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Relationship of Helicobacter pylori Infection to Arterial Stiffness in Japanese Subjects
(日本人におけるピロリ菌感染と動脈硬度との関係)

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Short running head: *H. pylori* and arterial stiffness

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Abstract

The role of *H. pylori* in the pathogenesis of atherosclerosis remains controversial, and the relationship between *H. pylori* and the early stage of atherosclerosis has not been fully investigated. We investigated the influence of *H. pylori* infection on arterial stiffness to clarify whether *H. pylori* infection is related to early-stage atherosclerosis. The subjects were 3412 males and 854 females. Anti-*H. pylori* antibody and C-reactive protein (CRP) level were measured. Arterial stiffness was evaluated using the brachial-ankle pulse wave velocity (PWV). In multivariate logistic repression analyses of male subjects, *H. pylori* seropositivity (odds ratio (OR) 1.27; (95% confidence interval, 1.05-1.52)) and *H. pylor* seropositivity with a high CRP value (>0.045 mg/dl) (OR 1.50 (1.14-1.98)) were significantly related to a high value of PWV. In the analyses of male subjects aged ≤49 years, *H. pylori* seropositivity (OR 1.40 (1.04-1.88)) and *H. pylori* seropositivity with a high CRP value (OR 1.81 (1.16-2.80)) were also significantly related to a high value of PWV. However, in male subjects aged ≥50 years and female subjects, no associations were found. These results suggest that inflammation following *H. pylori* infection contributes to the early stage of atherosclerosis in younger males.

Key Words: *Helicobacter pylori*; C-reactive protein; pulse wave velocity; arterial stiffness

Introduction

Atherosclerosis is now generally accepted to be an inflammatory disorder in the arterial wall (*1*). Japan has a relatively high prevalence of Helicobacter pylori among developed countries (*2*). It has been hypothesized that exposure to this agent promotes the initiation and progression of atherosclerosis. Seropositivity to *H. pylori* has been postulated to be a risk factor for cardiovascular and cerebrovascular disease (CVD) (*3, 4*). From an epidemiological perspective, however, the role of *H. pylori* in the pathogenesis of coronary artery disease remains controversial, and a recent meta-analysis revealed only limited evidence of a positive relationship (*5*).

Pulse wave velocity (PWV) is known to be an indicator of arterial stiffness (*6, 7*), and there have been many reports on PWV and the development of atherosclerotic disease (*8-10*). A simple method for noninvasive automatic measurement of brachial-ankle PWV (baPWV) has recently been developed. The technical simplicity and short sampling time of the new method make it more feasible for screening a large population than previous methods, such as carotid-femoral PWV.

However, the relation of *H. pylori* infection to arterial stiffness as a marker of early stage atherosclerosis has not been fully investigated. In this study, we investigated the influence of *H. pylori* infection on arterial stiffness to clarify whether *H. pylori* infection is related to early stage atherosclerosis.

Methods

Subjects

The subjects were local government employees (8229 men and 2194 women) aged 35 years or more who had their annual health checkup during the period from April 2003 through March 2004. We used a self-administered questionnaire including items on clinical history, family history, smoking, alcohol consumption, educational status, frequency of exercise, menopausal status, and hormone-replacement therapy. The questionnaires were distributed to the subjects in advance of their annual health checkup, and were collected at the checkup. Answers to the questionnaire and written consent to view health checkup data were obtained from 3907 men and 1044 women (response rate: men 47.5%, women 47.6%). A total of 685 subjects (495 men, 190 women) were excluded for the following reasons: past history of coronary disease or stroke (n=136; 124 men, 12 women), low ankle/brachial pressure index (<0.9, n= 12; 11 men, 1 woman), PWV not measured] (n= 600; 416 men, 184 women, or blood samples not measured (n=3; 3 women). The final study group thus consisted of 3412 male and 854 female subjects.

This study was conducted with all the subjects' written informed consent and approved by the institutional ethical board for epidemiological studies of Hokkaido University Graduate School of Medicine.

Data collection

Smoking habits were classified as “never”, “ex-smoker”, “1-19 cigarettes per day” or “≥20 cigarettes per day” for men; and “never”, “ex-smoker” or “current smoker” for women. The total average amount of alcohol consumed was calculated in grams per day, after taking into account the frequency, amount, and alcohol content for specific beverages. Alcohol consumption was categorized into “rarely or never”, “≤19.9g/day”, “20-39.9g/day”, “40-59.9g/day” or “≥60g/day” for men; and “rarely or never”, “≤19.9g/day” or “≥20/day” for women. The frequency of leisure time exercise (with perspiration) was categorized into “rarely or never”, “1-2 per week” or “≥3 per week”. Educational attainment was categorized into “high school education or less” or “more than high school education.” Women who reported that they were in the postmenopausal stage were defined as such, whether it was natural or surgically induced. If they were in the postmenopausal stage, we asked whether or not postmenopausal hormone replacement therapy had been received.

Anthropometric measures (height, body weight, and waist and hip circumferences) were recorded by a standardized protocol. The body mass index (BMI) was calculated as weight (kg)/height (m^2).

Blood samples were drawn from the antecubital vein of the seated subject with minimal tourniquet use after a 12-hour fast. Specimens were collected in siliconized glass vacuum tubes

containing sodium fluoride for blood glucose, and no additives for serum.

Total cholesterol (TC) levels were measured by an enzymatic method (Wako, Osaka, Japan). Triglyceride (TG) levels were measured by an enzymatic method (Daiichi Pure Chemicals, Tokyo, Japan), high density lipoprotein cholesterol (HDL-C) levels by a direct method (Daiichi Pure Chemicals), blood glucose levels by an amperometric method (ARKRAY, Kyoto, Japan), and uric acid (UA) by an enzymatic method (Daiichi Pure Chemicals).

CRP levels were measured by nephelometry, a latex particle-enhanced immunoassay (N Latex CRP II; Dade Behring, Tokyo, Japan). The assay could detect 0.004 mg/dl of CRP. Undetectable CRP values were recorded as 0.002 mg/dl. Serum concentrations were dichotomized with the gender-segregated median values (0.045 mg/dl for males and 0.025 mg/dl for females) as the cutoff point. Concentrations >0.045mg/dl for males and >0.025mg/dl for females were defined as high CRP, and others were defined as low CRP.

The anti-*H. pylori* antibody concentration was measured using an enzyme immunoassay (E plate; Eiken Chemical, Tokyo, Japan) (*11*)；an assay value < 10 U/ml was considered negative, and a value ≥ 10U/ml was considered positive.

All blood variables except for CRP and the anti-*H. pylori* antibody were measured at Daiichi Clinical Laboratories, Inc. (Sapporo, Japan), a commercial hematology laboratory, where the measurements of TC and HDL cholesterol were all standardized by the Lipid Standardized

Program of the Centers for Disease Control and Prevention (Atlanta, GA). CRP and the anti-*H. pylori* antibody were measured at Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan), a commercial hematology laboratory.

baPWV was measured using a volume-plethysmographic apparatus (Form PWV/AVI; model BP-203RPEII, Colin Co., Komaki, Japan) (*12-14*). This device records the phonocardiogram, electrocardiogram, and volume pulse form and arterial blood pressure at both the left and right brachia and ankles. baPWV was calculated by time-phase analysis between the right brachial and volume waveforms at both ankles. The distance between the right brachium and ankle was estimated based on body height. Yamashina et al. reported that baPWV was significantly correlated with arterial PWV measured directly by a catheter pressure transducer ($n=41$, $r=0.87$, $P<0.01$), and that the coefficients of variation of interobserver and intraobserver reproducibility were 8.4% and 10.0%, respectively (*12*). Since there was a significant positive correlation between left and right baPWV ($r=0.95$, $P<0.0001$), we used a mean right and left baPWV value during analysis.

Blood pressure, heart rate (HR), and the ankle brachial index (ABI) were measured using the pulse-wave velocimeter at the same time that PWV was measured. ABI was the ratio of ankle systolic blood pressure (SBP) to brachial SBP, and the right and left ABIs were measured simultaneously. In all the studies, baPWV was obtained after an at least 5-min rest.

Statistical analysis

All analyses were performed separately for men and women. The data of the subjects are presented as the mean ± SD, the median (and interquartile range) for variables with a skewed distribution, or percentages. Values in the two groups according to *H. pylori* serology were compared by the Student's unpaired *t*-test for variables with approximately normal distribution, by the Wilcoxon rank-sum test for variables with a skewed distribution, or by the χ^2-test for categorical data. Next, study subjects were categorized according to tertiles of PWV values with the sexes separated. Analysis of variance (ANOVA), the Kruskal-Wallis test, or the χ^2-test was used to compare data for these groups. Logistic regression analyses were used to evaluate whether *H. pylori* seropositivity and high CRP levels were predictors of high values of PWV (tertile three). As the next step, combined variables ("*H. pylori* seronegative and low CRP", "*H. pylori* seropositive or high CRP", "*H. pylori* seropositive and high CRP") were created, and their association with a high value of PWV was evaluated. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated before and after adjustment for potential confounders. The following potential confounders were included in the multivariate logistic regression models as independent variables: age, BMI, SBP, HR, TC, HDL, fasting glucose, log TG, UA, smoking status, alcohol consumption, frequency of exercise, educational attainment, medication for

hypertension, medication for hyperlipidemia, and medication for diabetes for men; and all of these variables and menopausal status for women. *P*-values <0.05 were considered to be statistically significant. All analyses were conducted using the SPSS software package Version 12 for Windows (SPSS Inc., Chicago, IL).

Results

Of the 3312 male subjects, 1586 (46.5%) had anti-*H. pylori* antibodies, and of the 854 female subjects, 358 (41.9%) had anti-*H. pylori* antibodies.

Characteristics of the groups according to *H. pylori* seropositivity are presented in Table 1 for males and Table 2 for females. In male subjects, the variables were significantly different between the *H. pylori* antibody seropositive and seronegative groups except for BMI, HR, UA, alcohol consumption, frequency of exercise, medication for hyperlipidemia, and medication for diabetes. In female subjects, the variables were significantly different between the *H. pylori* antibody seropositive and seronegative groups except for heart rate, CRP, smoking habit, frequency of exercise, medication for hypertension, medication for hyperlipidemia, medication for diabetes and current use of hormone-replacement therapy. In both genders, the mean PWV values were significantly higher in the *H. pylori* seropositive group than in the seronegative group.

Characteristics of the groups divided by PWV tertiles are shown in Table 3 for males and Table 4 for females. In male subjects, all variables except HDL cholesterol were significantly different among the PWV tertiles. In female subjects, all variables except smoking habit, frequency of exercise, and current use of hormone-replacement therapy were significantly different among the PWV tertiles. In both genders, there was a significant increase in prevalence

of *H. pylori* seropositivity across the PWV categories.

In unadjusted logistic regression analysis of all male subjects (Table 5), *H. pylori* seropositivity, high CRP, and the combination of *H. pylori* seropositivity and high CRP were significantly associated with a high value of PWV (high CRP: OR 1.57, 95%CI 1.37-1.82; *H. pylori* seropositivity: OR 1.49, 95%CI 1.29-1.72; the combination of *H. pylori* seropositivity and high CRP: OR 2.36, 95%CI 1.93-2.90). After being adjusted for age, BMI, SBP, HR, total cholesterol, HDL cholesterol, fasting glucose, log TG, uric acid, smoking status, alcohol consumption, frequency of exercise, educational attainment, and use of hypertension medication, hyperlipidemia medication, and diabetes medication, the associations with CRP disappeared (OR 1.18, 95%CI 0.97-1.44), but *H. pylori* seropositivity and the combination of *H. pylori* seropositivity and high CRP were significantly associated with a high value of PWV (*H. pylori* seropositivity: OR 1.27, 95%CI 1.05-1.52; the combination of *H. pylori* seropositivity and high CRP: OR 1.50, 95%CI 1.14-1.98).

Next we categorized subjects into two age groups ($\leq$49 and $\geq$50 years). In unadjusted logistic regression analyses of $\leq$49-year-old male subjects, *H. pylori* seropositivity, high CRP, and the combination of *H. pylori* seropositivity and high CRP were significantly associated with a high value of PWV (high CRP: OR 1.61, 95%CI 1.27-2.03; *H. pylori* seropositivity: OR 1.47, 95%CI 1.16-1.86; the combination of *H. pylori* seropositivity and high CRP: OR 2.36, 95%CI 1.69-3.30).

After being adjusted for cited possible confounders, the associations with CRP disappeared (OR 1.28, 95%CI 0.94-1.75), but *H. pylori* seropositivity and the combination of *[H. pylori]* seropositivity and high CRP were significantly associated with a high value of PWV (*H. pylori* seropositivity: OR 1.40, 95%CI 1.04-1.88; the combination of *H. pylori* seropositivity and high CRP: OR 1.81, 95%CI 1.16-2.80). In unadjusted logistic regression analyses of ≥50-year-old male subjects, high CRP, and the combination of *H. pylori* seropositivity and high CRP were significantly associated with a high value of PWV (high CRP: OR 1.53, 95%CI 1.26-1.86; the combination of *H. pylori* seropositivity and high CRP: OR 1.56, 95%CI 1.19-2.07), but *H. pylori* seropositivity was not (*H. pylori* seropositivity: OR 1.01, 95%CI 0.83-1.23). After being adjusted for possible confounders, OR: for the possible confounders described above, these associations disappeared (high CRP: OR 1.14, 95%CI 0.87-1.50; *H. pylori* seropositivity: OR 0.91, 95%CI 0.70-1.18; the combination of *H. pylori* seropositivity and high CRP: OR 1.04, 95%CI 0.71-1.52).

In unadjusted logistic regression analysis of all female subjects (Table 6), *H. pylori* seropositivity, high CRP, and the combination of *H. pylori* seropositivity and high CRP were significantly associated with a high value of PWV (high CRP: OR 2.30, 95%CI 1.72-3.09; *H. pylori* seropositivity: OR 1.60, 95%CI 1.19-2.16; the combination of *H. pylori* seropositivity and high CRP: OR 4.02, 95%CI 2.54-6.38). After adjusting for age, BMI, SBP, HR, total cholesterol, HDL cholesterol, fasting glucose, log TG, UA, smoking status, alcohol consumption, frequency of

exercise, educational attainment, medication of hypertension, medication of hyperlipidemia, medication of diabetes, and menopausal status, these associations disappeared (high CRP: OR 1.46, 95%CI 0.97-2.21; *H. pylori* seropositivity: OR 1.11, 95%CI 1.74-1.67; the combination of *H. pylori* seropositivity and high CRP: OR 1.66, 95%CI 0.89-3.09).

We also categorized subjects into two age groups (≤49 and ≥50 years). In unadjusted logistic regression analyses of female subjects ≤49 years old, high CRP and a combination of *H. pylori* seropositivity and high CRP were significantly associated with high value of PWV (high CRP: OR 1.81, 95%CI 1.26-2.60; the combination of *H. pylori* seropositivity and high CRP: OR 2.86, 95%CI 1.59-5.14), but *H. pylori* seropositivity was not (OR 1.34, 95%CI 0.92-1.96). After being adjusted for possible confounders, these were no associations with a high value of PWV (high CRP: OR 1.70, 95%CI 0.78-3.73; *H. pylori* seropositivity: OR 1.15, 95%CI 0.69-1.93; the combination of *H. pylori* seropositivity and high CRP: OR 1.41, 95%CI 0.85-2.33). In unadjusted logistic regression analyses of ≥50-year-old female subjects, high CRP and the combination of *H. pylori* seropositivity and high CRP were significantly associated with a high value of PWV (high CRP: OR 2.42, 95%CI 1.36-4.32; the combination of *H. pylori* seropositivity and high CRP: OR 2.74, 95%CI 1.15-6.52), but *H. pylori* seropositivity was not (OR 1.13, 95%CI 0.63-2.01). After being adjusted for possible confounders, there were no associations with a high value of PWV (high CRP: OR 1.87, 95%CI 0.86-4.08; *H. pylori* seropositivity: OR 1.07, 95%CI 0.50- 2.26; the

combination of *H. pylori* seropositivity and high CRP: OR 1.95, 95%CI 0.62-6.12).

Discussion

In this study, *H. pylori* seropositivity and a combination of *H. pylori* seropositivity and high CRP were significantly associated with an increase of arterial stiffness in males even after controlling for known and potential cardiovascular risk factors, particularly in those less than 50 years old. Therefore, it is probable that inflammation following *H. pylori* infection contributes to the early stage of atherosclerosis in younger males. It has been postulated that seropositivity to *H. pylori* is a risk factor for cardiovascular and cerebrovascular disease (*3, 4*). But a recent meta-analysis revealed only limited evidence of a positive relationship (*5*). Meanwhile, in a case control study of a Japanese population, *H. pylori* seropositivity was significantly associated with acute myocardial infarction (AMI) in subjects <55 years (OR 2.97), but was not associated with AMI for subjects aged ≥55 years (55-64 years: OR 1.03; >65 years: OR 0.79) (*15*). Another Japanese case-control study showed that *H. pylori* seropositivity was significantly associated with coronary heart disease (OR 1.34) (*16*). It has been reported that genetic polymorphisms were related to *H. pylori*-associated gastric cancer or peptic ulcer (*17, 18*). Thus, a racial difference in the susceptibility to atherosclerotic diseases conferred by *H. pylori* infection might be present through racial-specific genetic characteristics. Furthermore, there were significant associations between *H. pylori* seropositivity and atherosclerosis in the younger subjects of our study and in the subjects of the former Japanese case-control study *(16)*. This is probably because the

associations of most risk factors with CVD and coronary heart disease (CHD) tend to be stronger in younger than in older subjects (*19, 20*).

Adachi et al. reported that heart carotid PWV was higher in *H. pylori* seropositive than *H. pylori* seronegative young (<39-year-old) subjects (*21*). However, the sample size was rather small (683 males and 313 females) and the analysis was only adjusted by age, sex, BMI, smoking, and drinking habits; i.e., heart rate, blood pressure, TC, TG, HDL-C, and fasting glucose were not included in the model. Additionally, there was no data on the socioeconomic status of the subjects. Thus, the results were not adjusted for potential confounders.

The present study could not identify a causal role for *H. pylori* infection in the pathogenesis of arterial stiffness. However, it has been reported that *H. pylori* is detected not only in gastric mucosa but also in human atherosclerotic plaque (*22, 23*), and the expression of intercellular adhesion molecule-1 is higher in plaques containing *H. pylori* than in those which do not (*23*). *H. pylori* infection stimulates the production of proinflammatory cytokines such as tumor necrosis factor, interleukin-6, and interleukin-8 (*24, 25*), and the association of *H. pylori* with elevations of serum CRP and fibrinogen has been reported (*3, 26*). In this study, male subjects aged ≤49 years with both *H. pylori* seropositivity and high CRP had a relatively high OR (1.81). Therefore, it is considered that there is an effect on atherosclerosis when *H. pylori* infection induces its higher inflammatory response. Antioxidants have been shown to be decreased in subjects with *H. pylori*

(*27*). This could contribute to lipid peroxidation, which in turn could result in atherosclerosis (*28*).

In contrast, in adjusted analyses of female subjects, neither *H. pylori* seropositivity nor the combination of *H. pylori* seropositivity and high CRP was associated with arterial stiffness. The smaller sample size (a quarter of that of male subjects) reduced the statistical power. Also, it is well known that menopause aggravates the progression of atherosclerosis (*29*), and the CRP level of females is lower than that of males (*30*). In another previous study, the baPWV of females was significantly lower than that of males in subjects ≤55 years old (*13*), so *H. pylori* infection in younger females might have a limited effect on arterial stiffness.

A recent large study consisting of 5,732 Japanese showed that the overall prevalence of *H. pylori* seropositivity was 47.0% (*31*). The prevalence of *H. pylori* seropositivity in our male subjects resembled that of this previous report, but for females it was rather small, presumably because our female subjects were somewhat younger than our male subjects. Because this study examined IgG antibodies to *H. pylori*, which can reflect a previous infection, IgG seropositivity to *H. pylori* may not reflect active infection. But the relative sensitivity, specificity and rates of agreement between the results obtained using the enzyme immunoassay employed in the present study (E plate) and those obtained by the culture/rapid urease test have been reported to be 100%, 80.0%, and 97.1%, respectively (*12*). Thus the serological method to detect *H. pylori* infection should be selected for screening a large population.

Socioeconomic status is known to affect both the prevalence of *H. pylori* infection and the risk of atherosclerosis (*32*). We could not obtain the subjects' income data, but the subjects worked for one local government. We therefore believe that the subjects were socioeconomically similar, and our data were adjusted for educational attainment, so it was considered that the influence of socioeconomic status on the adjusted OR was practically nil.

The present study has several limitations. First, this study could not identify a causal role for *H. pylori* infection in the pathogenesis of arterial stiffness. Second, we measured only the seropositivity of *H. pylori* infection. However, as previously mentioned, we believe that the serological method to detect *H. pylori* infection is sufficient for a large population study. Third, we could not obtain information about incomes of the subjects. However, as previously mentioned, we considered that the influence of socioeconomic status on the adjusted OR was practically nil. Forth, statins lower the level of CRP (*33*, *34*). We knew whether the subjects were taking medication for hyperlipidemia or not, but we could not identify the name of their medications. Thus, the analyses were adjusted for medication for hyperlipidemia. Finally, the number of female subjects was rather small. A further study with a larger number of female subjects is thus required.

In summary, our results indicate that *H. pylori* seropositivity and *H. pylori* seropositivity with elevated CRP levels are significantly associated with an increase of arterial stiffness in males, particularly those less than 50 years old. Therefore, it is probable that inflammation following *H.*

pylori infection contributes to the early stage of atherosclerosis in younger males. Further prospective studies are needed to clarify the relationship between *H. pylori* infection and atherosclerotic diseases. Because *H. pylori* infection can be eradicated by a short course of combination antibiotic therapy, studies will be needed to determine whether its eradication reduces the incidence of atherosclerotic diseases.

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Table 1. Characteristics of *H. pylori* seropositive and seronegative male subjects

	H. pylori positive (n=1586)	*H. pylori* negative (n=1826)	*P*-value
Age (y)	50.3 ± 6.1	46.7 ± 7.0	<0.00001
BMI (kg/m^2)	23.9 ± 2.9	23.7 ± 2.9	0.06
SBP (mmHg)	124.4 ± 15.9	121.4 ± 14.6	<0.00001
DBP (mmHg)	79.2 ± 10.9	76.7 ± 10.7	<0.00001
Heart rate (bpm)	60.6 ± 9.4	60.6 ± 9.6	0.96
Total cholesterol (mg/dl)	209.1 ± 33.8	205.9 ± 32.9	<0.01
Triglycerides (mg/dl)	108 (77-156)	103 (74-150)	<0.05
HDL cholesterol (mg/dl)	55.5 ± 13.9	57.8 ± 14.7	<0.00001
Fasting glucose (mg/dl)	96.9 ± 22.2	94.9 ± 19.9	<0.01
Uric acid (mg/dl)	5.9 ± 1.2	5.9 ± 1.2	0.65
CRP (mg/dl)	0.047 (0.024-0.090)	0.042 (0.022-0.088)	<0.05
High CRP (>0.045mg/dl), (%)	51.8	47.8	<0.05
Smoking status (%)			
Never smoker	22.1	25.0	<0.001
Ex-smoker	30.1	23.5	
Current smoker			
1-19 cigarettes/day	9.7	11.0	
≥20 cigarettes/day	37.8	40.5	
Alcohol consumption (%)			
Rarely or never	27.8	26.4	0.06
≤19.9g/day	23.1	26.7	
20-39.9g/day	18.7	19.3	
40-59.9g/day	12.1	12.0	
≥60g/day	18.3	15.6	

Frequency of exercise (%)			
Rarely or never	54.6	54.4	0.74
1-2/week	28.6	29.6	
≥3week	16.8	16.0	
Educational attainment (%)			
High school education or less	61.7	53.5	<0.00001
More than high school education	38.3	46.5	
Medication of			
Hypertension (%)	11.0	7.8	<0.01
Hyperlipidemia (%)	5.4	4.7	0.39
Diabetes (%)	2.6	2.0	0.23
PWV (cm/s)	1397.2 ± 209.6	1343.9 ± 187.1	<0.00001

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage. BMI: body mass index; SBP: systolic blood pressure; DPB: diastolic blood pressure; CRP: C-reactive protein.

Table 2. Characteristics of *H. pylori* seropositive and seronegative female subjects

	H. pylori positive (n=358)	*H. pylori* negative (n=496)	*P*-value
Age (y)	48.7 ± 6.8	45.4 ± 7.1	<0.00001
BMI (kg/m^2)	22.2 ± 3.4	21.6 ± 3.3	<0.01
SBP (mmHg)	116.6 ± 16.4	113.1 ± 14.9	<0.005
DBP (mmHg)	70.3 ± 10.3	68.6 ± 10.2	<0.05
Heart rate (bpm)	59.8 ± 8.1	59.5 ± 8.2	0.61
Total cholesterol (mg/dl)	213.9 ± 30.3	204.3 ± 33.0	<0.0001
Triglycerides (mg/dl)	74 (53-97)	61 (48-88)	<0.0001
HDL cholesterol (mg/dl)	67.9 ± 14.9	71.0 ± 15.0	<0.005
Fasting glucose (mg/dl)	88.8 ± 13.8	88.7 ± 15.5	0.92
Uric acid (mg/dl)	4.6 ± 1.0	4.4 ± 1.0	0.06
CRP (mg/dl)	0.026 (0.014-0.053)	0.025 (0.011-0.052)	0.3
High CRP (>0.025mg/dl), (%)	50.3	49.6	0.84
Smoking status (%)			
Never smoker	65.6	67.5	0.77
Ex-smoker	9.8	8.5	
Current smoker	24.6	24	
Alcohol consumption (%)			
Rarely or never	51.1	44.4	<0.05
≤19.9g/day	36.9	36.7	
≥20g/day	12.0	19.0	
Frequency of exercise (%)			
Rarely or never	67.1	67.6	0.25
1-2/week	24.8	21.5	
≥3week	8.1	10.9	

Educational attainment (%)			
High school education or less	48.9	40.3	<0.05
More than high school education	51.1	59.7	
Medication of			
Hypertension (%)	5.9	3.2	0.06
Hyperlipidemia (%)	6.1	3.6	0.09
Diabetes (%)	0.8	1.0	0.8
Menopausal Status (%)			
Postmenopausal	47.5	33.5	<0.00001
Current use of hormone-replacement therapy (%)	1.4	2.4	0.29
PWV (cm/s)	1273.4 ± 184.4	1233.4 ± 176.0	<0.005

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage. BMI: body mass index; SBP: systolic blood pressure; DPB: diastolic blood pressure; CRP: C-reactive protein.

Table 3. Characteristics in tertiles of PWV in male subjects

	PWV category			*P*-value
	Tertile 1 (n=1139)	Tertile 2 (n=1141)	Tertile 3 (n=1132)	
PWV range (cm/s)	916-1266	1267-1413	1414-2468	
Age (y)	45.4 ± 6.6	48.2 ± 6.6	51.5 ± 5.9	<0.00001
BMI (kg/m^2)	23.4 ± 2.7	23.9 ± 2.9	24.2 ± 3.0	<0.00001
SBP (mmHg)	112.3 ± 9.0	121.0 ± 11.2	135.2 ± 10.4	<0.00001
DBP (mmHg)	70.4 ± 7.3	77.2 ± 8.4	86.1 ± 9.0	<0.00001
Heart rate (bpm)	57.4 ± 8.1	60.3 ± 9.0	64.2 ± 10.0	<0.00001
Total cholesterol (mg/dl)	204.0 ± 33.1	208.1 ± 32.6	209.9 ± 34.1	<0.00001
Triglycerides (mg/dl)	94 (67-137)	106 (75-153)	116 (84-168)	<0.00001
HDL cholesterol (mg/dl)	56.8 ± 14.5	56.7 ± 14.2	56.6 ± 14.5	0.93
Fasting glucose (mg/dl)	90.4 ± 11.4	94.2 ± 14.2	103.0 ± 30.2	<0.00001
Uric acid (mg/dl)	5.7 ± 1.1	5.9 ± 1.2	6.0 ± 1.2	<0.00001
CRP (mg/dl)	0.035 (0.019-0.071)	0.046 (0.023-0.092)	0.052 (0.028-0.109)	<0.00001
High CRP (>0.045mg/dl), (%)	40.9	50.7	57.4	<0.00001
Smoking status (%)				
Never smoker	23.4	24.5	23.1	<0.05

Ex-smoker	23.4	27.7	28.8	
Current smoker				
1-19 cigarettes/day	12.6	9.5	9.3	
≥20 cigarettes/day	40.6	38.4	38.8	
Alcohol consumption (%)				
Rarely or never	30.3	26.8	24.0	<0.00001
≤19.9g/day	29.1	25.4	20.5	
20-39.9g/day	18.4	20.0	18.6	
40-59.9g/day	9.9	12.4	13.9	
≥60g/day	12.3	15.3	23.0	
Frequency of exercise (%)				
Rarely or never	53.0	52.2	58.4	<0.05
1-2/week	30.7	30.2	26.5	
≥3week	16.2	17.5	15.1	
Educational attainment (%)				
High school education or less	52.2	59.2	60.6	<0.0001
More than high school education	47.8	40.8	39.4	
Medication of				
Hypertension (%)	0.8	7.1	20.1	<0.00001

Hyperlipidemia (%)	2.9	4.8	7.3	<0.00001
Diabetes (%)	0.5	1.8	4.7	<0.00001
H. pylori seropositive (%)	38.5	47.6	53.4	<0.00001

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage. BMI: body mass index; SBP: systolic blood pressure; DPB: diastolic blood pressure; CRP: C-reactive protein.

Table 4. Characteristics in tertiles of PWV in female subjects

	PWV category			*P*-value
	Tertile 1 (n=286)	Tertile 2 (n=284)	Tertile 3 (n=284)	
PWV range (cm/s)	893-1156	1157-1300	1301-2198	
Age (y)	43.2 ± 6.4	46.4 ± 6.7	50.7 ± 6.4	<0.00001
BMI (kg/m^2)	21.1 ± 2.8	21.8 ± 3.0	22.6 ± 4.0	<0.00001
SBP (mmHg)	103.3 ± 7.8	112.9 ± 10.4	127.5 ± 16.4	<0.00001
DBP (mmHg)	62.1 ± 6.3	68.9 ± 7.7	77.0 ± 10.4	<0.00001
Heart rate (bpm)	57.7 ± 6.8	59.2 ± 7.8	62.0 ± 9.2	<0.00001
Total cholesterol (mg/dl)	197.2 ± 30.0	209.3 ± 31.5	218.5 ± 31.7	<0.00001
Triglycerides (mg/dl)	56 (45-72)	70 (50-96)	78 (60-113)	<0.00001
HDL cholesterol (mg/dl)	71.6 ± 13.5	69.6 ± 15.5	67.8 ± 15.8	<0.05
Fasting glucose (mg/dl)	83.9 ± 6.8	87.9 ± 10.2	94.6 ± 21.2	<0.00001
Uric acid (mg/dl)	4.2 ± 0.9	4.5 ± 1.0	4.8 ± 1.1	<0.00001
CRP (mg/dl)	0.018 (0.009-0.036)	0.028 (0.012-0.052)	0.032 (0.019-0.074)	<0.00001
High CRP (>0.025mg/dl), (%)	34.6	52.1	61.3	<0.00001
Smoking status (%)				
Never smoker	66.8	66.2	67.3	0.86

Ex-smoker	7.7	9.5	9.9	
Current smoker	25.5	24.3	22.9	
Alcohol consumption (%)				
Rarely or never	47.9	43.0	50.7	<0.005
≤19.9g/day	42.0	35.9	32.4	
≥20g/day	10.1	23.1	16.9	
Frequency of exercise (%)				
Rarely or never	70.3	67.3	64.4	0.07
1-2/week	18.2	25.0	27.1	
≥3week	11.5	7.7	8.5	
Educational attainment (%)				
High school education or less	36.0	42.3	53.5	<0.0001
More than high school education	64.0	57.7	46.5	
Medication of				
Hypertension (%)	0.7	0.7	11.6	<0.00001
Hyperlipidemia (%)	1.0	2.1	10.9	<0.00001
Diabetes (%)	0.0	0.4	2.5	<0.005
Menopausal Status (%)				
Postmenopausal	22.0	34.2	62.0	<0.00001

Current use of hormone-replacement therpy (%)	2.1	2.5	1.4	0.66
H. pylori seropositive (%)	34.6	40.8	50.4	<0.001

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage. BMI: body mass index; SBP: systolic blood pressure; DPB: diastolic blood pressure; CRP: C-reactive protein.

Table 5. Association of *H. pylori* seropositivity, CRP levels, and their combination with high values of PWV (tertile three category) in male subjects

Parameter	Unadjusted OR (95%CI)	*P*-value	Adjusted OR[a] (95%CI)	*P*-value
All (n=3412)				
H. pylori seropositive	1.49 (1.29-1.72)	<0.00001	1.27 (1.05-1.52)	0.013
High CRP (>0.045mg/dl)	1.57 (1.37- 1.82)	<0.00001	1.18 (0.97-1.44)	0.10
Combination				
H. pylori seronegative and low CRP	Reference		Reference	
H. pylori seropositive or high CRP	1.62 (1.36-1.94)	<0.00001	1.25 (0.99-1.58)	0.062
H. pylori seropositive and high CRP	2.36 (1.93-2.90)	<0.00001	1.50 (1.14-1.98)	<0.005
≤49 years old (n=1759)				
H. pylori seropositive	1.47 (1.16-1.86)	<0.005	1.40 (1.04-1.88)	0.026
High CRP (>0.045mg/dl)	1.61 (1.27- 2.03)	<0.0001	1.28 (0.94-1.75)	0.12
Combination				
H. pylori seronegative and low CRP	Reference		Reference	

H. pylori seropositive or high CRP	1.65	(1.24-2.19)	<0.0001	1.31	(0.92-1.86)	0.13
H. pylori seropositive and high CRP	2.36	(1.69- 3.30)	<0.00001	1.81	(1.16-2.80)	<0.01
≥50 years old (n=1653)						
H. pylori seropositive	1.01	(0.83-1.23)	0.90	0.91	(0.70 -1.18)	0.47
High CRP (>0.045mg/dl)	1.53	(1.26-1.86)	<0.0001	1.14	(0.87- 1.50)	0.34
Combination						
H. pylori seronegative and low CRP		Reference			Reference	
H. pylori seropositive or high CRP	1.29	(1.00-1.67)	0.049	1.10	(0.78-1.54)	0.59
H. pylori seropositive and high CRP	1.56	(1.19-2.07)	<0.005	1.04	(0.71-1.52)	0.85

[a]Adjusted for age, body mass index, systolic blood pressure, heart rate, total cholesterol, HDL cholesterol, fasting glucose, log triglycerides, uric acid, smoking status, alcohol consumption, frequency of exercise, educational attainment, medication of hypertension, medication of hyperlipidemia, and medication of diabetes.

Table 6. Association of *H. pylori* seropositivity, CRP levls, and their combination with high values of PWV (tertile three category) in female subjects

Parameter	Unadjusted OR (95%CI)	*P*-value	Adjusted OR[a] (95%CI)	*P*-value
All (n=854)				
H. pylori seropositive	1.60 (1.19-2.16)	<0.005	1.11 (1.74-1.67)	0.63
High CRP (>0.025mg/dl)	2.30 (1.72-3.09)	<0.00001	1.46 (0.97-2.21)	0.07
Combination				
H. pylori seronegative and low CRP	Reference		Reference	
H. pylori seropositive or high CRP	1.68 (1.22-2.31)	<0.005	1.20 (0.77-1.86)	0.41
H. pylori seropositive and high CRP	4.02 (2.54-6.38)	<0.00001	1.66 (0.89-3.09)	0.11
≤49 years old (n=503)				
H. pylori seropositive	1.34 (0.92-1.96)	0.13	1.15 (0.69-1.93)	0.58
High CRP (>0.025mg/dl)	1.81 (1.26-2.60)	<0.005	1.70 (0.78-3.73)	0.18
Combination				
H. pylori seronegative and low CRP	Reference		Reference	

H. pylori seropositive or high CRP	1.32	(0.90-1.93)	0.15	1.12	(0.67-1.88)	0.66
H. pylori seropositive and high CRP	2.86	(1.59-5.14)	<0.001	1.41	(0.85-2.33)	0.18
≥50 years old (n=351)						
H. pylori seropositive	1.13	(0.63-2.01)	0.68	1.07	(0.50-2.26)	0.86
High CRP (>0.025mg/dl)	2.42	(1.36-4.32)	<0.005	1.87	(0.86-4.08)	0.12
Combination						
H. pylori seronegative and low CRP		Reference			Reference	
H. pylori seropositive or high CRP	1.48	(0.72-3.01)	0.28	1.45	(0.57-3.73)	0.43
H. pylori seropositive and high CRP	2.74	(1.15-6.52)	0.02	1.95	(0.62-6.12)	0.25

[a]Adjusted for age, body mass index, systolic blood pressure, heart rate, total cholesterol, HDL cholesterol, fasting glucose, log triglycerides, uric acid, smoking status, alcohol consumption, frequency of exercise, educational attainment, medication of hypertension, medication of hyperlipidemia, medication of diabetes, and menopausal status.

Abbreviations

CRP: C-reactive protein

PWV: pulse wave velocity

baPWV: brachial-ankle pulse wave velocity

OR: odds ratio

CVD: cerebrovascular disease

BMI: body mass index

TC: total cholesterol

TG: triglyceride

HDL-C: high density lipoprotein cholesterol

UA: uric acid

HR: heart rate

ABI: ankle brachial index

ANOVA: analysis of variance

CI: confidence interval

AMI: acute myocardial infarction (AMI)

CHD: coronary heart disease

SBP: systolic blood pressure

DPB: diastolic blood pressure