Atherosclerosis Plus 44 (2021) 25–30

Contents lists available at ScienceDirect

Atherosclerosis Plus

journal homepage: www.elsevier.com/locate/atherosclerosis

Association between Helicobacter pylori infection and carotid atherosclerosis in Chinese adults

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ARTICLE INFO

Article history:
Received 16 April 2021
Received in revised form
26 July 2021
Accepted 12 August 2021
Available online 13 August 2021

Keywords:
Helicobacter pylori
Carotid plaque
Atherosclerosis
Cardiovascular disease
Cerebrovascular disease

ABSTRACT

Background and aims: The role of *Helicobacter pylori* (*H. pylori*) infection in carotid atherosclerosis remains inconsistent and sometimes controversial. We aimed to determine whether *H. pylori* infection is associated with carotid atherosclerotic plaques in a large number of Chinese adults.

Methods: We recruited 108,210 Chinese adults who participated in a standard medical screening with both carotid ultrasonic examination and ^{13}C-urea breath test for *H.pylori* infection from two Chinese cohorts. A total of 93,915 adults were included in the analysis after excluding participants with cardiovascular disease (CVD) and carotid plaques at baseline. Hazard ratio (HR) for developing carotid plaques by *H. pylori* infection was analyzed using the Cox proportional hazard model, with socio-demographic and clinical factors adjusted. Findings across cohorts were pooled by meta-analyses.

Results: 11,208 (13.13%) participants occurred carotid plaques at a median follow-up of 20 months in the MN cohort, while 1279 (14.95%) participants occurred carotid plaques at a median follow-up of 24 months in the MJ cohort. Compare with participants without *H. pylori* infection, participants with *H. pylori* infection were more likely to occur carotid plaques. After adjusting for age, sex, annual personal income, body mass index, blood pressure, blood glucose, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, and estimated glomerular filtration rate, the HR was 1.04 (95%CI: 1.01−1.08). After further adjusting for education level, marital status, smoking status, alcohol drinking status, physical activity, and family history of CVD, the HR changed minimally. Additional sensitivity analyses confirmed the robustness of the results. Significant interactions of age, sex, blood pressure, blood glucose, or chronic inflammation were not observed in this research.

Conclusions: *H. pylori* infection was associated with carotid plaque onset in a large number of Chinese adults without previous CVD. These data suggested that the prevention of *H. pylori* infection may reduce the burden of carotid atherosclerosis.

Introduction

Stroke is the second leading cause of death and a major cause of disability worldwide [1]; carotid atherosclerosis plays a fundamental role in the occurrence of ischemic stroke [2]; atherosclerosis is considered a chronic inflammation-driven disease of the arterial wall [3]. Persistent infectious pathogens, such as *Helicobacter pylori* (*H. pylori*), have the possibility of promoting the development of

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https://doi.org/10.1016/j.athplu.2021.08.004

atherosclerosis [4] because *H. pylori* can either directly invade vessel walls [5] or indirectly induce endothelial dysfunction and dyslipidemia [6]. Thus, the association between *H. pylori* infection and atherosclerosis disease has received considerable attention [7,8].

H. pylori infection infects at least 50% of the world's population [9], and the infection rate of *H. pylori* ranges from 28.0% to 73.3% in different regions of China [10,11]. Atherosclerosis is a significant contributing factor to cardiovascular mortality. The relationship between *H. pylori* infection and carotid atherosclerosis remains unclear and controversial, with uncertain study results regarding a positive [12–14] or negative [15,16] association. Moreover, there are also some limitations in previous studies. For example, some results came from cross-section designs [12–14]. Other limitations included relatively small sample size [12,15,16] and insufficient adjustments for possible confounder variables, such as physical activities, C-reactive protein, or socioeconomic status [7,12,15-17]. Additionally, tests using the serum antibodies show lower specificity than that of the urea breath test (UBT).

In this study, we conducted two prospective cohorts to evaluate whether *H. pylori* infection, diagnosed by UBT, was associated with the risk of carotid atherosclerosis in a large number of Chinese adults.

Materials and methods

Study populations

A total of 108,210 adult participants met the inclusion criteria: (1) without previous atherosclerotic cardiovascular disease (CVD) and carotid atherosclerotic plaques, (2) participated in a standard medical screening at baseline from 2 Chinese cohorts (the MJ and Meinian (MN) cohorts). The study was approved by the Institutional Review Board (IRB)/Ethics Committee of Tiantan Hospital and the Peking University Institutional Review Board with a waiver of informed consent (IRB00001052-19077).

The MN cohort, scattered in 319 health screening centers in 31 provinces, autonomous regions, and municipalities in China, was managed by Meinian OneHealth Group. 97,874 adults with UBT results and carotid Doppler ultrasound measurements in 2017 and follow-up carotid Doppler ultrasound measurements during 2018–2019 were recruited in the MN cohort. We excluded 1308 participants with a history of CVD and 11,208 participants with carotid atherosclerosis at the baseline Doppler ultrasound check-up. Finally, 85,358 participants were included in the analysis.

The Beijing MJ cohort, located in Beijing city, was established by MJ Health Management Institution. Detailed information on lifestyle was collected through a structured questionnaire, and health status was examined by various medical equipment in the MJ cohort. 10,336 adults with UBT and carotid Doppler ultrasound measurements at baseline and follow-up carotid Doppler ultrasound measurements during 2010–2018 were recruited. We excluded 405 participants with self-reported CVD and 1374 participants with carotid plaques at the baseline Doppler ultrasound check-up. Finally, 8557 participants were included in this analysis.

H. pylori infection assessment

H. pylori infection was diagnosed by the ^{13}C-UBT. Participants first provided the initial baseline breath sample (after 8–12 h of overnight fasting) and were then required to take 75 mg of ^{13}C-urea (Urea-^{13}C Capsule Breath Test Kit). We collected the second breath sample after 30 min. The samples were analyzed by ^{13}C infrared spectroscopy. *H. pylori* infection was defined as positive if the difference between the two samples exceeded 4.0 parts per 1000 of $^{13}CO_2$ [18].

Atherosclerosis assessment

The common carotid arteries, the bifurcation, the external carotid arteries, and the internal carotid arteries were examined on each side with a linear 7.5 MHz probe Doppler ultrasound (Sonoscape S50, China) on the same day as the *H. pylori* infection assessment. Carotid atherosclerosis was defined as the presence of plaques in any of the aforementioned arterial segments. Incident atherosclerosis was characterized by the occurrence of new plaques in previously normal segments. The carotid atherosclerotic plaque was defined as a discrete, focal wall thickening $\geq$1.5 mm or focal thickening >50% greater than the surrounding intima-media thickness (IMT) [19]. IMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. Doppler ultrasonography was performed by an experienced ultrasonographer under a standardized protocol.

Measurements of covariates

All laboratories in this study completed a standardization and certification program. Blood samples were drawn by venipuncture after overnight fasting. Fasting blood glucose (FBG), total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, and high-sensitivity C-reactive protein (hs-CRP) were measured using automatic biochemical analyzers with commercially available reagents at the clinical biochemical laboratories in each center. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration creatinine equation [20]. Abnormal blood glucose was defined as a self-reported physician-diagnosis history of diabetes, currently treated with insulin or oral hypoglycemic agents, or fasting blood glucose $\geq$5.6 mmol/L. Bodyweight and height were measured by standard methods. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Systolic blood pressure and diastolic blood pressure was measured twice using a digital automatic blood pressure monitor after participants seated for at least 5 min. The mean values of the two readings were recorded. Abnormal blood pressure was defined as a self-reported physician-diagnosis history of hypertension, currently treated with antihypertensive agents, systolic blood pressure $\geq$120 mmHg, or diastolic blood pressure $\geq$80 mmHg.

In the MN cohort, chronic disease history was collected from a face-to-face interview between the physician and participants. As we did not collect relative data at an individual level, local gross domestic product per capita [21] was used to replace their income. Participants in the MJ cohort completed a self-administered questionnaire with demographic, socioeconomic, medical, and lifestyle information.

Statistical analysis

All analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina). A p-value of <0.05 was considered statistically significant. We plotted Schoenfeld residuals to ascertain that the proportional hazards assumption had not been violated. Then, the Cox proportional hazards model was used to investigate the association between *H. pylori* infection and the risk of developing carotid atherosclerosis. Missing data of covariate was coded as an extra category. In the first model, we adjusted for age and sex; in the second model, we further adjusted for annual personal income, BMI, blood pressure status, blood glucose status, TG, LDL-C, HDL-C, hs-CRP, and eGFR in both the MJ cohort and MN cohort. Findings across cohorts were pooled using inverse-

variance-weighted meta-analyses. The fixed-effects or random-effects model was chosen according to the between-studies variance as a percent of the total variance (if <50%, then the fixed-effects model was chosen). To minimize the residual error, we adjusted for education level, marital status, smoking status, alcohol drinking status, physical activity, and family history of CVD in the MJ cohort.

A series of sensitivity analyses were performed to test the robustness of the results. We excluded participants with self-reported cancer and peptic ulcer since both *H. pylori* infection and the risk of carotid atherosclerosis were associated with these diseases [22,23]. Further, we removed participants with missing values for covariates and ran complete case analyses. Additionally, we excluded participants who had a follow-up time of less than one year to minimize potential reverse causation caused by severe illness.

To explore whether the association was modified by age, sex, blood pressure status, blood glucose status, or chronic inflammation, we used likelihood ratio tests to examine potential interactions between *H. pylori* infection and these variables about carotid atherosclerosis risk, adjusting for aforementioned covariates. Multivariable-adjusted Cox proportional hazards model was also applied to the sub-population stratified by those variables.

Results

Baseline characteristics of the study populations

Table 1 shows participant characteristics. The prevalence of *H. pylori* infection was 40.1% (case number 34,259) in the MN cohort and 37.1% (case number 3176) in the MJ cohort. Overall, compared with participants without *H. pylori* infection, those with *H. pylori* infection were more likely to be women, current smokers, current drinkers, and people inactive in leisure-time physical activities. The infected population reported less than a 0.1million annual income or less than a high school education level. They were more likely to have a higher level of systolic blood pressure, diastolic blood pressure, BMI, FBG, and TG, but a lower level of HDL-C.

Findings from the MN and MJ specific cohorts

11,208 (13.13%) participants in the MN cohort occurred carotid plaques at a median follow-up of 20 months (range: 1–35 months; interquartile range 12–24 months), while 1279 (14.95%) participants in the MJ cohort occurred carotid plaques at a median follow-up of 24 months (range: 3–106 months; interquartile range 13–37 months (Table 2). Compare with participants without *H. pylori* infection, those with *H. pylori* infection had a higher risk of developing carotid plaques. In the MN cohort, after adjusting for age, sex, annual personal income, BMI, blood pressure status, blood glucose status, TG, LDL-C, HDL-C, hs-CRP, and eGFR, the hazard ratio (HR) for developing carotid atherosclerosis was 1.04, with a 95% confidence interval (95% CI) at 1.01–1.08. However, the corresponding HR was 1.03 (95% CI: 0.92–1.15) in the MJ cohort. The HR for developing carotid plaques was attenuated to 1.02 (95%CI: 0.91–1.14) after further adjustment for education, marital status, smoking status, alcohol drinking, physical activity, and family history of CVD.

Table 1
Basic characteristics according to *Helicobacter pylori* (*H. pylori*) infection status in the MN and MJ cohort[a].

	MN Cohort			MJ Cohort		
	H. pylori -	*H. pylori* +	P value	*H. pylori* -	*H. pylori* +	P value
No. of participants	51,099	34,259		5381	3176	
Age, years	45.0 ± 11.1	46.0 ± 10.8	<.001	44.9 ± 8.6	45.4 ± 8.7	<.001
Women	32,104 (62.8)	22,123 (64.6)	<.001	3235 (60.1)	2082 (65.6)	<.001
Personal income <0.1million ¥ /Y [b]	22,700 (44.4)	16,681 (48.7)	<.001	902 (16.8)	566 (17.8)	0.64
College education or above	–	–	–	3312 (61.5)	1882 (59.3)	<.001
Marital status	–	–	–	4315 (80.2)	2541 (80.0)	0.01
Current smoker	–	–	–	1361 (25.3)	987 (31.1)	<.001
Current drinker	–	–	–	1291 (24.0)	898 (28.3)	<.001
Family history of CVD	–	–	–	781 (14.5)	377 (11.9)	<.001
High physical activity [c]	–	–	–	1329 (24.7)	783 (24.7)	0.49
Self-reported cancer	496 (0.97)	239 (0.70)	<.001	52 (0.97)	28 (0.88)	0.69
Self-reported peptic ulcer	187 (0.37)	109 (0.32)	0.24	182 (3.38)	85 (2.68)	0.07
Self-reported hypertension	3789 (7.42)	2793 (8.15)	<.001	594 (11.0)	349 (11.0)	0.94
Self-reported diabetes	1247 (2.44)	923 (2.69)	0.02	175 (3.25)	125 (3.94)	0.10
SBP (mmHg)	122 ± 17	124 ± 18	<.001	113 ± 14	114 ± 14	0.002
DBP (mmHg)	75.7 ± 12.0	75.7 ± 12.0	<.001	72.3 ± 10.7	73.0 ± 11.0	0.006
BMI (kg/m^2)	24.4 ± 3.5	24.7 ± 3.5	<.001	24.3 ± 3.4	24.7 ± 3.4	<.001
FBG (mmol/L)	5.21 ± 1.16	5.27 ± 1.27	<.001	5.62 ± 0.82	5.69 ± 0.97	<.001
TG (mmol/L) [d]	1.67 (1.30)	1.73 (1.35)	<.001	1.48 (1.04)	1.55 (1.18)	0.002
TC (mmol/L)	4.89 ± 0.96	4.90 ± 0.96	0.44	4.79 ± 0.86	4.85 ± 0.87	<.001
LDL-C (mmol/L)	2.74 ± 0.79	2.74 ± 0.79	0.47	3.05 ± 0.79	3.12 ± 0.80	<.001
HDL-C (mmol/L)	1.35 ± 0.33	1.32 ± 0.32	<.001	1.38 ± 0.38	1.34 ± 0.37	<.001
eGFR ($ml/min/l.73m^2$)	102 ± 16	102 ± 15	0.10	102 ± 13	101 ± 13	0.04
hs-CRP (mg/L) [d]	3.00 (1.32)	3.00 (1.32)	0.90	0.70 (1.10)	0.70 (1.20)	0.002

Abbreviations: CVD, cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein.

[a] More detailed information was presented in sTable 1. The baseline characteristics were presented as mean with standard deviation (± Values) for normal distribution variables or as an absolute number with percentages [n (%) values] for categorical variables. P <0.05 was regarded as statistically significant.

[b] Data derived from "China city statistical yearbook-2018".

[c] Leisure-time physical activity categories based on the tertile of the product of intensity [metabolic equivalent value (MET;1 MET=1 kcal per h per kg of body weight)] and duration of exercise (h). Inactive, medium, and high physical activity indicated the low tertile (<0.79 MET-h), medium tertile (0.79-2.84 MET-h), and high tertile (≥2.85 MET-h).

[d] Described as median (interquartile range) for its skewed distribution.

Table 2
Hazard ratios for carotid plaque in participants with *Helicobacter pylori* (*H. pylori*) infection, according to the MN and MJ cohort.

	H. pylori -[a]	*H. pylori* + [a]	HR (95% CI)		
	Carotid plaque/total	Carotid plaque/total	Model 1[b]	Model 2[c]	Model 3[d]
MN cohort					
All participants	6433/51099	4775/34259	1.05 (1.02–1.09)	1.04 (1.01–1.08)	–
Without self-reported cancer [e]	6379/50603	4750/34020	1.05 (1.01–1.09)	1.04 (1.00–1.08)	–
Without self-reported cancer or peptic ulcer [f]	6346/50417	4733/33911	1.06 (1.02–1.10)	1.04 (1.00–1.08)	–
Without missing value [g]	5494/44600	4113/29712	1.07 (1.02–1.11)	1.06 (1.01–1.10)	–
Set 1-year lag [h]	5814/46317	4324/31168	1.06 (1.02–1.10)	1.05 (1.01–1.09)	–
MJ cohort					
All participants	762/5381	517/3176	1.05 (0.94–1.17)	1.03 (0.92–1.15)	1.02 (0.91–1.14)
Without self-reported cancer [i]	751/5329	514/3148	1.05 (0.94–1.18)	1.03 (0.92–1.16)	1.03 (0.92–1.15)
Without self-reported cancer or peptic ulcer [j]	711/5147	497/3063	1.05 (0.94–1.18)	1.04 (0.93–1.17)	1.03 (0.92–1.16)
Without missing value [k]	428/2912	294/1772	1.05 (0.93–1.18)	1.03 (0.91–1.15)	1.02 (0.90–1.14)
Set 1-year lag [l]	297/2007	215/1260	1.04 (0.87–1.24)	0.99 (0.83–1.19)	1.01 (0.84–1.21)

[a] Presented as case number/number of participants.
[b] Adjusted for age (<30, 30–39, 40–49, 50-59, 60–69, or ≥0 year), sex (women or men).
[c] Further adjusted for annual personal income (<0.1, 0.1–0.2, or ≥0.2 million), body mass index (<18.5, 18.5-23.9, 24.0-27.9, or ≥28 kg/m^2), blood pressure (normal, prehypertension, or hypertension), blood glucose (normal, prediabetes, or diabetes), triglycerides (tertile), low-density lipoprotein cholesterol (tertile), high-density lipoprotein cholesterol (tertile), high-sensitivity C-reactive protein (tertile), and estimated glomerular filtration rate (tertile).
[d] Further adjusted for education (illiteracy or elementary school, middle school, or college/university), marital status (married, separated, or never married), smoking status (never, former, or current), alcohol drinking (never, former, or current), physical activity (inactive, medium, or active), family history of cardiovascular disease (yes or no).
[e] Excluded 735 participants with self-reported cancer at baseline.
[f] Excluded 1030 participants with self-reported cancer or peptic ulcer at baseline.
[g] Excluded 71,018 participants with missing values in the covariate.
[h] Excluded 11,046 participants with follow time less than 12 months.
[i] Excluded 110 participants with self-reported cancer at baseline.
[j] Excluded 347 participants with self-reported cancer or peptic ulcer at baseline.
[k] Excluded 5290 participants with missing values in the covariate.
[l] Excluded 739 participants with follow time less than 12 months.

Pooled association between H. pylori infection and carotid atherosclerotic plaques risk

As showed in Table 3, the pooled HR was 1.05 (95% CI 1.02–1.09) after being adjusted for age and sex. After further adjusted for annual personal income, BMI, blood pressure status, blood glucose status, TG, LDL-C, HDL-C, hs-CRP, and eGFR, the pooled HR was still 1.04 (95%CI: 1.01–1.08). The relation between *H. pylori* infection at baseline and developing carotid plaques during follow-up was persistent after excluded participants with self-reported cancer or peptic ulcer. Similar results were observed when we were restricted to complete case analyses with an HR at 1.05 (95% CI: 1.01–1.10). After setting a one-year lag, the association trend continued, and the pooled multivariable-adjusted HR was 1.03 (95% CI: 0.95–1.12).

We did not observe significant interactions of age, sex, blood pressure status, blood glucose status, or chronic inflammation for the association (P for interaction >0.2 for all, sTable 2). Consistently, we observed that *H. pylori* infection increased carotid plaques in the sub-populations after being stratified by those variables mentioned above (Table 4).

Discussion

In the current study, we found that persons who had *H. pylori* infection had significantly increased the likelihood of carotid atherosclerosis, independent of potential confounders and known risk factors for carotid atherosclerosis in two prospective cohorts without previous CVD. Our finding persisted in a series of sensitivity analyses. The association between *H. pylori* infection and carotid atherosclerosis was significant and the HR of 1.04 indicated significant public health impact of the finding, especially both *H. pylori* infection and carotid atherosclerosis are highly prevalent in Chinese, especially excluding confounding from social and behavioral variables.

Our results were consistent with most previous studies in which the positive association between *H. pylori and* carotid atherosclerosis was suggested [17,24]. A prospective study by Zhang and colleagues reported that *H. pylori* infection might be an important risk factor for carotid atherosclerosis in young Chinese males under 50 during a 5-year follow-up [17]. Our study with a large sample size indicated that *H. pylori* infection was associated with a 4%

Table 3
Incidence[a] of carotid plaque according to *Helicobacter pylori* (*H. pylori*) infection status in the MN and MJ cohort and pooled multivariable-adjusted hazard ratios (HRs) for developing carotid plaques in participants with *H. pylori* infection.

Participants	MN cohort		MJ cohort		Pooled HR (95%CI)[b]	Pooled HR (95%CI)[c]
	H. pylori -	*H. pylori* +	*H. pylori* -	*H. pylori* +		
All participants	8.12	9.04	5.92	6.54	1.05 (1.02–1.09)	1.04 (1.01–1.08)
Without self-reported cancer	8.13	9.05	5.89	6.56	1.05 (1.02–1.09)	1.04 (1.01–1.08)
Without self-reported cancer or peptic ulcer	8.12	9.05	5.78	6.53	1.06 (1.02–1.09)	1.04 (1.01–1.08)
Without missing value	7.55	8.50	4.27	4.73	1.06 (1.02–1.11)	1.05 (1.01–1.10)
Set 1-year lag	8.09	9.00	5.69	6.27	1.05 (0.97–1.13)	1.03 (0.95–1.12)

[a] Presented per 100 person–year.
[b] Adjusted for age (<30, 30–39, 40–49, 50-59, 60–69, ≥70 year), sex (women, men).
[c] Further adjusted for annual personal income (<0.1, 0.1–0.2, or ≥ 0.2 million), body mass index (<18.5, 18.5-23.9, 24.0-27.9, or ≥ 28 kg/m^2), blood pressure (normal, prehypertension, or hypertension), blood glucose (normal, prediabetes, or diabetes), triglycerides (tertile), low-density lipoprotein cholesterol (tertile), high-density lipoprotein cholesterol (tertile), high-sensitivity C-reactive protein (tertile), and estimated glomerular filtration rate (tertile).

Table 4
Incidence of carotid plaque according to *Helicobacter pylori* (*H. pylori*) infection status in the MN and MJ cohort and pooled hazard ratios for risks of developing carotid plaque, stratified by sex, age, diabetes, hypertension status, body mass index, or reactive protein levels.

Sub-groups	MN cohort[a]		MJ cohort[a]		Pooled HR (95% CI)[b]
	H. pylori -	*H. pylori* +	*H. pylori* -	*H. pylori* +	
Women	1754 (6.03)	1267 (6.84)	224 (4.41)	132 (4.85)	1.07 (1.00, 1.15)
Men	4679 (9.34)	3508 (10.22)	538 (6.89)	385 (7.43)	1.03 (0.99, 1.08)
Age<60 year	4581 (6.38)	3438 (7.25)	661 (5.34)	434 (5.78)	1.08 (1.03, 1.12)
Age≥60 year	1852 (24.96)	1337 (24.70)	101 (19.80)	83 (21.34)	1.02 (0.95, 1.09)
BMI<25 (kg/m^2)	3000 (6.87)	2148 (7.72)	388 (4.91)	238 (5.46)	1.05 (1.00, 1.11)
BMI≥25 (kg/m^2)	3059 (9.77)	2354 (10.53)	373 (7.55)	278 (7.86)	1.03 (0.98, 1.08)
Normal blood glucose	4249 (6.85)	3148 (7.74)	314 (4.46)	186 (4.54)	1.04 (1.00, 1.09)
Abnormal blood glucose	2069 (13.20)	1535 (13.78)	445 (7.63)	331 (8.70)	1.04 (1.00, 1.09)
Normal blood pressure	1624 (5.02)	1177 (5.78)	365 (4.35)	239 (4.91)	1.07 (1.00, 1.15)
Abnormal blood pressure	4541 (10.38)	3407 (11.18)	396 (8.84)	276 (9.14)	1.03 (0.99, 1.08)
hs-CRP<1 (mg/L)	112 (8.96)	109 (10.40)	260 (5.20)	161 (5.25)	0.96 (0.81, 1.13)
hs-CRP≥3 (mg/L)	1005 (8.20)	851 (9.09)	204 (7.13)	167 (8.03)	1.06 (0.97, 1.15)

[a] Presented as case number (per 100 person−year).
[b] Adjusted for age (<30, 30−39, 40−49, 50-59, 60−69, or ≥70 year), sex (women or men), annual personal income (<0.1, 0.1−0.2, or ≥0.2 million), body mass index (<18.5, 18.5-23.9, 24.0-27.9, or ≥28 kg/m^2), blood pressure (normal, prehypertension, or hypertension), blood glucose (normal, prediabetes, or diabetes), triglycerides (tertile), low-density lipoprotein cholesterol (tertile), high-density lipoprotein cholesterol (tertile), high-sensitivity C-reactive protein (tertile), and estimated glomerular filtration rate (tertile).

increased risk for carotid atherosclerosis 1.04 (95%CI: 1.01−1.08). The finding is robust due to its large sample size. The diagnostic tests for *H. pylori* can be divided into endoscopic or nonendoscopic methods [25]. The serologic *H. pylori* IgG test, UBT, and fecal antigen assay are nonendoscopic. Although serologic antibody testing cannot distinguish current and past infection [26], previous studies found that *H. pylori* infection was associated with high carotid atherosclerosis risk when *H. pylori* infection was diagnosed by serologic *H. pylori* IgG test [27] or rapid urease test [14]. In the current study, we diagnosed *H. pylori* infection via UBT.

Carotid ultrasound examination is an ideal and sensitive noninvasive image modality to diagnose and monitor the progression of atherosclerosis [28]. A previous study found that *H. pylori* infection was related to the early stage of atherosclerosis, evaluated brachial-ankle pulse wave velocity [16]. Recently, it was reported that carotid IMT in participants with *H. pylori* infection was significantly thicker than those without infection [12]. This result is consistent with our discovery. Zhang et al. found that *H. pylori* infection was an independent risk factor for carotid atherosclerosis in males under 50 years, but not in older males or females [17]. However, we did not observe significant interactions of age for the association between *H. pylori* infection and carotid atherosclerosis.

Atherosclerosis is a pathologic process narrowing the coronary, cerebral, and peripheral arteries due to the formation of atheromatous plaques, which resulted in CVD [29]. The relation between *H. pylori* infection and cerebrovascular disease has been reported in various studies [17,30,31], which also supported our findings. A few previous studies did not show an association between *H. pylori* infection and atherosclerosis [13,27,32]. Certain factors should be taken into account in this circumstance. For example, in some researches, *H. pylori* infection was diagnosed via antibody test with lower specificity [27,32], which cannot distinguish a current infection from the past. Moreover, conventional cardiovascular risk factors, such as obesity, diabetes, hypertension, dyslipidemia, and lifestyle factors (e.g., smoking), still play a substantial role in the development of atherosclerosis. These factors were very likely to mask the effect of *H. pylori* infection.

H. pylori infection is acquired during childhood [33] and becomes persistent if not treated [34]. *H. pylori* may contribute to atherosclerosis disease through several pathways. It might directly invade the vessel wall and induce dyslipidemia [5,6,35]. Chronic *H. pylori* infection could trigger an immune response. Long-term exposure to such immune factors could lead to endothelial dysfunction and result in atherosclerosis [6]. Besides, endothelial dysfunction induced by Vacuolating cytotoxin A secreted from *H. pylori* [36], oxidative stress [37], molecular mimicry by the autoimmune response [38], and platelet aggregation [39] by *H. pylori* are all potential mechanisms of atherosclerosis. However, in the current study, we still observe the relation between *H. pylori* and carotid plaques even after adjusted CRP. We also observed that LDL-C levels in patients with *H. pylori* infection were higher than those without *H. pylori* infection, while HDL-C level was lower in patients with *H. pylori* infection than those without the infection.

The present study had several strengths. First, we did not investigate the incidence of cerebrovascular disease but rather the incidence of subclinical cerebrovascular atherosclerosis in a relatively large number of subjects with no history of CVD. Studying the association between *H. pylori* infection and subclinical atherosclerosis allows us to confirm that earlier vessel-wall changes might be induced by *H*. pylori infection. Second, the diagnosis of *H. pylori* infection in our study was established by the ^{13}C-UBT rather than serologic testing. Although there are several strengths, the present study still has limitations. First, it included only Chinese adults, thus may not be generalizable to other populations. However, the participants came from 319 health screening centers widely spread in 31 provinces, autonomous regions, and municipalities in China in the MN cohort, indicating the exhaustivity of the study population. Although detailed data about socioeconomic and lifestyle information were not collected in the MN cohort, we adjusted these covariates in the MJ cohort. The HR for developing carotid atherosclerosis almost kept the same. In addition, the follow-up time is less than 2 years, a longer follow-up time is needed.

Conclusions

In conclusion, *H. pylori* infection was associated with developing carotid atherosclerotic plaques, especially in a large number of Chinese adults without previous CVD. This study supports the possibility that the prevention of *H. pylori* might reduce the burden of carotid atherosclerosis.

Author contributions

All authors contributed to the conception and design of the study. LD, CJ, and YM contributed to the material preparation, data

collection, and analyses. The first draft of the manuscript was written by LD. All authors read, revised, and approved the final manuscript.

Funding

This work is supported by the Beijing Natural Science Foundation (7212029, 7212030) and the National Key R&D Program of China (No.2020YFC2003400).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2021.08.004.

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