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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

A cohort study on *Helicobacter pylori* infection associated with nonalcoholic fatty liver disease

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Abstract

Background Previous studies have suggested a link between *Helicobacter pylori* (*H. pylori*) infection and nonalcoholic fatty liver disease (NAFLD), yet large-scale longitudinal studies are lacking to elucidate this association.

Methods A cohort study of 17,028 adults without NAFLD at baseline, who participated in a repeated health screening examination including an *H. pylori*-specific immunoglobulin G antibody test, was conducted to evaluate the association between *H. pylori* and NAFLD development. Fatty liver was diagnosed by ultrasonography.

Results During the 83,130 person-years follow-up, participants with *H. pylori* infection had a higher rate of incident NAFLD than those who were uninfected. In a multivariable model adjusted for age, sex, body mass index, smoking status, alcohol intake, regular exercise, year of screening exam, and education level, the hazard ratio (HR) for NAFLD development in participants with *H. pylori* infection compared to those without infection was 1.21 [95% confidence interval (CI), 1.10–1.34]. The association persisted after further adjustment for metabolic variables, inflammatory marker, and liver enzymes. The association between *H. pylori* and NAFLD was still evident in an analysis using fatty liver index as a surrogate marker of NAFLD. In addition, the association between *H. pylori* infection and incident NAFLD did not differ across clinically relevant subgroups evaluated.

Conclusions *H. pylori* infection was significantly associated with the development of NAFLD, independent of metabolic and inflammatory risk factors. *H. pylori* infection may play a pathophysiologic role in NAFLD development, indicating that *H. pylori* eradication might play a role in reducing the risk of NAFLD.

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Keywords Nonalcoholic fatty liver disease · *Helicobacter pylori* · Cohort study · Incidence

Introduction

Helicobacter pylori (*H. pylori*) colonizes the stomach of at least half the world's population and is a key constituent of the human microbiome [1]. Infection is usually acquired early in life and persists throughout the host's life if untreated [2]. Clinical manifestations of *H. pylori* infection include peptic ulcer disease, gastric mucosa-associated lymphoid tissue, and non-cardia gastric adenocarcinoma [3]. *H. pylori* infection not only affects the stomach, but is also linked to a number of extra-gastric diseases, indicating that *H. pylori* may cause disease by a different biologic process far from the primary site of infection [4]. Notably, a number of studies showed the association between *H. pylori* infection and cardiovascular disease, type 2 diabetes mellitus, and metabolic syndrome [5–9].

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive fat infiltration of the liver in absence of significant alcohol intake or secondary causes for steatosis [10]. The clinical consequence of NAFLD is not limited to liver-related morbidity and mortality, but is also associated with cardiovascular disease, type 2 diabetes, and metabolic syndrome [11, 12]. The incidence of NAFLD is rapidly increasing, with huge clinical and economic burdens [13]. Identifying risk factors with potential therapeutic implications is important in managing NAFLD and decreasing these burdens.

Development of NAFLD is a complex process that includes genetic susceptibility and environmental exposures [14]. *H. pylori* infection has been suggested to play a role in NAFLD development with conflicting results [15–20]. A recent large-scale cross-sectional study of 13,737 adults reported that *H. pylori* is not associated with NAFLD [21]. In contrast, another study revealed that *H. pylori* infection was more frequently observed in 28 biopsy-proven NAFLD patients than in 25 healthy controls [19]. Large-scale, longitudinal studies are needed to elucidate the association between *H. pylori* infection and NAFLD. If proven to be a significant risk factor for NAFLD, *H. pylori* infection has a therapeutic potential, as it can be eradicated in most patients [22]. Therefore, we longitudinally studied the association between *H. pylori* infection and NAFLD in a large cohort of asymptomatic men and women.

Methods

Study population

This retrospective cohort study included healthy adults, aged 20 years or older, who participated in a comprehensive health-screening exam at the Center for Health Promotion of the Samsung Medical Center, South Korea, from January 2005 and December 2013 (Fig. 1). Since our objective was to evaluate the longitudinal association between *H. pylori* infection and NAFLD, we included subjects who underwent at least two screening exams, including abdominal ultrasonography (US), to assess fatty liver status and establish baseline *H. pylori* infection status ($n = 27{,}914$). We excluded 9778 participants who met any of the following exclusion criteria: fatty liver on baseline

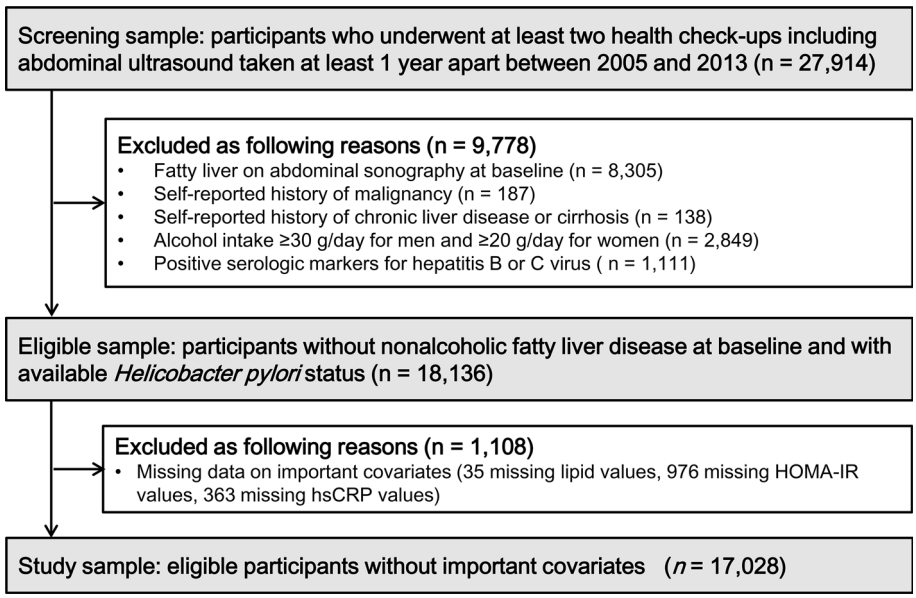

Fig. 1 Flow diagram of study participants

abdominal US ($n = 8305$); self-reported history of malignancy ($n = 187$); self-reported history of chronic liver disease or cirrhosis ($n = 138$); alcohol intake ≥30 g/day for men and ≥20 g/day for women ($n = 2849$); positive serologic markers for hepatitis B or C virus ($n = 1111$). We then excluded 1108 participants with missing data on important covariates, including the following metabolic and inflammatory parameters: 35 missing lipid values, 976 missing homeostasis model assessment of insulin resistance (HOMA-IR) values, 363 missing high-sensitivity C-reactive protein (hsCRP) values. Finally, 17,028 healthy participants without fatty liver at baseline and with available *H. pylori* status were analyzed. This study was approved by the Institutional Review Board of the Samsung Medical Center, and waived the requirement for informed consent, as we used only de-identified data routinely collected during health screening visits.

Data collection

The comprehensive health-screening program included demographic characteristics, anthropometric measurements, detailed physical examination, serum biochemical measurements, and a self-administered health questionnaire on smoking, alcohol consumption, physical activity, medication use, and personal medical history including diabetes mellitus, hypertension, dyslipidemia, and malignancy. Smoking status was categorized as never, former, or current smoker. Alcohol consumption was divided into mild (≤10 g/day) and modest (>10 g/day). Regular exercise was three or more times per week with moderately intense physical activity.

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, using an Inbody 720 machine (Biospace, Seoul, Korea) while wearing light clothing and with bare feet. Body mass index (BMI) was calculated as weight in kilograms/height in square meters (kg/m^2). Blood pressure was measured in the seated position after >5 min of quiet rest using an automated blood pressure monitor (Dinamap PRO 100; GE Healthcare, Milwaukee, WI, USA).

After a ≥12 h fast, blood samples were collected in the morning and analyzed by the hospital clinical laboratory. Serum levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured with a Hitachi 7600 (Hitachi, Tokyo, Japan) using enzymatic colorimetric and liquid-selective detergent methods. Serum insulin levels were measured using a radioimmunoassay method with the Packard Cobra II 5010 (Packard Instrument, Baltimore, MD, USA). Serum glucose levels were measured using the hexokinase/glucose-6-phosphate dehydrogenase method with a Hitachi 7600 Modular Dp-110 autoanalyzer (Hitachi, Tokyo, Japan). Serum hsCRP concentrations were measured using an immunoturbidimetric assay (CRPL3, Roche Diagnostics, Indianapolis, IN, USA). The inter- and intra-assay coefficients of variation for quality control specimens were <5% for blood variables. Serum immunoglobulin G (IgG) antibody to *H. pylori* was measured using an enzyme-linked immunosorbent assay (ELISA) (GAP test IgG kit, Bio-Rad Laboratories Inc., Hercules, CA, USA). Experienced radiologists unaware of the study aims performed the abdominal ultrasonography.

Variables

The primary outcome was incident NAFLD at follow-up, defined by US and exclusion for secondary causes. The US diagnosis of fatty liver was based on standard criteria, including diffuse increased echogenicity of liver parenchyma, liver-to-kidney contrast, deep beam attenuation, and bright vessel walls [23]. Since we excluded participants with excessive alcohol use and/or other chronic liver disease, cases of incident fatty liver were considered NAFLD. Exposure was *H. pylori* infection status, defined by positive ELISA using serum IgG antibody to *H. pylori*. Time was calculated from baseline to NAFLD development or last follow-up, whichever came first. For potential confounders, age, sex, BMI, smoking status, alcohol use, regular exercise, year of screening exam, and education level were collected. For potential confounders or mediators, we collected further metabolic and inflammatory variables, and liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), hsCRP, fasting blood glucose, triglycerides, LDL-C and HDL-C, and HOMA-IR calculated as [fasting insulin (μU/ml) fasting glucose (mg/dl)]/405 [24].

Statistical analysis

We used Cox regression models to estimate adjusted hazard ratios (aHRs) with 95% confidence intervals (CIs) for incident NAFLD associated with baseline *H. pylori* seropositivity. Multivariable models were initially adjusted for age (/year) and sex, and then further adjusted for potential confounding factors including year of screening exam, BMI (/kg/m^2), smoking status (never vs. past vs. current smoker), alcoholic intake (mild vs. modest), regular exercise (yes vs. no), and education level (elementary school or less vs. middle or high school vs. college or higher). Finally, we fitted additional models adjusted for inflammatory and metabolic factors, and liver function factors that could be potential confounders or mediators of the association between *H. pylori* infection and incident

NAFLD. Model 1 was adjusted for the confounding factors and hsCRP (/mg/dl). Model 2 was further adjusted for HOMA-IR (continuous). Model 3 was adjusted for model 2 plus fasting blood glucose (/mg/dl), triglycerides (/mg/dl), LDL-C (/mg/dl), and HDL-C (/mg/dl). Finally, model 4 was adjusted for model 3 plus AST (/U/l), ALT (/U/l), and GGT (/U/l).

and hsCRP (<0.1 vs. ≥0.1 mg/dl). Interactions between subgroups were tested using likelihood ratio tests comparing models with and without multiplicative interaction terms.

We also examined the association of *H. pylori* infection with fatty liver index (FLI) as a surrogate marker of NAFLD [28, 29]. The FLI was calculated as follows:

$$\mathrm{FLI} = \frac{(\mathrm{e}0.953 * \mathrm{loge}\ (\mathrm{triglycerides}) + 0.139 * \mathrm{BMI}\ + 0.718 * \mathrm{loge}\ (\mathrm{ggt}) + 0.053 * \text{waist circumference}\ -15.745)}{(1 + \mathrm{e}0.953 * \mathrm{loge}\ (\mathrm{triglycerides}) + 0.139 * \mathrm{BMI} + 0.718 * \mathrm{loge}\ (\mathrm{ggt}) + 0.053 * \text{waist circumference} - 15.745)} \times 100$$

To identify significant factors among the potential mediators, we calculated the mediation effect of metabolic parameters on the association between *H. pylori* infection and the risk of NAFLD, if metabolic abnormalities met the following three criteria for being a potential mediator: (1) *H. pylori* infection was associated with metabolic abnormalities, (2) *H. pylori* infection was significantly associated with the risk of NAFLD, controlling for the metabolic variables, (3) metabolic abnormalities must be significantly related to the risk of NAFLD, and (4) the addition of metabolic variables to the regression model both attenuated the association between *H. pylori* infection and risk of NAFLD. In addition, we calculated the attenuation of excess risk after adjustment for metabolic variables and inflammatory markers.

We defined the percentage of excess risk mediated (PERM) [25–27] with HRs as follows:

$$\mathrm{PERM} = [\mathrm{HR}_{(\text{confounder adjusted})} - \mathrm{HR}_{(\text{confounder and mediator adjusted})}] / [\mathrm{HR}_{(\text{confounder adjusted})}] - 1 \times 100$$

From this equation, we estimated the attenuation or indirect effect of the mediators on NAFLD risk. To estimate the empirical distribution of PERM, we randomly generated 5000 datasets using the bootstrap method and calculated PERM [$\mathrm{HR}_{(\text{confounder adjusted})}$, $\mathrm{HR}_{(\text{confounder and mediator adjusted})}$] by fitting Cox proportional hazard models for each dataset. Finally, we used the 50 (median), 2.5, and 97.5th percentiles of the 5000 bootstrap estimates of PERM as the point estimate of PERM and 95% CI.

We conducted subgroup analyses to identify interactions between *H. pylori* infection and clinically relevant groups, defined by age (<50 vs. ≥50 years), sex (men vs. women), smoking status (non-current vs. current smokers), alcohol intake (mild vs. moderate drinkers), regular exercise (no vs. yes), BMI (<25 vs. ≥25 kg/m^2), HOMA-IR (<2.5 vs. ≥2.5), and hsCRP (<0.1 vs. ≥0.1 mg/dl). Interactions between subgroups were tested using likelihood ratio tests comparing models with and without multiplicative interaction terms.

A FLI <30 was considered as indicative of no fatty liver disease, with a FLI ≥30 or ≥60 was considered as indicative of fatty liver disease [30]. We used the same inclusion and exclusion criteria to evaluate the association using FLI, defining fatty liver disease by the FLI rather than abdominal US. Detailed information is provided in Supplementary Materials. A p value <0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

The mean (SD) age of the 17,028 study participants was 49.3 (9.3) years. Baseline prevalence of *H. pylori* infection was 58.2% ($n = 9918$). Participants with *H. pylori* seropositivity were more likely to be older, male, and current smokers, as well as to have hypertension and higher BMI, total cholesterol, LDL-C, triglycerides, AST, ALT, GGT, and HOMA-IR, a lower education level, and lower levels of HDL-C than those without seropositivity (Table 1). Characteristics of the participants at the end of follow-up are presented in supplementary Table 1.

During the 83,130 person-years follow-up, NAFLD developed in 3381 participants, with an incidence rate of 40.7 per 1000 person-years. The incident rates (per 1000 person-years) of NAFLD by *H. pylori* serostatus were 37.2 for *H. pylori*-seropositive participants and 43.2 for those without seropositivity ($p < 0.001$; Table 2). The median follow-up period was 5.1 years (interquartile range 2.8–7.2). The age- and sex-adjusted HR (95% CI) for incident NAFLD comparing *H. pylori*-seropositive participants to those without *H. pylori* seropositivity was 1.14 (1.06–1.22; $p < 0.001$). The association persisted after further adjustment for year of screening exam, BMI, smoking status, alcohol intake, regular exercise, and education level (HR 1.21, 95% CI 1.10–1.34; $p < 0.001$). In the multivariable analysis, significant risk factors, other

Table 1 Characteristics of study participants at baseline

	All (n = 17,028)	*H. pylori* (−) (n = 7,110)	*H. pylori* (+) (n = 9,918)	p value
Age (years)	49.3 ± 9.3	48.3 ± 9.9	50.0 ± 8.7	<0.001
Male (%)	51.6	49.5	53.1	<0.001
BMI (kg/m^2)	23.0 ± 2.6	22.9 ± 2.6	23.1 ± 2.5	<0.001
Current smoker (%)	16.4	17.2	15.8	0.003
Modest alcohol intake (%)	13.3	12.7	13.7	0.08
Regular exercise (%)	36.7	36.4	36.9	0.69
Education level (%)				<0.001
Low	8.2	7.9	8.5	
Medium	28.1	26.0	29.6	
High	63.7	66.1	61.9	
Year of screening exam (%)				0.227
2005	58.5	57.9	58.9	
2006	30.1	30.8	29.5	
2007	11.4	11.3	11.6	
Systolic BP (mmHg)	112.6 ± 15.4	112.1 ± 15.1	113.0 ± 15.6	<0.001
Diastolic BP (mmHg)	68.8 ± 10.5	68.3 ± 10.3	69.1 ± 10.5	<0.001
Antihypertensive medication	7.8	7.2	8.3	0.014
Hypertension[a]	12.1	11.2	12.7	0.003
FBG (mg/dl)	89.6 ± 13.8	89.5 ± 13.7	89.7 ± 13.9	0.068
Hypoglycemic medication	1.9	1.7	2.0	0.253
Diabetes[a]	2.5	2.3	2.6	0.227
Total cholesterol (mg/dl)	189.2 ± 32.2	187.2 ± 32.3	190.6 ± 32.1	<0.001
LDL-C (mg/dl)	123.3 ± 29.6	120.5 ± 29.6	125.3 ± 29.5	<0.001
HDL-C (mg/dl)	59.5 ± 14.2	60.4 ± 14.5	58.8 ± 13.9	<0.001
Triglycerides (mg/dl)	97 (72–136)	96 (71–135)	98 (72–136)	0.005
Dyslipidemia medication	3.2	2.9	3.4	0.004
Dyslipidemia[a]	23.3	22.7	23.7	0.161
AST (U/l)	21 (18–24)	20 (18–24)	21 (18–25)	<0.001
ALT (U/l)	17 (13–23)	17 (13–23)	18 (14–23)	0.004
GGT (U/l)	19 (13–32)	19 (13–31)	20 (13–32)	0.02
hsCRP (mg/dl)	0.06 (0.04–0.11)	0.06 (0.04–0.11)	0.06 (0.04–0.11)	0.28
HOMA-IR	1.74 (1.35–2.22)	1.76 (1.37–2.26)	1.72 (1.34–2.19)	0.007

Values are expressed as mean ± standard deviation, percentages, or median (interquartile range)

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-glutamyltransferase, *hsCRP* high-sensitivity C-reactive protein, *HOMA-IR* homeostasis model assessment of insulin resistance

[a] Definitions: hypertension, SBP ≥140 mmHg or DBP ≥90 mmHg or use of antihypertensive medication; dyslipidemia, HDL-C <40 mg/dl or triglyceride ≥150 mg/dl or use of dyslipidemia medication; diabetes, FBS ≥126 mg/dl or use of hypoglycemic medication

than *H. pylori*, for NAFLD development were increasing age, BMI, alcohol intake, systolic blood pressure, fasting glucose, LDL-C, triglycerides, AST, ALT, hsCRP, HOMA-IR, and hypoglycemic medication. Protective factors for NAFLD were high education level, HDL-C, and dyslipidemia medication (Table 3).

To explore whether the increased incidence of NAFLD with *H. pylori* infection was mediated by metabolic risk factors, insulin resistance, inflammatory marker, or liver enzymes, we conducted additional analyses adjusted for potential mediators (Table 4). The association persisted after adjustment for hsCRP as an inflammatory marker (Model 1) or HOMA-IR (Model 2). After adjustment for metabolic variables such as fasting blood glucose, triglycerides, LDL-C, and HDL-C (Model 3), the association between *H. pylori* infection and incident NAFLD was attenuated but remained statistically significant, suggesting mediation by these metabolic factors.

Table 2 Development of nonalcoholic fatty liver disease (NAFLD) by *H. pylori* status

	Person-years	Number of incident cases	Incidence density (per 1000 person-years)	Age- and sex-adjusted HR (95% CI)	Multivariable-adjusted HR[a] (95% CI)
H. pylori (−)	34,960.7	1301	37.2	1.00 (reference)	1.00 (reference)
H. pylori (+)	48,169.3	2080	43.2	1.14 (1.06–1.22)	1.21 (1.10–1.34)

H. pylori Helicobacter pylori, *HR* hazard ratio, *CI* confidence intervals

[a] Estimated from Cox proportional hazard models adjusted for age, sex, body mass index, year of screening exam, smoking status, alcohol intake, regular exercise, and education level

Table 3 The risk of nonalcoholic fatty liver disease (NAFLD) development in the univariate and multivariate analyses

	Univariate analysis		Multivariate analysis[a]	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age	1.02 (1.02–1.03)	<0.001	1.01 (1.01–1.02)	<0.001
Male sex	1.73 (1.62–1.86)	<0.001	1.08 (0.94–1.24)	0.292
BMI	1.31 (1.29–1.33)	<0.001	1.20 (1.18–1.23)	<0.001
Current smoking	1.88 (1.72–2.07)	<0.001	0.96 (0.81–1.15)	0.672
Modest alcohol intake	1.62 (1.48–1.76)	<0.001	1.17 (1.01–1.35)	0.04
Regular exercise	0.54 (0.50–0.58)	<0.001	0.69 (0.62–0.77)	<0.001
High education level				
Low	1.00 (reference)		1.00 (reference)	
Medium	0.81 (0.70–0.93)	0.003	0.82 (0.68–1.00)	0.056
High	0.79 (0.68–0.92)	0.003	0.78 (0.64–0.97)	0.02
Year of screening exam				
2005	1.00 (reference)		1.00 (reference)	
2006	1.08 (0.99–1.17)	0.054	0.92 (0.78–1.09)	0.332
2007	1.45 (0.93–2.26)	0.102	1.17 (0.88–1.34)	0.394
Systolic BP (mmHg)	1.01 (1.01–1.02)	<0.001	1.01 (1.01–1.02)	<0.001
Antihypertensive medication	0.95 (0.86–1.04)	0.264	0.91 (0.79–1.04)	0.169
FBG (mg/dl)	1.01 (1.01–1.02)	<0.001	1.01 (1.00–1.01)	0.002
Hypoglycemic medication	1.11 (0.93–1.32)	0.269	1.38 (1.06–1.80)	0.017
LDL-C (mg/dl)	1.01 (1.00–1.01)	<0.001	1.01 (1.00–1.01)	<0.001
HDL-C (mg/dl)	0.96 (0.95–0.96)	<0.001	0.98 (0.97–0.98)	<0.001
Triglycerides (mg/dl)	1.01 (1.01–1.02)	<0.001	1.01 (1.00–1.01)	<0.001
Dyslipidemia medication	0.72 (0.63–0.83)	<0.001	0.80 (0.66–0.99)	0.038
AST (U/l)	1.01 (1.01–1.02)	<0.001	1.02 (1.01–1.03)	<0.001
ALT (U/l)	1.01 (1.01–1.02)	<0.001	1.03 (1.02–1.03)	<0.001
GGT (U/l)	1.01 (1.01–1.02)	<0.001	1.00 (0.99–1.00)	0.23
hsCRP (mg/dl)	1.31 (1.22–1.42)	<0.001	1.31 (1.22–1.42)	<0.001
HOMA-IR	1.58 (1.50–1.67)	<0.001	1.09 (1.01–1.17)	0.03
H. pylori (+)	1.16 (1.08–1.25)	<0.001	1.16 (1.05–1.30)	0.005

H. pylori Helicobacter pylori, *HR* hazard ratio, *CI* confidence intervals

[a] Estimated from Cox proportional hazard models adjusted for all variables

We calculated the PERM of the inflammatory markers and metabolic variables tested in adjusted models 1, 2, and 3 (Table 5). Among these variables, LDL-C and HDL-C were significant mediators, accounting for 4 and 8% of the total effect of *H. pylori* infection on NAFLD development, respectively. A combination lipid profile, including LDL-C, HDL-C, and triglycerides, accounted for 11% (4–28) of the excess risk of *H. pylori* on NAFLD. Dyslipidemia was a partial mediator in the relationship of *H. pylori* infection and NAFLD, with the HR for NAFLD being reduced in terms of absolute size but remaining different from zero when mediation was introduced. In contrast, hsCRP, fasting blood glucose, and HOMA-IR were not significant mediators for the association.

To evaluate the consistency of the effect of *H. pylori* on NAFLD, we performed subgroup analyses of factors

Table 4 Mediation analysis of the association between *H. pylori* status and the development of nonalcoholic fatty liver disease (NAFLD)

	H. pylori (−)	*H. pylori* (+)	*p* value
Model 0, aHR[a] (95% CI)	Reference	1.21 (1.10–1.34)	<0.001
Model 1, aHR[a] (95% CI)	Reference	1.21 (1.10–1.35)	<0.001
Model 2, aHR[a] (95% CI)	Reference	1.31 (1.14–1.52)	0.002
Model 3, aHR[a] (95% CI)	Reference	1.16 (1.06–1.30)	0.005
Model 4, aHR[a] (95% CI)	Reference	1.16 (1.05–1.30)	0.005

Model 0: adjusted for age, sex, body mass index, year of screening exam, smoking status, alcohol intake, regular exercise, and education level

Model 1: Model 0 plus adjustment for hsCRP

Model 2: Model 1 plus adjustment for HOMA-IR

Model 3: Model 2 plus adjustment for systolic blood pressure, use of antihypertensive medications, fasting blood glucose, use of hypoglycemic medications, triglycerides, LDL-C, HDL-C, and use of dyslipidemia medications

Model 4: Model 3 plus adjustment for AST, ALT, and GGT

H. pylori Helicobacter pylori, *aHR* adjusted hazard ratio, *CI* confidence intervals, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyl transferase

[a] Estimated from Cox proportional hazard models

Table 5 HRs and excess risk of *Helicobacter pylori* mediated thorough different combinations of metabolic risk factors

Metabolic mediators	*H. pylori* (+)	
	HR[a] (95% CI)	Excess risk mediated (%, 95% CI)
None	1.21 (1.10–1.34)	
hsCRP	1.21 (1.10–1.35)	3% (−22, 20)
HOMA-IR	1.31 (1.14–1.52)	24% (−23, 73)
FBG	1.21 (1.09–1.34)	1% (−4, 5)
LDL-C	1.19 (1.08–1.32)	4% (1, 9)
HDL-C	1.18 (1.07–1.32)	8% (1, 16)
Triglycerides	1.21 (1.10–1.35)	0% (−5, 5)
LDL-C, HDL-C, and triglycerides	1.17 (1.06–1.31)	11% (4, 28)

H. pylori Helicobacter pylori, *HR* hazard ratio, *CI* confidence intervals, *hsCRP* high-sensitivity C-reactive protein, *FBG* fasting blood glucose, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance

[a] Estimated from Cox proportional hazard models adjusted for age, sex, body mass index, year of screening exam, smoking status, alcohol intake, regular exercise, and education level

affecting NAFLD development (Fig. 2). The pre-specified subgroup analysis did not show heterogeneity of risk of incident NAFLD from *H. pylori* infection, or significant interactions by age (<50 vs. ≥50 years), sex (men vs. women), smoking status (non-current vs. current smokers), alcohol intake (mild vs. moderate drinkers), regular exercise (<3 vs. ≥3 times per week), BMI (<25 vs. ≥25 kg/m^2), metabolic syndrome (no vs. yes), HOMA-IR (<2.5 vs. ≥2.5), and hsCRP (<0.1 vs. ≥0.1 mg/dl).

The association of *H. pylori* with incident NAFLD was still evident in analysis using the FLI (Supplementary materials and Table 2).

Discussion

In this large cohort study of the association between *H. pylori* infection and risk of incident NAFLD, we found that participants with *H. pylori* infection were at higher risk of NAFLD development compared to uninfected individuals. This association persisted even though further adjustment for metabolic risk factors, inflammatory markers, or liver enzymes. Although adjustment for metabolic variables, such as fasting blood glucose, LDL-C, HDL-C, and triglycerides, reduced the association between *H. pylori* infection and incident NAFLD, the association did remain statistically significant. Among the metabolic variables, lipid metabolism markers, such as LDL-C, HDL-C, and the combination of LDL-C, HDL-C, and triglycerides were significant mediators of the association between *H. pylori* infection and NAFLD. This association was still evident in the analysis using FLI and was similar across all subgroups. Our findings indicate that *H. pylori* infection might represent one further hit contributing to NAFLD pathogenesis.

To our knowledge, this is the first large-scale cohort study to evaluate the association between *H. pylori*

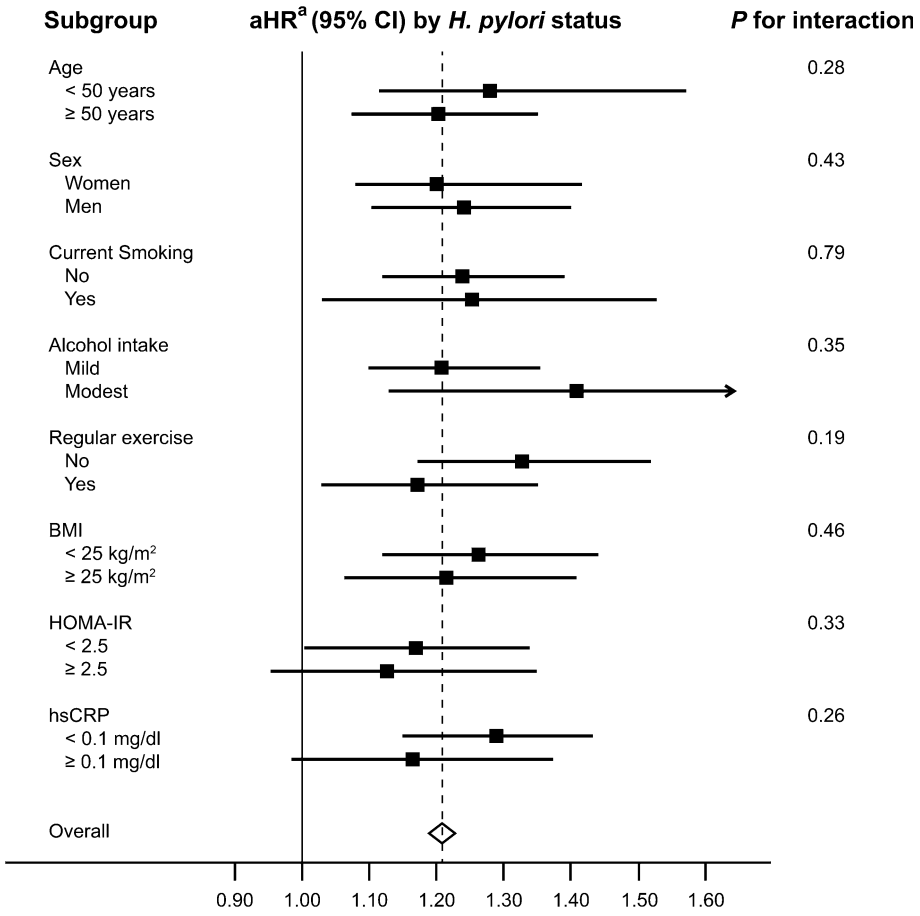

Fig. 2 Development of nonalcoholic fatty liver disease (NAFLD) according to *H. pylori* status in clinically relevant subgroups. [a]Estimated from Cox proportional hazard models. Multivariable model was adjusted for age, sex, BMI, year of screening exam, smoking status, alcohol intake, regular exercise, and education level. *H. pylori Helicobacter pylori*, *BMI* body mass index, *HOMA-IR* homeostasis model assessment of insulin resistance, *hsCRP* high-sensitivity C-reactive protein

infection and the risk of developing NAFLD. *H. pylori* infection is associated with living in crowded conditions and low socioeconomic status [31, 32]. Metabolic syndrome and NAFLD are usually related to socioeconomic status [33, 34]. We thus conducted this study, paying particular attention to potential confounders, including age, sex, education level, smoking status, alcohol consumption, physical inactivity, and year of screening exam. We also performed further adjustment for metabolic variables and inflammatory markers. Even after adjustment for established NAFLD risk factors and the potential confounders, *H. pylori* infection was significantly associated with incident NAFLD.

The pathogenetic link between *H. pylori* infection and NAFLD is debated and clinical data are limited. Several previous studies reported an association between *H. pylori* infection and NAFLD [6, 16–19, 21]. A recent large-scale cross-sectional study of 13,737 Japanese adults reported that *H. pylori* infection is not associated with fatty liver disease [21]. Another Korean cross-sectional analysis of 3663 participants found that *H. pylori* is not a risk factor for NAFLD [18]. In contrast, Polyzos et al. reported that *H. pylori* infection was more frequently observed in 28 NAFLD patients than in 25 healthy controls [19]. Another study of 130 Japanese participants revealed that the prevalence of nonalcoholic steatohepatitis is higher in *H. pylori*-infected participants than in non-infected participants [17]. The cross-sectional design of the studies limited assessment of causality; the sample size was small in two studies, which showed positive associations. This large-scale longitudinal study suggests that association between *H. pylori* and NAFLD is not just an epiphenomenon and *H. pylori* could be a significant contributor to NAFLD pathogenesis.

Although the exact mechanism linking *H. pylori* infection and NAFLD needs to be studied in more detail, there are several studies describing a potential role of *H. pylori* in the predisposition to and causation of NAFLD. *H. pylori* infection is linked to chronic inflammation, insulin resistance, diabetes, dyslipidemia, and metabolic syndrome [7–9, 16, 35–37]. These variables are also risk factors for NAFLD [38]. In this study, we tested inflammatory and metabolic variables that could be potential mediators linking *H. pylori* infection and NAFLD. Among these variables, hsCRP, fasting blood glucose, and HOMA-IR were not significant mediators, while lipid markers attenuated the association and accounted for one quarter of the excess risk of *H. pylori* on NAFLD. These findings suggest

that *H. pylori* infection contributes to development NAFLD by altered lipid metabolism. NAFLD is closely linked to abnormal lipid metabolism, resulting in increased hepatic accumulation of triacylglycerol [39]. Notably, *H. pylori* infection can alter lipid profiles, including low HDL-C, high LDL-C and triglycerides [8, 9, 40]. The unexplained risk by the metabolic mediators might be caused by other mechanisms such as *H. pylori* induced gastric atrophy leading to loss of acid, subsequent small intestinal bacterial overgrowth (SIBO), increased intestinal permeability, and increased portal endotoxins [15, 41]. It is reported that SIBO may reflect qualitative and quantitative changes in the microbiota, leading to intestinal barrier disruption, subsequent bacterial translocation, and development of portal endotoxemia. As a result, endotoxin-mediated cytokines were increased in the liver and enhanced hepatic inflammation and fibrosis [42, 43]. Future studies will be required to evaluate this possibility.

Several limitations need to be considered in interpreting our findings. First, we defined NAFLD by US after exclusion of secondary causes for steatosis. US may lead to an incorrect diagnosis of NAFLD in 10 to 30% of cases [44]. However, many population-based studies have relied on US to diagnose fatty liver [45, 46] and the results did not differ when fatty liver was defined using the FLI. Second, *H. pylori* infection status was evaluated solely with serum IgG to *H. pylori* measured by ELISA, without other laboratory assessments, such as a rapid urease test or urease breath test. Accordingly, the serologic test cannot discriminate accurately between current and past infections. This may not necessarily be a disadvantage, as past infection may even be more relevant for disease pathogenesis. In addition, the serum IgG antibody test to *H. pylori* is inexpensive mass-screening tool that can be used easily in areas with a high prevalence of *H. pylori* infection. Prevalence of *H. pylori* infection in Korea from a nationwide multicenter study was 59.6%; this result did not differ from the 58.2% in our data [47, 48]. Third, we did not have the information of *H. pylori* eradication. Effect of *H. pylori* eradication on the risk of NAFLD cannot be assessed and the results might have been influenced by *H. pylori* eradication. However, in Korea, according to our national health policy and guidelines, indications for *H. pylori* eradication include peptic ulcer disease, infection after endoscopic resection for early gastric cancer and gastric mucosa-associated lymphoid tissue [49, 50]. Accordingly, we expected that most asymptomatic participants with *H. pylori* seropositivity had not received medication of *H. pylori* eradication. Also, we excluded participants who were diagnosed with gastric malignancy in this study. Finally, this study focused on healthy men and women who underwent routine health check-ups; thus, it may be difficult to generalize our findings to other populations.

This study is strengthened by the cohort design used, the relatively large sample size and exclusion of baseline NAFLD, which allowed us to identify causal associations. This is usually impossible in cross-sectional studies. Additional strengths include the use of high-quality, standardized clinical and laboratory methods.

In conclusion, this study showed that *H. pylori* infection was associated with an increased risk of NAFLD development in asymptomatic participants without baseline NAFLD. This effect might be exerted through markers of lipid metabolism. These findings support the hypothesis that *H. pylori* may contribute to the pathogenesis of NAFLD. Further investigations are needed to determine whether *H. pylori* eradication can improve NAFLD risk.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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