study participants, 30,086 (42.0%) were female and 41,547 (58.0%) were male. Among all subjects, the prevalence of *H. pylori*

infection in this study was 34.5% (n = 24,745). Table 1 summarizes the clinical and biochemical characteristics of the participants based on their *H. pylori* infection status. Males with a high BMI (*H. pylori*- vs. *H. pylori*+ : 23.8 ± 3.4 vs. 24.1 ± 3.4) and high blood pressure (systolic BP: 124.3 ± 19.5 vs. 125.9 ± 20.0; diastolic BP: 77.8 ± 11.4 vs. 78.9 ± 11.8) were more likely to be *H. pylori* positive. Compared with the *H. pylori*- group, the *H. pylori*+ group had a more adverse lipid profile, including higher levels of TC (*H. pylori*- vs. *H. pylori*+ : 4.80 ± 0.92 vs. 4.86 ± 0.92), TG (*H. pylori*- vs. *H. pylori*+ : 1.58 ± 1.39 vs. 1.65 ± 1.50), and LDL-C (*H. pylori*- vs. *H. pylori*+ : 2.76 ± 0.77 vs. 2.82 ± 0.78), and a lower level of HDL-C (*H. pylori*- vs. *H. pylori*+ : 1.43 ± 0.34 vs. 1.41 ± 0.34). Moreover, the serum ALT level (*H. pylori*- vs. *H. pylori*+ : 26.5 (15–31) vs. 27.0 (15–31)), and GGT (*H. pylori*- vs. *H. pylori*+ : 30.2 (14–33) vs. 31.0 (15–35)) in the *H. pylori*+ group were higher than those in the *H. pylori*- group. Moreover, the FBG level (*H. pylori*- vs. *H. pylori*+ : 5.05 ± 1.27 vs. 5.18 ± 1.47), and HbA1c (*H. pylori*- vs. *H. pylori*+ : 5.48 ± 0.75 vs. 5.55 ± 0.83) were higher in the *H. pylori*+ group (Table 1). In the matched samples (n = 29,974), there was no difference in the incidence of hypertension (P = 0.129), diabetes (P = 0.565), or dyslipidemia (P = 0.887) between the two groups (*H. pylori*- and *H. pylori*+). Further analysis showed that there was no significant difference in the incidence of NAFLD between the *H. pylori*- and *H. pylori*+ groups (P = 0.716) (Table 1).

The prevalence of NAFLD and *H. pylori*

The prevalence of NAFLD and the *H. pylori* infection status are shown in Fig. 1. The prevalence of NAFLD in the *H. pylori*+ group (total: 34.8%; mild: 27.1%, moderate: 7.5%, severe: 0.2%) was significantly higher than that in the *H. pylori*- group (total: 31.3%; mild: 24.6%, moderate: 6.5%, severe: 0.2%) (P < 0.001). Further stratified analysis by sex revealed that the prevalence of NAFLD with *H. pylori*+ in males was 44.4% (mild: 33.7%, moderate: 10.4%, severe: 0.3%), which was higher than that in the *H. pylori*- group (total: 41.1%; mild: 31.7%, moderate: 9.2%, severe: 0.3%) (P < 0.001). Moreover, the prevalence of NAFLD in female with *H. pylori*+ (total: 21.1%, mild: 17.7%, moderate: 3.3%, severe: 0.07%) was significantly higher than *H. pylori*- group (total: 17.9%, mild: 15.0%, moderate: 2.8%, severe: 0.03%) (P < 0.001).

Associations between NAFLD and *H. pylori* infection status

The relationship between *H. pylori* infection and NAFLD prevalence was analyzed using logistic regression analysis. In the full samples (n = 71,633), univariate analysis showed that *H. pylori* infection increased the risk of NAFLD (OR = 1.172, 95% CI = 1.135–1.211, P < 0.001) (Table 2). Subsequently, we performed a multicollinearity analysis with the significant variables (variance inflation factor test, VIF) and found that there was multicollinearity between total cholesterol (VIF = 13.778) and LDL-C (VIF = 10.511). In contrast, there was no multicollinearity among the other variables (VIF < 10; triglyceride, VIF = 3.968; FBG, VIF = 3.377; HbA1c, VIF = 3.486). We then excluded total cholesterol from the multivariate regression model. The multivariate analysis showed no

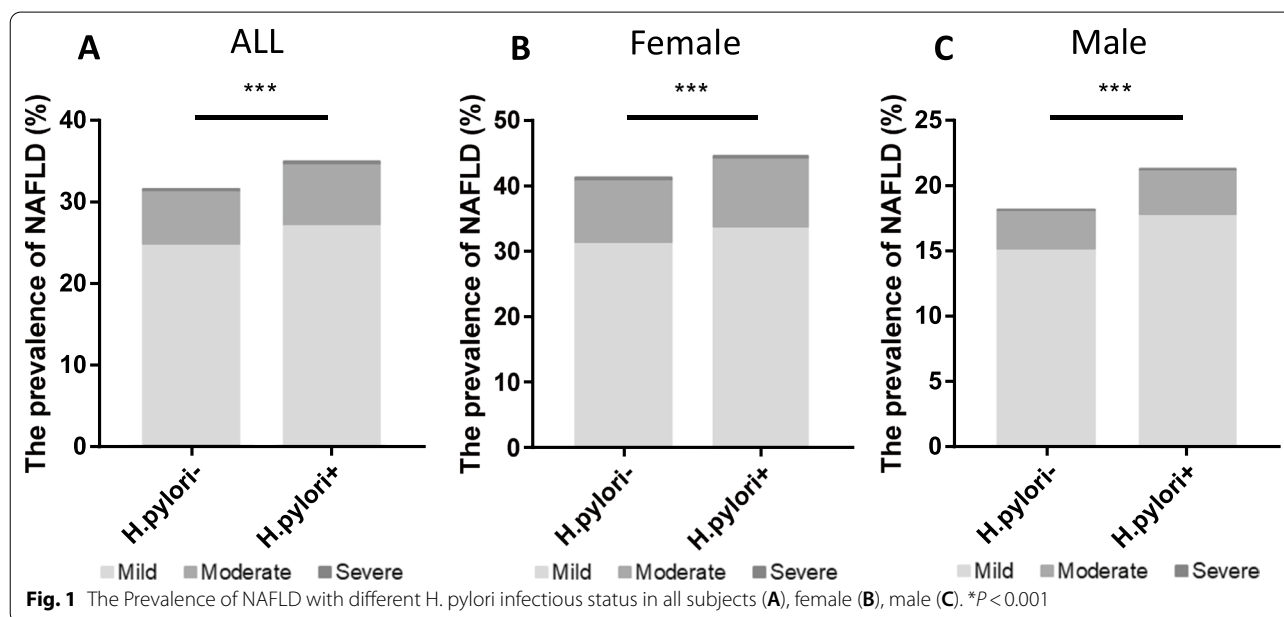


Table 2 The risk of NAFLD development in the univariate and multivariate analyses

| Variables | Full sample (n = 71,633) | | | | Matched sample (n = 29,974) | | | |
|--------------------------|--------------------------|---------|------------------------------------|---------|-----------------------------|---------|------------------------------------|----------------|
| | Univariate analysis | | Multivariate analysis ^a | | Univariate analysis | | Multivariate analysis ^b | |
| | OR (95%CI) | P value | OR (95%CI) | P value | OR (95%CI) | P value | OR (95%CI) | P value |
| Age | 1.02 (1.02–1.02) | <0.001 | 1.02 (1.01–1.02) | <0.001 | 1.01 (1.01–1.02) | <0.001 | 1.01 (1.01–1.02) | <0.001 |
| Male sex (%) | 3.13 (3.03–3.24) | <0.001 | 1.09 (1.01–1.18) | 0.022 | 0.35 (0.33–0.36) | <0.001 | 0.98 (0.90–1.07) | 0.601 |
| BMI (kg/m ²) | 1.62 (1.61–1.63) | <0.001 | 1.44 (1.42–1.45) | <0.001 | 1.59 (1.57–1.61) | <0.001 | 1.42 (1.40–1.44) | <0.001 |
| Systolic BP (mmHg) | 1.03 (1.03–1.03) | <0.001 | 1.00 (0.99–1.00) | <0.001 | 1.03 (1.03–1.03) | <0.001 | 1.00 (0.99–1.00) | 0.003 |
| Diastolic BP (mmHg) | 1.07 (1.06–1.07) | <0.001 | 1.02 (1.02–1.03) | <0.001 | 1.06 (1.06–1.06) | <0.001 | 1.02 (1.02–1.03) | <0.001 |
| FBG (mM) | 1.46 (1.44–1.49) | <0.001 | 1.03 (0.99–1.07) | 0.087 | 1.43 (1.39–1.46) | <0.001 | 1.05 (1.01–1.10) | 0.018 |
| HbA1C (%) | 1.98 (1.92–2.04) | <0.001 | 1.23 (1.15–1.30) | <0.001 | 1.83 (1.76–1.90) | <0.001 | 1.22 (1.13–1.30) | <0.001 |
| Total cholesterol (mM) | 1.45 (1.42–1.47) | <0.001 | – | – | 1.35 (1.31–1.39) | <0.001 | – | – ^c |
| LDL-C (mM) | 1.57 (1.53–1.60) | <0.001 | 1.35 (1.30–1.40) | <0.001 | 1.44 (1.40–1.49) | <0.001 | 1.34 (1.28–1.39) | <0.001 |
| HDL-C (mM) | 0.07 (0.07–0.08) | <0.001 | 0.36 (0.32–0.39) | <0.001 | 0.09 (0.08–0.10) | <0.001 | 0.37 (0.33–0.41) | <0.001 |
| Triglycerides (mM) | 2.53 (2.47–2.58) | <0.001 | 1.46 (1.42–1.50) | <0.001 | 2.32 (2.25–2.39) | <0.001 | 1.46 (1.41–1.50) | <0.001 |
| AST (IU/L) | 1.05 (1.04–1.05) | <0.001 | 0.97 (0.97–0.97) | <0.001 | 1.04 (1.04–1.04) | <0.001 | 0.97 (0.97–0.98) | <0.001 |
| ALT (IU/L) | 1.05 (1.05–1.05) | <0.001 | 1.03 (1.03–1.03) | <0.001 | 1.04 (1.04–1.05) | <0.001 | 1.03 (1.03–1.03) | <0.001 |
| GGT (IU/L) | 1.03 (1.03–1.03) | <0.001 | 1.00 (1.00–1.00) | <0.001 | 1.02 (1.02–1.03) | <0.001 | 1.00 (1.00–1.00) | <0.001 |
| Scr (μM) | 1.02 (1.02–1.02) | <0.001 | 0.99 (0.99–1.00) | <0.001 | 1.02 (1.02–1.02) | <0.001 | 1.00 (0.99–1.00) | <0.001 |
| BUN (mM) | 1.11 (1.10–1.13) | <0.001 | 0.99 (0.97–1.01) | 0.275 | 1.10 (1.08–1.12) | <0.001 | 0.99 (0.97–1.02) | 0.546 |
| <i>H. pylori</i> (+)(%) | 1.17 (1.14–1.21) | <0.001 | 1.02 (0.97–1.08) | 0.446 | 1.01 (0.96–1.06) | 0.716 | 1.00 (0.94–1.06) | 0.898 |

^a Estimated from Logistic regression analysis and adjusted for Age, sex, BMI, Systolic BP, Diastolic BP, FBG, HbA1C, LDL-C, HDL-C, Triglycerides, AST, ALT, GGT, Scr, BUN and *H. pylori*+ (%)

^b Estimated from Logistic regression analysis and adjusted for Age, sex, BMI, Systolic BP, Diastolic BP, FBG, HbA1C, LDL-C, HDL-C, Triglycerides, AST, ALT, GGT, Scr, BUN and *H. pylori*+ (%)

^c Multicollinearity analysis with the significant variables (variance inflation factor test, VIF) was conducted and Total cholesterol was excluded from the multivariate regression model (VIF = 12.104)

H. pylori, *Helicobacter pylori*; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; HbA1C, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; Scr, serum creatinine; BUN, blood urea nitrogen; NAFLD, nonalcoholic fatty liver disease; OR, odd ratio; CI, confidence intervals

significant association between *H. pylori* infection and NAFLD (OR = 1.022, 95% CI = 0.967–1.079, *P* = 0.446) (Table 2). In the matched samples (n = 29,974), the univariate analysis (OR = 1.01, *P* = 0.716) and the multivariate analysis (OR = 1.00, *P* = 0.898) showed *H. pylori* infection was not the risk of NAFLD development (Table 2). As shown in Table 3, after adjusting for age, sex, SBP, DBP, Scr, BUN, FBG, HbA1C, LDL-C, HDL-C, and triglycerides, *H. pylori*+ was still associated with an increased risk of NAFLD (model 1, *P* < 0.001; model 2, *P* < 0.001; model 3, *P* < 0.001; model 4, *P* = 0.033). Conversely, when other confounding factors (BMI) were further adjusted (model 5), the results showed that there was no appreciable relationship between *H. pylori* infection and NAFLD (*P* = 0.574). Furthermore, Fig. 2 shows that in the subgroups stratified by sex (female: 0.991, *P* = 0.863; male: 1.039, *P* = 0.250), age (< 60: 1.028, *P* = 0.354; ≥ 60: 0.992, *P* = 0.910), BMI (< 25: 1.004, *P* = 0.926; ≥ 25: 1.045, *P* = 0.220), hypertension (No: 1.034, *P* = 0.280; Yes: 0.965, *P* = 0.563), diabetes (No: 1.018, *P* = 0.533; Male: 0.969, *P* = 0.759), and dyslipidemia

Table 3 The risk of NAFLD according to the infection of *H. pylori*

| | OR ^a | 95% CI | P value |
|---------|-----------------|-------------|---------|
| Model 0 | 1.196 | 1.147–1.247 | <0.001 |
| Model 1 | 1.160 | 1.110–1.212 | <0.001 |
| Model 2 | 1.129 | 1.079–1.181 | <0.001 |
| Model 3 | 1.112 | 1.062–1.165 | <0.001 |
| Model 4 | 1.055 | 1.004–1.109 | 0.033 |
| Model 5 | 1.016 | 0.962–1.072 | 0.574 |

Model 0 is unadjusted

Model 1 is adjusted for age, sex

Model 2 is further adjusted for SBP, DBP, Scr and BUN

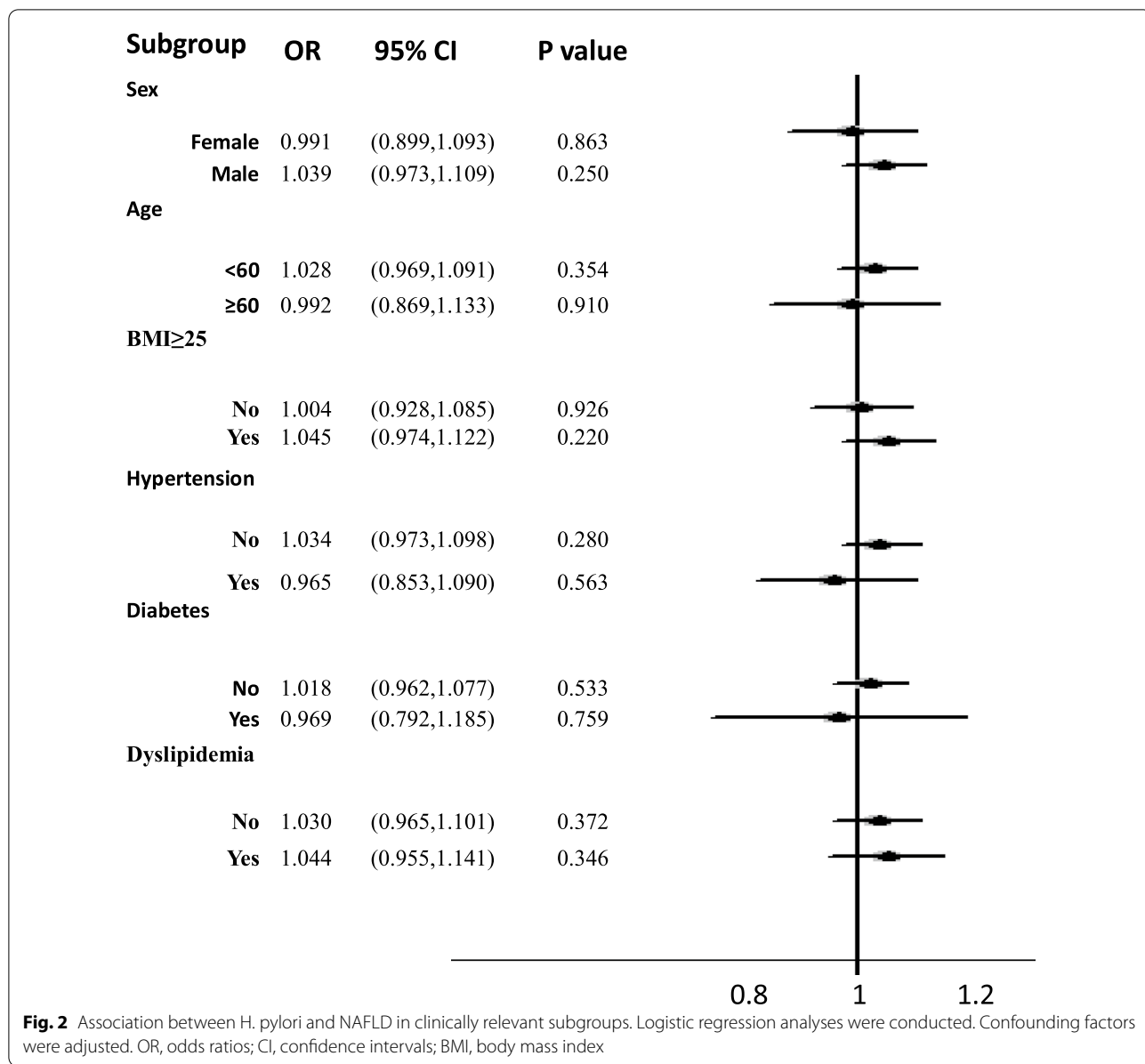
Model 3 is further adjusted for FBG and HbA1C

Model 4 is further adjusted for LDL-C, HDL-C, Triglycerides

Model 5 is further adjusted for BMI

^a Estimated from Logistic regression analysis

H. pylori, *Helicobacter pylori*; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; HbA1C, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Scr, serum creatinine; BUN, blood urea nitrogen; NAFLD, nonalcoholic fatty liver disease; N, number of subjects; OR, odd ratio; CI, confidence intervals



(No: 1.030, $P=0.372$; Yes: 1.044, $P=0.346$), no significant correlation between *H. pylori* positivity and an increased risk of NAFLD was detected.

Association between different grades of NAFLD and *H. pylori* infection

The relationship between *H. pylori* infection and different grades of NAFLD was further explored. The results showed that *H. pylori* infection is not an independent risk factor for the different grades of NAFLD (mild: OR = 1.014, $P=0.619$; moderate: OR = 0.976, $P=0.668$; severe: OR = 0.861, $P=0.602$) after adjusting

for confounding factors (Table 4). In addition, *H. pylori* infection was not a risk factor for liver function damage in patients with non-alcoholic fatty liver disease (AST, $P=0.911$; ALT, $P=0.237$; GGT, $P=0.776$) (Table 5). Regarding lipid metabolism, there were no significant differences in TC ($P=0.627$), LDL-C ($P=0.100$), and HDL-C ($P=0.746$) levels between the *H. pylori*+ and the *H. pylori*- groups among patients with NAFLD. *H. pylori* infection was not a risk factor for liver and kidney damages, abnormal carbohydrate metabolism, or abnormal lipid metabolism in patients with severe NAFLD ($P>0.05$).

Table 4 Odds ratios of *H. pylori* infection in different grades of NAFLD

| Variable | <i>H. pylori</i> – | <i>H. pylori</i> + | Crude OR (95% CI) | P | Adjust OR (95% CI) ^a | P |
|----------|--------------------|--------------------|---------------------|---------|---------------------------------|-------|
| NAFLD | 14,678 (31.30%) | 8617 (34.82%) | 1.172 (1.135–1.211) | < 0.001 | 1.016 (0.962–1.072) | 0.574 |
| No | 32,210 (68.70%) | 16,128 (65.17%) | 1.000 | | 1.000 | |
| Mild | 11,549 (24.63%) | 6711 (27.12%) | 1.161 (1.120–1.202) | < 0.001 | 1.014 (0.960–1.072) | 0.619 |
| Moderate | 3044 (6.49%) | 1852 (7.48%) | 1.215 (1.143–1.291) | < 0.001 | 0.976 (0.875–1.089) | 0.668 |
| Severe | 85 (0.18%) | 54 (0.21%) | 1.269 (0.902–1.785) | 0.172 | 0.861 (0.490–1.512) | 0.602 |

^a Adjusted for Age, sex, BMI, SBP, DBP, Scr, BUN, FBG, HbA1C, HDL-C, LDL-C, Triglycerides

H. pylori, *Helicobacter pylori*; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; HbA1C, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Scr, serum creatinine; BUN, blood urea nitrogen; NAFLD, nonalcoholic fatty liver disease; OR, odd ratio; CI, confidence intervals

Discussion

In this study, we aimed to explore the relationship between *H. pylori* infection and the risk of NAFLD in the Chinese population. Our results showed that 34.5% of the participants were infected with *H. pylori*, and that *H. pylori* positivity was not an independent risk factor for NAFLD after adjusting for age, sex, BMI, and other factors.

The results showed that the levels of BMI, blood pressure, FBG, HbA1c, total cholesterol, LDL-C, and triglycerides were significantly higher in the *H. pylori*+ group than in the negative group, while the level of HDL-C was lower. In recent years, research has been conducted on the association between *H. pylori* infection and unfavorable metabolic characteristics. In a cross-sectional study, 7417 participants were enrolled to assess the relevance between *H. pylori* infection and HbA1c levels. The results indicated that among adult participants without diabetes, *H. pylori* infection demonstrated significant positive correlation with HbA1c level [22]. In addition, a study of 3,578 subjects showed a positive correlation between *H. pylori* infection and metabolic syndrome, especially in women [23]. Although the mechanisms by which *H. pylori* infection relates to metabolic abnormalities have not been fully elucidated, intestinal microflora and their effects on the innate immune system, intestinal endothelial barrier function, production of intestinal metabolites, and fermentation of indigestible carbohydrates could be potential mechanisms.

NAFLD is considered a manifestation of liver metabolic syndrome. In this study, the *H. pylori* positivity rate was higher in the NAFLD group than in the healthy controls (37.9% vs. 33.8%). In recent years, an increasing number of studies have reported a relationship between *H. pylori* infection and NAFLD. A cross-sectional study reported that the infection rate of *H. pylori* in the NAFLD group was significantly higher than that in healthy controls [24]. Another cohort study showed that compared with participants without *H. pylori*

infection, *H. pylori* positive patients were at higher risk of developing NAFLD, independent of inflammatory and metabolic risk factors [13]. A recent meta-analysis indicated that in middle-aged individuals, *H. pylori* infection has obvious relevance with increased prevalence and incidence of NAFLD. The review further suggested that more prospective studies and studies investigating mechanisms are essential to better clarify the possible relationship between *H. pylori* infection and NAFLD [25]. Nevertheless, the correlation between them remains controversial. For example, a large retrospective study in Japan claimed that *H. pylori* positivity was not related to NAFLD [14]. Similarly, another retrospective cross-sectional study involving 4,030 Korean participants showed that *H. pylori* infection was not a risk factor for NAFLD based on the hepatic steatosis index [26]. Recently, another cross-sectional study found that there was no significant association between *H. pylori* infection and NAFLD, regardless of age, sex, BMI, and diabetes status [27]. These studies suggest that *H. pylori* may not play a vital role in the development of NAFLD. In our univariate analysis, *H. pylori* infection was an obvious risk factor for the development of NAFLD. Importantly, after adjusting for BMI, lipid profiles, FBG, and HbA1c levels, there was no independent correlation between them. These results suggest that BMI, lipid profiles, FBG, and HbA1c may mediate the association between *H. pylori* infection and NAFLD. *H. pylori* positivity was associated with higher BMI, dyslipidemia, and blood glucose levels, which were significant risk factors for NAFLD.

The existing controversy between *H. pylori* infection and NAFLD may be owing to the different individuals involved in sample populations, and various diagnostic methods used for *H. pylori* infection and NAFLD. For example, studies in Japan, South Korea, and China have proposed different conclusions [13, 26, 28]. These differences may be owing to different dietary patterns, lifestyles, and socioeconomic factors.

Table 5 The relationship between *H.pylori* infection and liver function, renal function, carbohydrate metabolism, lipid metabolism in nonalcoholic fatty liver patients

| Parameter (n, %) | NAFLD (23,295) | | | Mild-NAFLD (18,260) | | | Moderate-NAFLD (4896) | | | Severe-NAFLD (139) | | |
|-----------------------------|----------------------------|--------------------------|---------|----------------------------|--------------------------|---------|--------------------------|--------------------------|---------|------------------------|------------------------|---------|
| | <i>H.pylori</i> − (14,678) | <i>H.pylori</i> + (8617) | P value | <i>H.pylori</i> − (11,549) | <i>H.pylori</i> + (6711) | P value | <i>H.pylori</i> − (3044) | <i>H.pylori</i> + (1852) | P value | <i>H.pylori</i> − (85) | <i>H.pylori</i> + (54) | P value |
| | | | | | | | | | | | | |
| Liver function | | | | | | | | | | | | |
| AST increased | 893 (6.2%) | 529 (6.2%) | 0.911 | 467 (4.1%) | 261 (3.9%) | 0.582 | 382 (12.7%) | 217 (11.9%) | 0.393 | 20 (24.7%) | 11 (21.6%) | 0.833 |
| ALT increased | 4492 (30.7%) | 2572 (29.9%) | 0.237 | 2796 (24.3%) | 1456 (21.8%) | <0.001 | 1643 (54.2%) | 976 (52.9%) | 0.391 | 53 (63.1%) | 40 (75.5%) | 0.139 |
| GGT increased | 3580 (24.8%) | 2123 (25.0%) | 0.776 | 2456 (21.6%) | 1382 (20.9%) | 0.242 | 1093 (36.5%) | 677 (37.0%) | 0.712 | 31 (38.3%) | 17 (33.3%) | 0.584 |
| Renal function | | | | | | | | | | | | |
| Scr increased | 53 (0.4%) | 25 (0.3%) | 0.411 | 39 (0.3%) | 22 (0.3%) | 0.995 | 14 (0.5%) | 3 (0.2%) | 0.130 | 0 | 0 | - |
| BUN increased | 824 (5.6%) | 550 (6.4%) | 0.014 | 670 (5.8%) | 415 (6.2%) | 0.299 | 149 (4.9%) | 104 (5.6%) | 0.287 | 5 (6.0%) | 4 (7.4%) | 0.737 |
| Carbohydrate metabolism | | | | | | | | | | | | |
| FBG increased | 1097 (8.5%) | 810 (10.6%) | <0.001 | 727 (7.1%) | 532 (9.0%) | <0.001 | 361 (13.4%) | 262 (15.8%) | 0.033 | 9 (11.1%) | 4 (7.7) | 0.568 |
| HbA1C increased | 1286 (10.7%) | 930 (12.8%) | <0.001 | 880 (9.3%) | 611 (10.8%) | 0.003 | 395 (16.0%) | 311 (19.9%) | 0.002 | 11 (18.3%) | 8 (22.2%) | 0.792 |
| Lipid metabolism | | | | | | | | | | | | |
| Total cholesterol increased | 1533 (10.5%) | 918 (10.7%) | 0.627 | 1149 (10.0%) | 679 (10.1%) | 0.721 | 375 (12.3%) | 230 (12.4%) | 0.929 | 9 (10.7%) | 9 (16.7%) | 0.315 |
| LDL-C increased | 1114 (7.6%) | 706 (8.2%) | 0.100 | 849 (7.4%) | 540 (8.1%) | 0.087 | 259 (8.5%) | 163 (8.8%) | 0.714 | 6 (7.1%) | 3 (5.6%) | 1.000 |
| HDL-C decreased | 2177 (14.7%) | 1291 (15.0%) | 0.746 | 1500 (13.0%) | 873 (13.0%) | 0.964 | 652 (21.4%) | 398 (21.5%) | 0.943 | 25 (29.8%) | 20 (37.0%) | 0.457 |
| Triglycerides increased | 4953 (33.8%) | 3049 (35.4%) | 0.012 | 3433 (29.8%) | 2118 (31.6%) | 0.010 | 1484 (48.8%) | 902 (48.8%) | 0.976 | 36 (42.9%) | 29 (53.7%) | 0.226 |

H. pylori, *Helicobacter pylori*; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; HbA1C, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; Scr, serum creatinine; BUN, blood urea nitrogen; NAFLD, nonalcoholic fatty liver disease

All of these factors may affect the composition of the commensal gut microbiota. In some studies, the status of *H. pylori* infection was determined by ELISA using serum IgG antibodies against *H. pylori*. However, serological tests do not accurately discriminate between current and past infections [13]. In our current study and other studies, *H. pylori* infection was defined by the urea breath test, which demonstrates current infectivity [18]. Another aspect to consider is the diagnostic method used for NAFLD. In this study, NAFLD was defined by ultrasonography, while other studies defined NAFLD via biopsy or hepatic steatosis index [29]. Besides, a recent study showed that *H. pylori* infection contributes to the progression from NAFLD to non-alcoholic steatohepatitis (NASH) [28]. Taken together, the specific relationship between *H. pylori* infection and NAFLD is worth exploring in the future.

Our study has several limitations. First, this research was a cross-sectional study with its intrinsic restrictions. As such, only an association between *H. pylori* infection status and NAFLD could be suggested and not a cause–effect inference. Second, we used ultrasonography instead of liver biopsies to diagnose NAFLD, which is not sensitive enough to detect mild liver steatosis. However, because of its high sensitivity and specificity for hepatic steatosis examination, this non-invasive method is widely applied in both clinical practice and epidemiological research [30]. Third, a previous study showed that the prevalence of *H. pylori* infection was 66% in rural Chinese populations and 47% in urban Chinese populations, which is higher than that in our study (34.5%) [31]. We did not exclude patients with a history of *H. pylori* eradication or PPI medication owing to the limited clinical data in this study, which may have contributed to the false negatives. However, all included samples were the baseline results of the patient's first physical examination, which would have reduced the error to some extent. Fourth, although virulence factors of *H. pylori* may influence the degree of NAFLD, they were neglected because individuals undergoing routine health examinations were included in this retrospective study.

In conclusion, our study showed that *H. pylori* infection was not independently associated with NAFLD in China. Further cohort studies involving liver biopsies are required to determine whether *H. pylori* eradication helps to decrease the risk of NAFLD.

Abbreviations

H. pylori: *Helicobacter pylori*; BMI: Body mass index; BP: Blood pressure; FBG: Fasting blood glucose; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; Scr: Serum creatinine; BUN: Blood urea nitrogen; NAFLD: Nonalcoholic fatty liver disease;

¹³C-UBT: ¹³C urea breath test; PSM: Propensity score matching; TC: Total cholesterol; TG: Triglyceride; VIF: Variance inflation factor.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12876-022-02148-6>.

Additional file 1: Figure S1. Flow diagram of study.

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Authors' contributions

WW and FM performed most of the data collection/analysis and wrote the manuscript; G-R helped in most of the data collection, analysis and manuscript revision; Z-YR and Z-JC helped in some of the data collection and analysis; L-R, X-SP performed the project design, supervision, and manuscript revision. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data generated and analyzed in the current study are not publicly available because of appropriate protection of patient personal information but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Wuhan Union Hospital Ethics Committee and the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (IORG No: IORG0003571) and performed in accordance with the Declaration of Helsinki. The data are anonymous, and the requirement for informed consent was therefore waived.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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