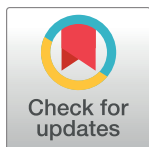


RESEARCH ARTICLE

# Current *Helicobacter pylori* infection is significantly associated with subclinical coronary atherosclerosis in healthy subjects: A cross-sectional study

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## Abstract

*Helicobacter pylori* is a gastrointestinal pathogen known to be associated with cardiovascular disease (CVD). However, most analyses about the effect of *H. pylori* infection have been done in patients with a history of CVD but not in healthy subjects. We evaluated the association between *H. pylori* infection and subclinical atherosclerosis by using cardiac multidetector computed tomography (MDCT) in healthy subjects without previous CVD. From December 2007 to February 2014, 463 subjects who underwent the rapid urease test (CLO test), pulse-wave velocity (PWV) measurement, and MDCT for a self-referred health check-up were enrolled to this study. *Helicobacter pylori* infection was defined on the basis of CLO test positivity on endoscopic gastric biopsy. Significant coronary artery stenosis was defined as  $\geq 50\%$  stenosis in any of the major epicardial coronary vessel on MDCT. The CLO-positive subjects had a lower high-density lipoprotein-cholesterol (HDL-cholesterol) level compared to the CLO-negative subjects. The incidence of significant coronary stenosis was higher in the CLO-positive group (7.6% vs. 2.9%,  $P = 0.01$ ). Furthermore, the number of subjects with coronary artery calcium score  $> 0$  and  $\log\{(\text{number of segments with plaque}) + 1\}$  were also significantly higher in the CLO-positive group. However, there was no statistical difference in the number of subjects with coronary artery calcium score  $> 100$ , the prevalence of any plaque nor the plaque characteristics (calcified, mixed, or soft). Pulse-wave velocity (PWV) was neither associated with CLO test positivity. The CLO-positive group was 3-fold more likely to have significant coronary artery stenosis even after adjusting for confounding factors (adjusted odds ratio 2.813, 95% confidence interval 1.051–7.528,  $P = 0.04$ ). In a healthy population, current *H. pylori* infection was associated with subclinical but significant

coronary artery stenosis. The causal relationship between *H. pylori* infection and subclinical atherosclerosis in a “healthy” population remains to be investigated in the future.

## Introduction

Several studies have reported that cardiovascular disease (CVD) may be associated with certain microorganisms such as *Chlamydia pneumonia*, cytomegalovirus, or *Helicobacter pylori* [1]. Those reports have suggested that the pathogens might directly invade the vessel wall, leading to localized vascular inflammation [2]. Additionally, not only localized but also systemic inflammation by such pathogens might indirectly induce endothelial dysfunction and dyslipidemia, resulting in CVD [3].

*Helicobacter pylori* is a gram-negative bacterium that lives in the stomach and a carcinogen leading to stomach cancer [4,5]. The infection rate of *H. pylori* is especially high in Asians [6]. Because local and systemic inflammation by microbes and infectious agents is known to be crucial in CVD [7,8], the relationship between *H. pylori* infection and CVD has received considerable attention especially in Asian countries [9–12].

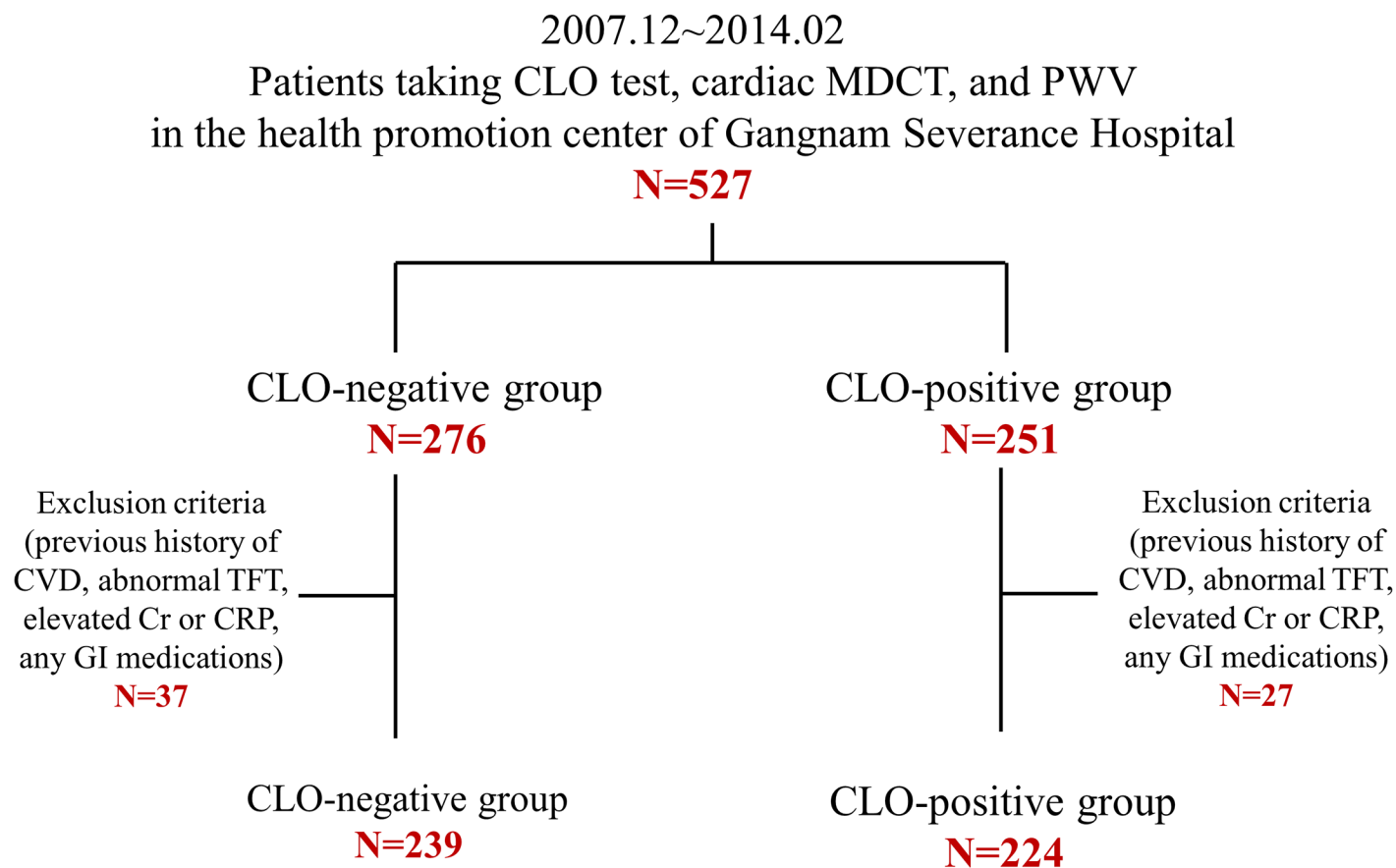
*H. pylori* has been suggested as a possible contributor to CVD progression but the results from the previous studies were quite controversial. Some reports showed that the seroprevalence of *H. pylori* was not correlated with coronary artery disease and they insisted that the infection status of *H. pylori* did not determine the risk of CVD [13–16]. In contrast, other reports demonstrated a meaningful higher prevalence of *H. pylori* infection among patients with previous history of CVD and supported a possible connection between *H. pylori* and the development of CVD [12,17–21]. In healthy populations without previous CVD, however, it still remains unclear whether *H. pylori* infection is significantly associated with subclinical atherosclerosis. The individuals with subclinical atherosclerosis are at a higher risk of developing clinical atherosclerosis including coronary heart disease compared to the individuals without subclinical diseases [22–24]. If the association between *H. pylori* and subclinical atherosclerosis is significant, earlier diagnosis and possibly eradication of *H. pylori* might be necessary for preventing atherosclerosis progression, especially in the high-risk population. Additionally, most studies on *H. pylori* infection and CVD have relied on antibody tests for defining the *H. pylori* infection status [17,18,20], the results of which cannot differentiate between current versus past infections. Additionally, tests using the serum antibodies demonstrate lower specificity than that of the rapid urease test (CLO test) [25,26].

In this study, we aimed to evaluate whether current *H. pylori* infection, diagnosed by using an endoscopic CLO test, was associated with subclinical atherosclerosis using pulse-wave velocity (PWV) and cardiac multidetector computed tomography (MDCT) in a healthy population.

## Methods

### Study design and population

This study included 527 consecutive subjects undergoing esophagogastroduodenoscopy with the CLO test, PWV, and MDCT as part of the self-referred health check-up program at Gangnam Severance Hospital Health Promotion Center, Seoul, Korea from December 2007 to February 2014. All participants were divided into two study groups according to the CLO positivity. To avoid confounding bias, we excluded possible confounding medical conditions known to be associated with the health outcome. The exclusion criteria were as follows: (i)



**Fig 1. Schematic diagram and overall flow of study participants.** Abbreviations: CLO test, rapid urease test; cardiac MDCT, cardiac multidetector computed tomography; PWV, pulse-wave velocity; CVD, cardiovascular disease; TFT, thyroid function test; Cr, creatinine; CRP, C-reactive protein; GI medications, gastrointestinal medications.

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history of coronary artery disease, (ii) significant hypothyroidism/hyperthyroidism (thyroid-stimulating hormone  $>4.5$   $\mu$ IU/mL or free thyroxine  $>2.0$  ng/dL), (iii) significant renal insufficiency (creatinine  $>1.5$  mg/dL), (iv) significant CRP (C-reactive protein) elevation ( $>10.0$  mg/L) and (v) subjects with any GI (gastrointestinal) medications. Since we intended to investigate the association between *H. pylori* infection and subclinical atherosclerosis in healthy population, subjects with previous coronary artery disease were excluded. Subjects with thyroid dysfunction and renal insufficiency were omitted as changes in thyroid or renal function influence the level of serum lipids along with coronary atherosclerosis [27–29]. We excluded the subjects with elevated CRP levels as various pro-inflammatory conditions and diseases can affect the progression of atherosclerosis [30,31]. We further excluded the subjects taking GI medications for gastritis or gastric ulcer. Finally, 463 participants were included in the current analysis (Fig 1). The protocol of this study was approved by the institutional review board of Gangnam Severance Hospital.

### Anthropometric and biochemical measurements

We reviewed the participants' questionnaire at the time of the health check-up, which included medical history, concomitant medication use, and other medico-social history information. After an overnight fasting for  $\geq 8$  hours, the fasting plasma glucose, total cholesterol, HDL-

cholesterol, triglyceride, calcium, and C-reactive protein levels were checked from the peripheral venous blood samples by means of adequate enzymatic methods (Hitachi 7600–120 automated chemistry analyzer; Hitachi, Tokyo, Japan). The Friedewald formula was used to calculate the low-density lipoprotein-cholesterol (LDL-cholesterol) level.

### Coronary artery calcium score, coronary artery stenosis, and coronary plaque measurement with MDCT

Coronary artery calcium scores (CACs), coronary artery stenosis, and the number, presence and the characteristics of intracoronary plaques were evaluated by using an MDCT scanner (Philips Brilliance 64; Philips Medical System, Best, the Netherlands). A standard prospective electrocardiogram-gating protocol with a step-and-shoot technique (64 × 0.625 mm slice section collimation, 420-ms rotation time, 120-kV tube voltage, and 210-mAs tube current) was used. The CACS was measured (Extended Brilliance Workspace BW V4.5.2.4031, Philips Medical System) and described as Agatston scores. The CACS was interpreted as having either no coronary calcium (CACS 0 vs. >0) or severe coronary calcium (CACS ≤100 vs. >100). Coronary arteries were segmented to 3 major arteries and 12 small branches. Significant coronary artery stenosis was defined as at least ≥50% stenosis in any coronary artery/branch. A plaque was defined as a structure >1 mm<sup>2</sup> within and/or adjacent to the vessel lumen, and classified according to the presence/proportion of intraplaque calcification. The plaques were divided into calcified (calcium [≥130 Hounsfield units] content ≥50% of the whole plaque), mixed (calcium content <50% of the whole plaque) or soft (no calcium content). Log{(number of segments with plaque) + 1} was used to analyze the number of segments with plaque.

### Pulse-wave velocity

Brachial-ankle PWV (ba-PWV) was assessed by using a volume plethysmographic instrument (VP-1000; Omron Healthcare Corporation, Kyoto, Japan). The ba-PWV was estimated as the value of brachial-ankle distance divided by the blood transit time. An automatic device recorded electrocardiograms, phonocardiograms and the blood pressure (BP) at both the brachial artery and the posterior tibial artery after a stable rest for ≥5 min in supine position. The average value of the right and left sides was defined as the mean PWV.

### Rapid urease test

Biopsy of the gastric mucosa was performed to assess the *H. pylori* infection status with a commercial CLO kit (ASAN Pharm. Co., Seoul, Korea). Each gastric mucosal biopsy specimen was immediately transferred into the CLO kit and analyzed after 30 min of reaction to determine the test positivity according to the color change of the reaction kit.

### Statistical analyses

Continuous variables with normal distribution are presented as mean ± standard error (SE), and those with a skewed distribution, such as CACS or the number of coronary segments with any plaque, were log transformed for analyses. Categorical variables are presented as absolute numbers and percentages. Intergroup comparisons with age adjustment were performed by using analysis of covariance for continuous variables and the generalized estimating equation for categorical variables. Adjusted odds ratios (ORs) for significant coronary artery stenosis in the CLO-positive group compared with the negative group were estimated with logistic regression analysis models. The SPSS statistical package (version 20.0; SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A *P*-value of ≤0.05 was considered statistically significant.

## Results

### Clinical and biochemical characteristics of the study population

A total of 463 subjects (336 men, 127 women, mean age  $54.2 \pm 8.5$  years) were analyzed for the current study and divided into two groups according to the CLO test result. There were 224 (48.4%) CLO-positive and 239 (51.6%) CLO-negative subjects, respectively. The clinical and biochemical characteristics of the study subjects are presented in Table 1. The minimum and maximum age of this whole study population was 31 and 80 years, respectively (33–80 years for the CLO-negative group; and 31–77 years for the CLO-positive group). The mean age was different between the two groups ( $53.2 \pm 7.9$  years for the CLO-positive group vs.  $55.3 \pm 8.9$  years for the CLO-negative group,  $P = 0.01$ ). Therefore, comparisons throughout this study were performed after adjustment for age as a confounding variable. The CLO-positive group had a lower mean HDL-cholesterol level ( $46.6 \pm 0.8$  mg/dL vs.  $49.6 \pm 0.8$  mg/dL,  $P = 0.01$ ) and a higher mean triglyceride level ( $134.5 \pm 5.7$  mg/dL vs.  $116.4 \pm 5.5$  mg/dL,  $P = 0.02$ ). There were no differences in the prevalence of diabetes and hypertension nor the use of antidiabetic, antihypertensive, and antiplatelet agents between the two groups. Subjects in the CLO-positive group were more likely to take a lipid-lowering agent compared to those in the CLO-negative group (15.6% vs. 10.5%,  $P = 0.05$ ).

**Table 1. Clinical and biochemical characteristics of study subjects with age adjustment.**

	Total N = 463	CLO-negative N = 239	CLO-positive N = 224	P-value
<b>Demographics</b>				
Age (yr)	$54.2 \pm 8.5$	$55.3 \pm 8.9$	$53.2 \pm 7.9$	0.01
minimum and maximum	31–80	33–80	31–77	
Male (N, %)	336 (72.6)	165 (69.0)	171 (76.3)	0.07
Hypertension (N, %)	213 (46.0)	113 (47.3)	100 (44.6)	0.89
Diabetes (N, %)	79 (17.1)	39 (16.3)	40 (17.9)	0.35
Diabetes medication (N, %)	46 (9.9)	27 (11.3)	19 (8.5)	0.58
Lipid lowering agent (N, %)	60 (13.0)	25 (10.5)	35 (15.6)	0.05
Antiplatelet agent (N, %)	76 (16.4)	42 (17.6)	34 (15.2)	0.84
<b>Anthropometrics</b>				
Systolic BP (mmHg)	$128.2 \pm 16.7$	$128.2 \pm 1.1$	$128.2 \pm 1.1$	0.97
Diastolic BP (mmHg)	$80.1 \pm 9.9$	$79.8 \pm 0.6$	$80.4 \pm 0.7$	0.51
BMI (Kg/m <sup>2</sup> )	$24.3 \pm 3.1$	$24.1 \pm 0.2$	$24.5 \pm 0.2$	0.26
<b>Laboratory indices</b>				
Fasting glucose (mg/dL)	$100.6 \pm 24.8$	$98.9 \pm 1.6$	$102.5 \pm 1.7$	0.12
Total cholesterol(mg/dL)	$191.3 \pm 36.3$	$193.2 \pm 2.3$	$189.3 \pm 2.4$	0.25
Triglyceride (mg/dL)	$125.2 \pm 84.8$	$116.4 \pm 5.5$	$134.5 \pm 5.7$	0.02
HDL-cholesterol (mg/dL)	$48.2 \pm 12.5$	$49.6 \pm 0.8$	$46.6 \pm 0.8$	0.01
LDL-cholesterol (mg/dL)	$116.8 \pm 32.9$	$119.1 \pm 2.1$	$114.3 \pm 2.2$	0.12
Calcium (mg/dL)	$9.1 \pm 0.52$	$9.1 \pm 0.03$	$9.0 \pm 0.03$	0.37
CRP (mg/L)	$1.2 \pm 1.32$	$1.2 \pm 0.09$	$1.2 \pm 0.09$	0.77

BP, blood pressure; BMI, body mass index; HDL-cholesterol, high density lipoprotein-cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; CRP, C-reactive protein; DM, diabetes mellitus. A one-way analysis of variance was used to evaluate the difference of continuous variables between CLO positive and negative subjects with age adjustment. The generalized estimating equation was used to compare categorical variables between the CLO positive and negative groups with age adjustment. Continuous variables are presented as mean  $\pm$  standard error or the value of minimum and maximum. Dichotomous variables are presented as the number of subjects with the percentage of subjects in the parenthesis.  $P < .05$  was regarded as statistically significant.

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## Differences in PWV, CACS, and incidence of coronary stenosis/ intracoronary plaque between the CLO-positive versus the CLO-negative subjects

The presence of coronary artery calcium (CACS >0) was significantly higher in the CLO-positive group (36.7% vs. 32.5%,  $P = 0.05$ ) than in the CLO-negative group. However, there were no statistical differences in the presence of severe coronary artery calcium (CACS >100), the mean value of  $\log(\text{CACS} + 1)$  or the mean PWV between the CLO-positive and CLO-negative subjects. There was also no difference between the two groups not only in the prevalence of any plaque but also in the characteristics of plaque (calcified, mixed, or soft). However, the number of segments with plaque was higher in the CLO-positive group ( $0.22 \pm 0.02$  vs.  $0.17 \pm 0.02$ ,  $P = 0.03$ ). More importantly, the incidence of significant coronary artery stenosis ( $\geq 50\%$  stenosis of any major vessel) was significantly higher in the CLO-positive subjects than in the CLO-negative subjects (7.6% vs. 2.9%,  $P = 0.01$ ) (Table 2).

## Elevated adjusted risk for significant coronary artery stenosis in CLO-positive subjects

To investigate whether current *H. pylori* infection is an independent risk factor for subclinical atherosclerosis, we evaluated the adjusted risk for significant coronary artery stenosis in the CLO-positive subjects compared with the CLO-negative subjects by analyzing odds ratio (Table 3 and Fig 2). The CLO-positive subjects were 2.72-fold more likely to have significant coronary artery stenosis compared to the CLO-negative subjects. This risk became more significant after adjusting for age and sex (adjusted OR 3.431, 95% confidence interval 1.343–8.765,  $P = 0.01$ ) and also after adjusting for several additional factors that may influence coronary artery stenosis, such as systolic blood pressure (BP), fasting glucose, HDL-cholesterol, anti-hypertension/diabetic medications, lipid-lowering agents, and antiplatelet agents (adjusted OR 2.813, 95% confidence interval 1.051–7.528,  $P = 0.04$ ).

Table 2. Difference of subclinical atherosclerosis between the CLO-negative and CLO-positive subjects with age adjustment.

	Total N = 463	CLO-negative N = 239	CLO-positive N = 224	P-value
PWV (cm/s)	1422.4 ± 234.3	1419.9 ± 13.1	1425.0 ± 13.5	0.79
CACS				
CACS >0 (n, %)	157 (34.5)	76 (32.5)	81 (36.7)	0.05
CACS >100 (n, %)	51 (11.2)	27 (11.5)	24 (10.9)	0.49
Log (CACS +1)	0.57 ± 0.87	0.51 ± 0.05	0.64 ± 0.05	0.08
Plaque				
Any plaque (%)	104 (22.5)	52 (21.8)	52 (23.2)	0.32
Log[(Number of segments with plaque)+1]	0.20 ± 0.26	0.17 ± 0.02	0.22 ± 0.02	0.03
Calcified plaque (%)	58 (12.5)	29 (12.1)	29 (12.9)	0.38
Mixed plaque (%)	41 (8.9)	19 (7.9)	22 (9.8)	0.25
Soft plaque (%)	32 (6.9)	17 (7.1)	15 (6.7)	0.99
Coronary artery stenosis (n, %)	24 (5.2)	7 (2.9)	17 (7.6)	0.01

PWV, pulse-wave velocity; CACS, coronary artery calcium score. A one-way analysis of variance was used to evaluate the difference of PWV or number of coronary segments involved between CLO negative and positive subjects with age adjustment. The generalized estimating equation was used to evaluate the difference of dichotomous variables between the two groups with age adjustment. Continuous variables are presented as mean ± standard error. Dichotomous variables are presented as the number of subjects with the percentage of subjects in the parenthesis.  $P < .05$  was regarded as statistically significant.

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Table 3. Odds ratio for significant coronary artery stenosis.

	Odds ratio (95% CI)		P-value
	CLO-negative N = 239	CLO-positive N = 224	
Model 1	1.000 (reference)	2.722 (1.107–6.694)	0.03
Model 2		3.431 (1.343–8.765)	0.01
Model 3		3.330 (1.285–8.629)	0.01
Model 4		2.813 (1.051–7.528)	0.04

Model 1: Not adjusted

Model 2: adjusted for age and sex

Model 3: adjusted for age, sex, HDL-cholesterol

Model 4: adjusted for age, sex, systolic BP, fasting glucose, HDL-cholesterol, anti-hypertension medication, anti-diabetic medication, lipid lowering agent, anti-platelet agent

CI, confidence interval; BP, blood pressure; HDL-cholesterol, high density lipoprotein-cholesterol. Data are presented as odds ratios with the CLO-negative group as a reference.

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## Discussion

In this study, we demonstrated that current *H. pylori* infection is significantly associated with significant coronary artery stenosis in a healthy population. The elevated risk of coronary artery stenosis in *H. pylori*-infected subjects was associated with a lower HDL-cholesterol level. This is the first report demonstrating that active/current *H. pylori* infection can be a risk for subclinical atherosclerosis in healthy subjects without previous CVD.

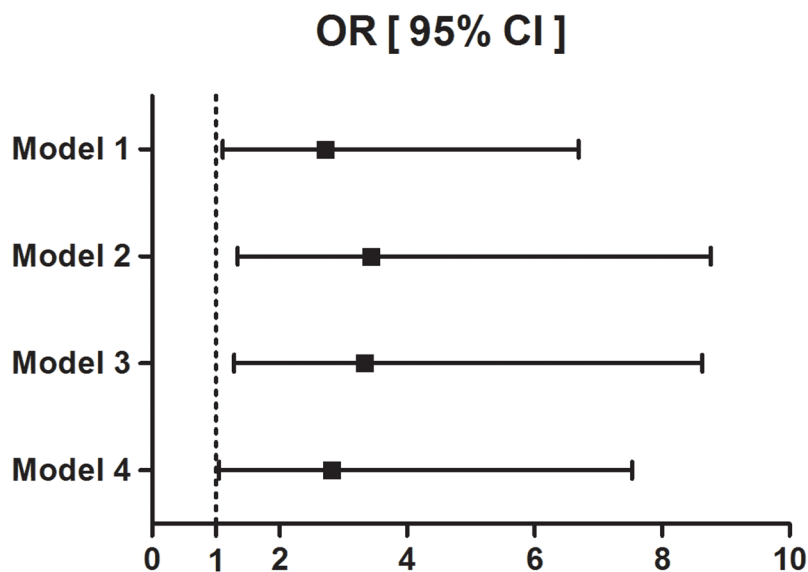


Fig 2. Odds ratio for significant coronary artery stenosis according to the CLO test. Logistic regression was used for calculating odds ratios with 95% confidence intervals. The reference group comprised the CLO-negative subjects.

Model 1: Not adjusted

Model 2: adjusted for age and sex

Model 3: adjusted for age, sex, HDL-cholesterol

Model 4: adjusted for age, sex, systolic BP, fasting glucose, HDL-cholesterol, anti-hypertension medication, anti-diabetic medication, lipid lowering agent, anti-platelet agent

Abbreviations: OR, odds ratio; CI, confidence interval; BP, blood pressure; HDL-cholesterol, high density lipoprotein-cholesterol.

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The diagnostic tests for *H. pylori* can be divided into invasive and noninvasive methods. The serologic *H. pylori* IgG test, urea breath test, and stool antigen assay are noninvasive, whereas the CLO test can only be performed with stomach tissue obtained from invasive endoscopic biopsy [25]. As the serologic *H. pylori* IgG test is fast, cheap, and noninvasive, most of the studies on *H. pylori* infection and CVD were performed using this test [17,18,20,32–35]. However, conflicting results exist between the serology and the prevalence of CVD, with some studies showing a significant association between *H. pylori* seropositivity and CVD [12,17,18,20,32] while others showing no such association [33–35]. One of the possible reasons for these conflicting results may be certain limitations of the serologic test in detecting *H. pylori* infection itself [36]. Most importantly, serologic antibody testing cannot distinguish current and past infection.[26] Owing to this limitation, the serologic test has been mostly used to investigate merely the association between the prevalence of *H. pylori* infection and CVD [18,32,37]. In contrast, a positive CLO test means a current infection [26]. If current *H. pylori* infection is associated with subclinical atherosclerosis, it can be inferred that the eradication of *H. pylori* infection might help prevent the progression of atherosclerosis. In this context, this study provides a theoretical basis for studying the effect of *H. pylori* eradication on preventing the future progression of atherosclerosis.

However, the CLO test is not without limitations. For example, although atrophic gastritis is explicitly associated with *H. pylori* in many cases, the CLO test may fail to detect the presence of *H. pylori* because of a decreased bacterial burden in atrophic gastritis, particularly in the presence of intestinal metaplasia [26]. Nevertheless, the CLO test can provide more accurate and reliable results than the serologic test [25]. The specificity of the CLO test is 95–100%, whereas the serologic test has variable specificity ranging from 76% to 96% [25]. Moreover, the CLO test has a higher sensitivity (80–95%) than the serologic test (75–85%) [26]. Hence, our data provide more accurate evidence for examining the association between *H. pylori* infection and CVD compared with previous studies with the serologic test.

Several studies have reported the correlation between the prevalence of *H. pylori* infection and overt coronary artery disease [11,17–21]. However, no study has demonstrated the association between current *H. pylori* infection and subclinical coronary atherosclerosis. This study is distinct from previous studies because we did not observe CVD events but the incidence of subclinical coronary atherosclerosis in a relatively large number of healthy subjects without previous CVD. Atherosclerosis is a pathologic process narrowing the coronary, cerebral, and peripheral arteries due to the formation of atheromatous plaques [38]. Studying the association between *H. pylori* infection and subclinical atherosclerosis allows investigators to confirm that earlier vessel-wall changes can be induced by *H. pylori* infection. Some reports have shown the association between *H. pylori* infection and the degree of carotid atherosclerosis, but not with that of coronary atherosclerosis [39–41]. To our knowledge, this is the first study that employed the CLO test to detect current *H. pylori* infection, and linked this test with direct visualization of the coronary artery with cardiac MDCT in a relatively large number of healthy subjects. The significant correlation between *H. pylori* infection and subclinical coronary atherosclerosis in this study might be able to support *H. pylori* eradication as a potential cardiovascular prevention strategy.

How *H. pylori* infection can induce atherosclerosis has not been clearly established yet [10,29]. In this study, HDL-cholesterol was lower and triglyceride was higher in the CLO-positive group. Some data suggest that dysregulated lipid metabolism, including a low HDL-cholesterol level, may accelerate atherosclerosis in patients with *H. pylori* infection [10,42–45]. *Helicobacter pylori* carries lipopolysaccharide and can upregulate certain cytokines in the host, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [9,10,35]. Inhibition of lipoprotein lipase by cytokines, such as TNF- $\alpha$ , may mobilize lipids from the tissues [46], leading to low serum HDL-



cholesterol levels [47]. Besides perturbing the lipid metabolism, *H. pylori* infection may also directly induce atherosclerosis. Endothelial dysfunction induced by vacuolating cytotoxin A secreted from *H. pylori* [48,49], molecular mimicry by the autoimmune response [50], enhanced systemic inflammation [51], oxidative stress [52], and platelet aggregation [53] by *H. pylori* are all potential mechanisms of atherosclerosis that have been reported to be directly induced by *H. pylori* infection. The possibility of *H. pylori* induced-atherosclerosis independent of HDL-cholesterol levels is also supported by our data showing that *H. pylori* itself is associated with a higher incidence of significant coronary artery stenosis despite adjusting for HDL-cholesterol level. This significant association between *H. pylori* and significant coronary artery stenosis, irrespective of disturbed HDL-cholesterol levels, suggests that *H. pylori* infection may aggravate coronary atherosclerosis through both lipid metabolism-dependent and independent pathways.

CACS has also been used for suggesting the association between *H. pylori* seropositivity and coronary atherosclerosis. In a previous study, the degree of coronary atherosclerosis assessed by CACS was significantly associated with *H. pylori* seropositivity [32]. In our study, the presence of coronary artery calcium (CACS >0) was significantly higher in the CLO-positive group. On the other hand, there were no statistical differences in the presence of severe coronary artery calcium (CACS >100) and the mean value of log(CACS + 1) between the CLO-positive and CLO-negative subjects. Considering that CACS is more specific in subjects older than 60 years [54,55], the relationship between CACS and *H. pylori* infection needs to be further investigated in a larger study population.

The link between *H. pylori* infection and increased arterial PWV has been documented previously [56,57]. As chronic *H. pylori* infection is thought to induce systemic inflammation through cytokines such as interleukin-6 [44,51], not only coronary but also peripheral vascular changes can occur [58]. However, the mean PWV was not different according to the CLO positivity in our study. These inconsistent results may possibly be because of the differences in the PWV measurement sites (heart-carotid vs. brachial-ankle) and differences in the study population, as well as the diagnostic tool for *H. pylori* infection [56,57]. In addition, considering the previous reports that bacterial DNA of *H. pylori* was identified in coronary atherosclerotic plaque [10,59,60], *H. pylori* might be able to aggravate coronary atherosclerosis by direct invasion into the coronary vessels preferentially compared to other vessels regardless of systemic inflammation.

Our study has several limitations. First, this study lacks the measurement of cytotoxin-associated gene-A (CagA), which has been known to determine the pathogenicity of *H. pylori* in atherosclerosis [61–63]. It would be necessary to investigate this in future studies. Other limitations of this study are characteristics of the study population. As Gangnam Severance hospital is located in an affluent part of the capital city, the demographic and the socio-economic characteristics might be different from the general population. The individuals in our study population were more likely to be male, aged and upper white-collar workers prominently [64,65]. Lastly, we could not discriminate the subjects who had *H. pylori* eradication treatment previously because this was not included in the questionnaire of the health check-up. Nevertheless, our study is powerful and unique in demonstrating the association between subclinical atherosclerosis and *H. pylori* infection compared to other studies because our dataset is composed of extensive data encompassing MDCT, PWV, and CLO-test, the results of which are difficult to gather comprehensively.

In conclusion, current *H. pylori* infection is associated with subclinical but significant coronary artery stenosis in a healthy population. This result suggests the possibility that *H. pylori* eradication might be worthwhile in preventing coronary artery disease. Further studies are warranted to investigate whether CLO test positivity could be a new marker for the assessment

of future coronary artery disease risk and the underlying mechanism of how *H. pylori* infection may lead to coronary atherosclerosis.

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