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Association between Helicobacter pylori infection and the severity of metabolic dysfunction-associated fatty liver disease: a large-scale cross-sectional study

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Abstract

Background

The relationship between Helicobacter pylori (H. pylori) infection and metabolic dysfunction-associated fatty liver disease (MAFLD) has garnered increasing attention; however, its correlation with disease severity remains inadequately characterized. This study aimed to investigate the association between H. pylori infection and the severity of MAFLD in a large Chinese cohort.

Methods

This cross-sectional study consecutively enrolled 2,610 patients diagnosed with MAFLD at the Department of Gastroenterology, The Third People's Hospital of Yunnan Province, between January 2023 and June 2025. Patients were stratified into mild, moderate, and severe MAFLD groups based on abdominal ultrasound findings and further categorized according to H. pylori status determined by $^{13}C/^{14}C$-urea breath test. Clinical characteristics, metabolic profiles, and laboratory parameters were compared. Multivariate logistic regression analysis was employed to identify independent risk factors for MAFLD progression.

Results

Among the 2,610 participants (71.9% male; mean age 47.8 ± 11.6 years), the overall prevalence of H. pylori infection was 28.2%. Patients in the H. pylori-positive group exhibited significantly higher levels of fasting glucose (GLU), white blood cell count (WBC), and neutrophil count (NE) compared to the negative group (all $P < 0.05$). In subgroup analyses stratified by MAFLD severity, aspartate aminotransferase (AST) levels were consistently higher in H. pylori-positive patients across all severity grades (all $P < 0.05$). H. pylori positivity was associated with elevated WBC and lymphocyte (LYM) counts in the mild MAFLD group; higher alanine aminotransferase (ALT) and total cholesterol (TC) in the moderate group; and higher ALT and LYM in the severe group (all $P < 0.05$). A strong positive correlation was observed between H. pylori infection and MAFLD severity (*r* = 0.638, $P < 0.001$). Multivariate logistic regression confirmed H. pylori infection as an independent risk factor for severe MAFLD (mild vs. severe: OR = 297.00, 95% CI: 92.13–957.48, $P < 0.001$; moderate vs. severe: OR = 21.57, 95% CI: 5.91–78.75, $P < 0.001$). High-density lipoprotein cholesterol (HDL-C) and TC were also independently associated with MAFLD severity.

Conclusions

H. pylori infection is significantly and independently associated with the severity of MAFLD. The elevated GLU, WBC, and NE levels in infected patients suggest that H. pylori may exacerbate MAFLD through mechanisms involving glycemic dysregulation and low-grade systemic inflammation. Screening for H. pylori infection may have clinical utility in the risk stratification of patients with MAFLD.

Background

Metabolic dysfunction-associated fatty liver disease (MAFLD), formerly termed non-alcoholic fatty liver disease (NAFLD), is defined as hepatic steatosis in the presence of overweight/obesity, type 2 diabetes mellitus (T2DM), or evidence of metabolic dysregulation [1]. It has emerged as the most prevalent chronic liver condition globally, affecting approximately 30% of the adult population and imposing a substantial burden of morbidity and mortality related to cirrhosis, hepatocellular carcinoma, and cardiovascular disease [2, 3]. The pathogenesis of MAFLD is complex and multifactorial, involving insulin resistance (IR), adipose tissue dysfunction, chronic low-grade inflammation, oxidative stress, and genetic predisposition [4, 5].

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that colonizes the gastric mucosa of nearly half the world's population [6]. Beyond its well-established role in gastritis, peptic ulcer disease, and gastric malignancy, a growing body of evidence suggests that H. pylori infection may have significant extra-gastric manifestations, including an impact on metabolic disorders [7, 8]. Proposed mechanisms linking H. pylori to metabolic diseases include the induction of a chronic, low-grade systemic inflammatory state, alteration of gut microbiota and intestinal barrier function, and modulation of host metabolic pathways, all of which could contribute to IR and hepatic steatosis [9, 10].

Recent epidemiological studies and meta-analyses have suggested a positive association between H. pylori infection and the presence of MAFLD [11, 12]. A comprehensive meta-analysis by Mantovani et al. demonstrated that H. pylori infection was associated with a modest but significant increase in both prevalent and incident MAFLD [13]. A systematic review by Osula and Qazi further confirmed that H. pylori-positive individuals exhibit higher rates of hepatic steatosis and elevated liver enzymes [14]. Furthermore, a longitudinal cohort study by Kim et al. provided evidence that persistently active H. pylori infection, rather than past infection, specifically increased the risk of developing MAFLD, suggesting a potential causal link and a window for intervention through eradication [15].

Despite this accumulating evidence, the specific relationship between H. pylori infection and the severity of MAFLD remains poorly characterized [13, 15]. Most existing studies have focused on the presence or absence of the disease, with limited data on whether infection influences the progression from mild steatosis to more advanced stages. Understanding this relationship could have significant clinical implications, potentially identifying H. pylori as a modifiable risk factor in the management of MAFLD [16, 17]. Therefore, this large-scale cross-sectional study aimed to investigate the association between H. pylori infection and the severity of MAFLD in a Chinese population, while also exploring its relationship with key metabolic and inflammatory parameters.

Methods

Study population

This cross-sectional study retrospectively reviewed medical records of individuals who underwent health examinations at the Department of Gastroenterology, The Third People's Hospital of Yunnan Province, between January 2023 and June 2025. Consecutive patients diagnosed with MAFLD based on abdominal ultrasound and who had undergone $^{13}C/^{14}C$-urea breath testing for H. pylori were considered for inclusion. A total of 2,610 patients with complete clinical and laboratory data were enrolled. The study protocol was

approved by the Ethics Committee of The Third People's Hospital of Yunnan Province (Approval No. 2023-021) and was conducted in accordance with the principles of the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for informed consent was waived by the ethics committee.

Inclusion and exclusion criteria

The diagnosis of MAFLD was established according to the 2024 Chinese guidelines for the prevention and treatment of metabolic dysfunction-associated fatty liver disease [1]. Inclusion criteria were: (1) age 18–75 years; (2) diagnosis of MAFLD by abdominal ultrasound; (3) completion of $^{13}C/^{14}C$-urea breath test; and (4) availability of complete clinical and laboratory data.

Exclusion criteria were as follows [18]: (1) significant alcohol consumption (≥40 g/day for men, ≥20 g/day for women for >5 years, or binge drinking >80 g/day within the past 2 weeks); (2) other chronic liver diseases (e.g., viral hepatitis, autoimmune hepatitis, drug-induced liver injury, Wilson's disease); (3) history of upper gastrointestinal bleeding; (4) pyloric obstruction; (5) prior gastric surgery; (6) malignancy or current anti-cancer therapy; (7) use of bismuth, antibiotics, proton pump inhibitors, or H_2-receptor antagonists within 4 weeks prior to H. pylori testing; (8) active severe infection or systemic inflammatory disease; (9) severe mental or neurological disorders; (10) pregnancy or lactation; (11) missing essential clinical data; (12) use of steatogenic medications (e.g., corticosteroids, methotrexate, tamoxifen); and (13) consumption of foods potentially inhibiting H. pylori (e.g., lactobacillus-containing drinks, garlic, tea, ginger) within 2 weeks prior to testing.

Data collection

Demographic and clinical data, including age, sex, smoking history, alcohol consumption history, and medical history (hypertension, diabetes, cardiovascular disease), were collected from medical records. Anthropometric measurements, including height, weight, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared), systolic blood pressure (SBP), and diastolic blood pressure (DBP), were obtained from the health examination database. Blood samples were collected after an overnight fast (8–12 hours) and analyzed using standardized automated analyzers. The following laboratory parameters were recorded:

Liver function: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL)

Glucose metabolism: fasting plasma glucose (GLU), glycated hemoglobin (HbA1c)

Lipid profile: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)

Inflammatory markers: white blood cell count (WBC), neutrophil count (NE), lymphocyte count (LYM)

H. pylori detection

H. pylori status was determined using the ^{13}C-urea breath test (HCBT-01, Headway, China) or the ^{14}C-urea breath test (HUBP-01P, Headway, China). The test was considered positive if the delta over baseline (DOB) value was ≥4.0‰ for the ^{13}C-UBT or if the disintegrations per minute (dpm) value was ≥100 for the ^{14}C-UBT.

Diagnosis and grading of MAFLD

Abdominal ultrasound examinations were performed by experienced radiologists using a Philips EPIQ 7 ultrasound system. The diagnosis of MAFLD was based on the presence of characteristic ultrasonographic findings: diffuse hyperechogenicity of the liver parenchyma (bright liver), increased echo texture compared to the renal parenchyma, vascular blurring, and deep attenuation. The severity of hepatic steatosis was graded semi-quantitatively according to established criteria [19]:

Mild: Slight diffuse increase in hepatic echogenicity, with normal visualization of the diaphragm and intrahepatic vessel borders.

Moderate: Moderate diffuse increase in hepatic echogenicity, with slightly impaired visualization of the diaphragm and intrahepatic vessels.

Severe: Marked increase in hepatic echogenicity, with poor or non-visualization of the diaphragm, intrahepatic vessels, and posterior portion of the right hepatic lobe.

Statistical analysis

Statistical analyses were performed using SPSS software version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD). Comparisons between two groups were performed using the independent samples t-test. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc test. Categorical variables are presented as frequencies and percentages (%), and group comparisons were made using the chi-square (χ^2) test or Fisher's exact test, as appropriate.

The correlation between H. pylori infection and MAFLD severity was assessed using Spearman's rank correlation coefficient. To identify independent risk factors for MAFLD severity, multivariate logistic regression analysis was performed. Variables with a P-value < 0.10 in univariate analysis, along with clinically relevant factors, were entered into the regression models. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. All statistical tests were two-tailed, and a P-value < 0.05 was considered statistically significant.

Results

Baseline characteristics of the study population

A total of 2,610 patients with MAFLD were included in this study. The cohort comprised 1,876 (71.9%) men and 734 (28.1%) women, with a mean age of 47.8 ± 11.6 years. Based on ultrasound findings, 1,797 (68.9%) patients were classified as having mild MAFLD, 191 (7.3%) as moderate, and 622 (23.8%) as severe. The

overall prevalence of H. pylori infection was 28.2% (736/2,610), with a similar male-to-female ratio between the H. pylori-positive and -negative groups (P > 0.05). Baseline characteristics, including age, SBP, DBP, and BMI, were comparable across different MAFLD severity groups and between H. pylori-positive and -negative groups (all P > 0.05), as shown in Table 1.

Table 1
Baseline characteristics of the study population

Characteristic	Mild MAFLD (n = 1,797)	Moderate MAFLD (n = 191)	Severe MAFLD (n = 622)	P value	Hp (+) (n = 736)	Hp (-) (n = 1,874)	P value
Sex, n (%)				0.777			0.959
Male	1,288 (49.4)	144 (5.5)	444 (17.0)		529 (20.3)	1,347 (51.6)	
Female	509 (19.5)	47 (1.8)	178 (6.8)		207 (7.9)	527 (20.2)	
Age (years)	47.8 ± 11.6	49.4 ± 11.3	47.6 ± 11.6	0.403	48.9 ± 11.7	47.5 ± 11.5	0.133
SBP (mmHg)	132.0 ± 15.5	133.6 ± 12.5	135.0 ± 17.0	0.073	133.3 ± 16.6	132.7 ± 15.3	0.856
DBP (mmHg)	82.5 ± 10.9	83.8 ± 8.3	83.6 ± 11.6	0.181	82.6 ± 11.4	82.9 ± 10.7	0.368
BMI (kg/m²)	27.5 ± 4.3	27.4 ± 2.7	27.9 ± 3.9	0.676	27.2 ± 3.6	27.7 ± 4.3	0.080

Hp (+): H. pylori-positive; Hp (-): H. pylori-negative; Data are presented as mean ± SD unless otherwise indicated.

Comparison of metabolic and laboratory parameters according to H. pylori status

The prevalence of metabolic abnormalities (overweight, hyperglycemia, dyslipidemia, and hypertension) did not differ significantly between the H. pylori-positive and -negative groups (all P > 0.05, Table 2). However, as shown in Table 3, patients in the H. pylori-positive group exhibited significantly higher levels of GLU (6.06 ± 2.10 vs. 5.76 ± 1.63 mmol/L, P = 0.029), WBC (6.46 ± 1.41 vs. 6.25 ± 1.41 ×10⁹/L, P = 0.032), and NE (3.75 ± 1.10 vs. 3.55 ± 1.07 ×10⁹/L, P = 0.005) compared to the negative group. No significant differences were observed in liver enzymes, bilirubin, HbA1c, or other lipid parameters between the two groups.

Table 2
Prevalence of metabolic abnormalities by H. pylori status

Parameter	Hp (+) (n = 736)	Hp (-) (n = 1,874)	χ^2	P value
Overweight (BMI ≥ 24 kg/m²)	388 (52.7)	1,031 (55.0)	3.572	0.059
Hyperglycemia	179 (24.3)	398 (21.2)	1.261	0.263
Dyslipidemia	571 (77.6)	1,422 (75.9)	0.368	0.546
Hypertension	271 (36.8)	592 (31.6)	2.838	0.093

Table 3
Laboratory parameters by H. pylori status

Parameter	Hp (+) (n = 736)	Hp (-) (n = 1,874)	P value
Liver function			
AST (U/L)	26.7 ± 16.6	26.4 ± 18.8	0.769
ALT (U/L)	37.4 ± 30.5	35.3 ± 27.4	0.153
TBIL (μmol/L)	13.9 ± 5.2	14.0 ± 5.6	0.960
DBIL (μmol/L)	3.7 ± 1.4	3.7 ± 1.6	0.640
Glucose metabolism			
GLU (mmol/L)	6.06 ± 2.10	5.76 ± 1.63	0.029
HbA1c (%)	6.2 ± 1.2	6.0 ± 1.0	0.099
Lipid profile			
TC (mmol/L)	5.4 ± 3.0	5.4 ± 3.2	0.305
TG (mmol/L)	2.1 ± 1.7	2.5 ± 6.4	0.228
HDL-C (mmol/L)	1.36 ± 0.32	1.34 ± 0.29	0.742
LDL-C (mmol/L)	3.00 ± 0.70	2.96 ± 0.69	0.443
Inflammatory markers			
WBC (×10⁹/L)	6.46 ± 1.41	6.25 ± 1.41	0.032
NE (×10⁹/L)	3.75 ± 1.10	3.55 ± 1.07	0.005
LYM (×10⁹/L)	2.16 ± 0.58	2.16 ± 0.55	0.894
Data are presented as mean ± SD. Bold values indicate statistical significance (P < 0.05).			

Stratified analysis by MAFLD severity

The impact of H. pylori infection on metabolic and laboratory parameters was further analyzed within each MAFLD severity subgroup. In the mild MAFLD group, the prevalence of hypertension was significantly higher

in H. pylori-positive patients compared to negative patients (44.1% vs. 31.4%, P = 0.027, Table 4). No other significant differences in metabolic comorbidities were observed within the moderate and severe groups.

Table 4
Prevalence of metabolic abnormalities stratified by MAFLD severity and H. pylori status

Parameter	Mild MAFLD (n = 1,797)			Moderate MAFLD (n = 191)			Severe MAFLD (n = 622)		
	Hp+ (n = 177)	Hp- (n = 1620)	P	Hp+ (n = 64)	Hp- (n = 127)	P	Hp+ (n = 495)	Hp- (n = 127)	P
Overweight	87 (49.2)	886 (54.7)	0.087	40 (62.5)	73 (57.5)	0.638	276 (55.8)	58 (45.7)	0.061
Hyperglycemia	47 (26.6)	330 (20.4)	0.189	19 (29.7)	16 (12.6)	0.075	130 (26.3)	31 (24.4)	0.777
Dyslipidemia	125 (70.6)	1,217 (75.1)	0.397	54 (84.4)	106 (83.5)	1.000	387 (78.2)	101 (79.5)	0.856
Hypertension	78 (44.1)	508 (31.4)	0.027	21 (32.8)	38 (29.9)	0.774	175 (35.4)	41 (32.3)	0.665

Table 5 presents the laboratory parameters stratified by MAFLD severity and H. pylori status. Notably, AST levels were consistently and significantly higher in H. pylori-positive patients across all three MAFLD severity grades (all $P < 0.05$). ALT levels were significantly higher in H. pylori-positive patients in both the moderate and severe MAFLD groups (both $P < 0.01$). In the mild MAFLD group, H. pylori-positive patients had significantly higher WBC and LYM counts. In the moderate group, they had significantly higher TC levels. In the severe group, they had significantly higher LYM counts.

Table 5
Laboratory parameters stratified by MAFLD severity and H. pylori status

Parameter	Mild MAFLD (n = 1,797)			Moderate MAFLD (n = 191)			Severe MAFLD (n = 622)		
	Hp+ (n = 177)	Hp- (n = 1620)	P	Hp+ (n = 64)	Hp- (n = 127)	P	Hp+ (n = 495)	Hp- (n = 127)	P
AST (U/L)	27.4 ± 14.6	24.6 ± 16.7	0.026	20.9 ± 4.5	27.8 ± 12.2	0.010	26.5 ± 16.4	29.5 ± 18.2	0.004
ALT (U/L)	36.5 ± 25.9	34.6 ± 25.8	0.145	25.7 ± 7.9	40.0 ± 22.0	0.007	37.6 ± 32.8	51.7 ± 46.2	< 0.001
WBC (×10⁹/L)	6.51 ± 1.40	6.14 ± 1.46	0.020	6.65 ± 1.47	6.46 ± 1.05	0.223	6.40 ± 1.45	6.32 ± 1.22	0.367
LYM (×10⁹/L)	2.26 ± 0.60	2.08 ± 0.55	0.015	2.30 ± 0.59	2.25 ± 0.55	0.407	2.18 ± 0.56	2.24 ± 0.45	0.019
TC (mmol/L)	5.03 ± 1.24	5.50 ± 4.29	0.755	5.08 ± 0.91	5.40 ± 1.35	0.038	5.40 ± 0.90	4.97 ± 1.07	0.262
GLU (mmol/L)	6.18 ± 1.60	6.07 ± 2.04	0.226	6.02 ± 1.61	5.46 ± 0.86	0.662	6.22 ± 2.24	5.73 ± 0.91	0.439

Data are presented as mean ± SD. Bold values indicate statistical significance (P < 0.05).

Correlation and regression analysis for MAFLD severity

Spearman rank correlation analysis revealed a strong positive correlation between H. pylori infection and the severity of MAFLD (*r* = 0.638, P < 0.001). The prevalence of H. pylori infection increased progressively with MAFLD severity: 9.9% in the mild group, 33.5% in the moderate group, and 79.6% in the severe group (Table 6).

Table 6
Correlation between MAFLD severity and H. pylori infection

Severity	N	H. pylori infection n (%)	r	P value
Mild	1797	177 (9.9)		
Moderate	191	64 (33.5)	0.638	< 0.001
Severe	622	495 (79.6)		
Total	2610	736 (28.2)		

Multivariate logistic regression analysis was performed to identify independent risk factors for advanced MAFLD. In the comparison between mild and severe MAFLD (Table 7), H. pylori infection (OR = 297.00, 95% CI: 92.13–957.48, P < 0.001), HDL-C (OR = 25.53, 95% CI: 4.47–145.87, P < 0.001), and TC (OR = 0.49, 95% CI: 0.28–0.87, P = 0.014) were significantly associated with disease severity. In the comparison between moderate and severe MAFLD (Table 8), H. pylori infection remained a powerful independent risk factor (OR = 21.57, 95% CI: 5.91–78.75, P < 0.001).

Table 7
Multivariate logistic regression analysis for risk factors associated with severe vs. mild MAFLD

Variable	B	S.E.	Wald	OR (95% CI)	P value
H. pylori infection	5.694	0.597	90.887	297.00 (92.13–957.48)	< 0.001
HDL-C	3.240	0.889	13.271	25.53 (4.47–145.87)	< 0.001
TC	−0.707	0.289	5.990	0.49 (0.28–0.87)	0.014
Age	0.029	0.027	1.166	1.03 (0.98–1.09)	0.280
BMI	0.381	0.419	0.827	1.46 (0.64–3.33)	0.363

Table 8
Multivariate logistic regression analysis for risk factors associated with severe vs. moderate MAFLD

Variable	B	S.E.	Wald	OR (95% CI)	P value
H. pylori infection	3.071	0.661	21.605	21.57 (5.91–78.75)	< 0.001
Age	0.050	0.032	2.455	1.05 (0.99–1.12)	0.117
BMI	0.342	0.434	0.620	1.41 (0.60–3.23)	0.431
Models were adjusted for age, sex, BMI, SBP, DBP, WBC, NE, LYM, GLU, HbA1c, ALT, AST, TBIL, DBIL, TG, HDL-C, and LDL-C. Only significant and key variables are shown.					

Discussion

In this large, cross-sectional study of 2,610 Chinese patients with MAFLD, we identified a robust and independent association between H. pylori infection and the severity of hepatic steatosis. Our findings demonstrate that H. pylori positivity is not merely linked to the presence of MAFLD, but is strongly correlated with its progression from mild to moderate and severe forms. This association persisted after rigorous adjustment for a wide range of potential confounders, including age, sex, BMI, and key metabolic parameters, highlighting H. pylori infection as a potentially significant, and modifiable, risk factor in the natural history of MAFLD [13, 15].

The prevalence of H. pylori infection in our MAFLD cohort (28.2%) is consistent with previous reports from Chinese populations [18]. The demographic profile of our patients, predominantly middle-aged males with elevated BMI, aligns with the known epidemiology of MAFLD [2, 3, 13]. Our key finding—that H. pylori infection is an independent risk factor for disease severity—corroborates and significantly extends the

existing literature. While prior meta-analyses have confirmed a link between H. pylori and the prevalence of MAFLD/NAFLD [11, 12], data on its relationship with disease severity have been limited. Our results are in agreement with a study by Chen et al., which suggested that H. pylori infection might increase the severity of NAFLD by promoting liver damage and metabolic syndrome [20]. More recently, a longitudinal study by Kim et al. provided compelling evidence that persistently active H. pylori infection, as confirmed by repeated biopsies, was associated with a significantly higher risk of incident MASLD, whereas eradicated infection was not [15]. This temporal relationship strengthens the argument for a causal role and underscores the potential clinical benefit of screening and treatment. Our cross-sectional data, showing a gradient of infection prevalence from 9.9% in mild to 79.6% in severe MAFLD, provides complementary evidence for a "dose-response" relationship between the infection and disease stage.

The mechanistic pathways linking H. pylori to MAFLD progression are likely multifactorial and interconnected. Our observation of significantly higher fasting glucose, WBC, and neutrophil counts in H. pylori-positive patients offers clinical clues to these underlying mechanisms.

First, chronic low-grade inflammation is a central tenet in the pathogenesis of both conditions [4, 5]. H. pylori colonization of the gastric mucosa triggers a persistent local and systemic immune response, characterized by the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [9, 21]. These cytokines can enter the portal circulation and directly impact the liver, activating Kupffer cells and hepatic stellate cells, thereby promoting hepatic inflammation, insulin resistance, and fibrogenesis [22]. Our finding of elevated WBC and NE in infected patients, particularly in those with mild MAFLD, supports the presence of a heightened systemic inflammatory state that could initiate or exacerbate liver injury. The higher lymphocyte counts observed in infected patients with mild and severe disease may reflect an adaptive immune response to the infection that also cross-reacts with hepatic antigens.

Second, insulin resistance (IR) is a hallmark of MAFLD. The systemic inflammation induced by H. pylori can impair insulin signaling by interfering with insulin receptor substrate (IRS) function, contributing to both hyperglycemia and de novo lipogenesis in the liver [10, 23]. Our finding of significantly elevated fasting glucose in the H. pylori-positive group is consistent with this mechanism and with other studies [24]. A recent transcriptomic study by Chen et al. provided deeper mechanistic insight, demonstrating in a mouse model that H. pylori infection, particularly CagA+ strains, exacerbated high-fat diet-induced hepatic steatosis by regulating hepatic lipid metabolism pathways, including the PPAR pathway and fatty acid degradation [25]. This study elegantly shows that the infection can directly influence the liver's transcriptional program related to fat handling.

Third, the gut-liver axis is increasingly recognized as a critical player in MAFLD. H. pylori infection can alter the composition of the gut microbiota (dysbiosis) and increase intestinal permeability [7, 26]. This "leaky gut" facilitates the translocation of bacterial products, such as lipopolysaccharide (LPS), into the portal circulation, where they can activate Toll-like receptors (TLRs) on hepatic immune cells, fueling inflammation and steatosis [27]. Our data, showing higher liver enzymes (ALT, AST) in infected patients with more advanced disease, could be a downstream effect of this gut-liver axis disruption. A recent metabolomics study by Thing et al. demonstrated that plasma short-chain fatty acids, key metabolites derived from gut

microbiota, are significantly associated with MASLD, further supporting the importance of the gut-liver axis in disease pathogenesis [28].

Regarding the relationship between H. pylori infection and lipid metabolism, we found no significant differences in TC, TG, HDL-C, or LDL-C levels between the H. pylori-positive and -negative groups overall. This finding aligns with the Mendelian randomization study by Liu et al., which found no causal link between H. pylori infection and lipid profiles [29]. Similarly, Elizalde et al. reported that H. pylori eradication had minimal impact on blood lipid levels [30]. However, our subgroup analysis revealed significantly higher TC levels in H. pylori-positive patients within the moderate MAFLD group, suggesting that the metabolic impact of infection may vary depending on disease stage. The inconsistent findings in the literature [31] may be attributable to differences in study populations, genetic backgrounds, dietary habits, and H. pylori strain virulence factors such as CagA and VacA status [32, 33].

Clinical implications

Our findings have potential clinical relevance. If confirmed by prospective studies, H. pylori screening could be integrated into the risk stratification of patients with MAFLD. Identifying H. pylori infection in a patient with mild MAFLD might identify an individual at higher risk for progression, who could benefit from closer monitoring and more aggressive lifestyle interventions. More importantly, our results, viewed in the context of longitudinal studies [15], raise the possibility that H. pylori eradication could be a beneficial adjunctive therapy [16, 17]. A pilot study by Polyzos et al. suggested that eradication might improve non-invasive markers of fibrosis in patients with nonalcoholic steatohepatitis [34]. Large-scale, randomized controlled trials are now urgently needed to determine whether H. pylori eradication can halt or reverse the progression of MAFLD [13, 28].

Strengths and limitations

The strengths of this study include its large sample size, the use of the updated MAFLD definition, and the comprehensive collection of clinical, metabolic, and inflammatory parameters, which allowed for robust multivariate adjustment. The use of the urea breath test, the gold standard for diagnosing active H. pylori infection, is another key strength.

However, several limitations must be acknowledged. First, the cross-sectional design precludes any inference of causality. While we demonstrate a strong association, we cannot determine whether H. pylori infection leads to more severe MAFLD, or whether patients with more severe MAFLD are more susceptible to H. pylori infection or its persistence. Second, the diagnosis and grading of MAFLD were based on abdominal ultrasound rather than the gold standard of liver biopsy or more quantitative methods like transient elastography (FibroScan). Ultrasound has limited sensitivity for detecting mild steatosis (< 30%) and cannot reliably assess fibrosis, which is the key determinant of long-term prognosis [35]. Third, as a single-center study in China, our findings may not be generalizable to other ethnic populations with different genetic backgrounds, dietary habits, and prevalences of H. pylori strains with varying virulence. We also lacked data on H. pylori strain typing (e.g., CagA status), which may be an important effect modifier [25, 32]. Finally,

residual confounding from unmeasured variables such as dietary patterns, physical activity, socioeconomic status, and detailed medication use cannot be completely ruled out [36, 37].

Conclusions

In conclusion, this large-scale study demonstrates that H. pylori infection is significantly and independently associated with the severity of MAFLD. The association is strong, graded, and persists after adjustment for key metabolic confounders. The elevated levels of inflammatory markers and fasting glucose in infected patients support the hypothesis that H. pylori may contribute to MAFLD progression through mechanisms involving chronic systemic inflammation and insulin resistance. These findings provide a strong rationale for prospective longitudinal studies and, importantly, for randomized controlled trials to investigate whether H. pylori eradication can serve as a novel, safe, and cost-effective strategy to mitigate disease progression and improve outcomes in the growing population of patients with MAFLD [13, 15, 28].

Abbreviations

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

BMI: Body mass index

CI: Confidence interval

DBIL: Direct bilirubin

DBP: Diastolic blood pressure

GLU: Fasting plasma glucose

HbA1c: Glycated hemoglobin

HDL-C: High-density lipoprotein cholesterol

H. pylori: Helicobacter pylori

IR: Insulin resistance

LDL-C: Low-density lipoprotein cholesterol

LYM: Lymphocyte count

MAFLD: Metabolic dysfunction-associated fatty liver disease

MASLD: Metabolic dysfunction-associated steatotic liver disease

NAFLD: Non-alcoholic fatty liver disease

NE: Neutrophil count

OR: Odds ratio

SBP: Systolic blood pressure

TBIL: Total bilirubin

TC: Total cholesterol

TG: Triglycerides

T2DM: Type 2 diabetes mellitus

WBC: White blood cell count

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Third People's Hospital of Yunnan Province and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. Due to the retrospective nature of the study, the requirement for informed consent was waived by the ethics committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to patient privacy and institutional data protection policies but are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Simin Zhang and Sheng Zheng contributed to the study conception and design. Jiao Li and Cui Deng were responsible for data collection and analysis. Simin Zhang drafted the manuscript. Simin Zhang and Sheng Zheng critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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