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ORIGINAL ARTICLE

CagA-Positive *Helicobacter pylori* Strains Enhanced Coronary Atherosclerosis by Increasing Serum OxLDL and HsCRP in Patients with Coronary Heart Disease

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Abstract
Background The role of infection in the pathogenesis of atherosclerosis is still a matter of debate.
Aims This study aimed to investigate the effect of CagA-positive *Helicobacter pylori* (*H. pylori*) strains infection on coronary atherosclerosis in patients with coronary heart disease and to elucidate how cytotoxin-associated gene A (CagA)-positive *H. pylori* strains infections were involved in coronary heart disease by examining the levels of serum lipid, high-sensitivity C-reactive protein (hsCRP) and oxidized low-density protein (oxLDL).
Methods Recruited for this study were 159 patients with coronary heart disease. The severity of coronary heart disease was estimated by calculating the Gensini score. Serum oxLDL and hsCRP were examined in all subjects. Current *H. pylori* infection was determined in all participants by means of a modified (13C) urea breath test (>200 dpm classified as positive). IgG antibodies against CagA protein were analyzed by enzyme immunoassays. Antibody titers against CagA (≥8 U/ml) were classified as positive. All subjects were divided into three groups, including an uninfected group ($n = 30$), an *H. pylori*$^{+}$CagA^{-} group ($n = 69$), and an *H. pylori*$^{+}$CagA^{+} group ($n = 60$).
Results Significant differences were found among the three groups in levels of total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, serum hsCRP, oxLDL, and severity of coronary atherosclerosis ($p < 0.05$). The levels of total cholesterol, LDL, apolipoprotein B, serum hsCRP, oxLDL were significantly elevated and the severity of coronary atherosclerosis was significantly increased in *H. pylori*$^{+}$CagA^{+} group ($p < 0.05$).
Conclusions More serious coronary atherosclerosis was observed in CHD patients with *H. pylori*$^{+}$CagA^{+} infection. *H. pylori*$^{+}$CagA^{+} infection might be involved in coronary atherosclerosis by modifying serum lipids, enhancing LDL oxidation, and activating the inflammatory responses.

Keywords *Helicobacter pylori* · Coronary atherosclerosis · Coronary heart disease · Lipid · C-reactive protein · Oxidized low-density protein

Introduction

At present, *Helicobacter pylori* (*H. pylori*) infection is the main cause of chronic gastritis, peptic ulcer, and gastric mucosa-associated lymphoid tissue lymphoma (MALT), as well as being a risk factor for gastric cancer [1]. In recent years, several studies have demonstrated the association between *H. pylori* infection and coronary heart disease (CHD) [2–4]. Conversely, subsequent publications showed controversial results in the correlation between *H. pylori* infection and coronary atherosclerosis. Some studies indicated that there was no correlation between *H. pylori* infection and coronary atherosclerosis [5–7]. In recent years, several studies have demonstrated that only cytotoxin-associated gene A (CagA)-positive *H. pylori* could increase the risk of atherosclerosis except CagA-negative *H. pylori*.

Despite that a growing number of studies showed that *H. pylori* infection could increase the levels of serum cytokines and hsCRP and modifying lipid profile in

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patients [8–12], consensus on the possible effects of *H. pylori* infection on high-sensitivity C-reactive protein (hsCRP) and oxidized low-density protein (oxLDL) have not been achieved [7, 13–15]. The pathogenetic mechanisms of *H. pylori* infection contributing to atherosclerosis remain unclear.

This study was to investigate the effect of CagA-positive *Helicobacter pylori* strains infection on coronary atherosclerosis in patients with coronary heart disease and to elucidate how CagA-positive *Helicobacter pylori* strains infection were involved in coronary heart disease by examining the levels of serum lipid, hsCRP, and oxLDL.

Materials and Methods

Study Population

A total of 159 CHD patients [115 men, 44 women, aged 30–80 (63.37 ± 9.94) years] from the Cardiovascular Department were enrolled in this study from October 2006 to November 2007. We excluded the individuals with acute infection diseases, chronic inflammatory diseases, liver diseases, thyroid diseases, autoimmune diseases, acute or chronic heart failure, stroke, renal failure, lung diseases, and patients who had taken antibiotic drugs in the 6 months preceding the study. All patients were fully informed about the objective of the study and agreed to participate. Those patients with CHD who were recruited showed at least one coronary stenosis 50% at catheterization in the last 6 months prior to entering the study. The severity of coronary heart disease was also estimated by calculating the Gensini score [16]. In this scoring system, a greater reduction of the luminal diameter is assigned a high score and a proximal lesion in the left anterior descending or the left circumflex artery is assigned a higher score than a distal lesion.

Subjects were divided into three groups: uninfected group ($n = 30$), *H. pylori*$^{+}$CagA^{-} group ($n = 69$), and *H. pylori*$^{+}$CagA^{+} group ($n = 60$) according to the 13C urea breath test and the titers of IgG antibodies against CagA. Subjects underwent a completed standardized questionnaire on current and past exposure to candidate vascular risk factors. All the enrolled patients were successfully treated with anti-platelet drugs (aspirin and clopidogrel were used alone or dually), anti-ischemic, statin, antihypertension drugs, proton pump inhibitors, and so on. Among the three groups, there was no significant difference in these medicines administration.

Laboratory Analysis

All participants underwent blood sampling and a 13C urea breath test (>200 dpm was classified as positive) performed by the same trained team of interviewers. Serum samples were gained from peripheral venous blood in the second day after hospitalization. After being centrifuged, the serum samples were stored at −80° for 4 weeks before being examined. IgG antibodies against CagA protein (DIA.PRO, Diagnostic Bioprobes S.r.l, Milan, Italy) were analyzed by enzyme immunoassays. Antibody titers CagA (≥8 U/ml) were classified as positive according to the instructions of the manufacturer. The measurement of plasma ox-LDL (TPI Corporation, American Comfort Conditioning Company, Arizona, USA) levels was performed with a sandwich enzyme-linked immunosorbent assay (ELISA) method that was previously described [17]. Serum fibrinogen, lipid, urine acid, and high-sensitivity C-reactive protein (hsCRP) were measured with the Hitachi Biochemistry Analyzer (Hitachi, 717OA, Automatic Analyzer, Tokyo, Japan).

Statistics Analysis

Statistical analyses were performed using SPSS software for Windows (Version 11.0). Data were expressed as $\bar{x} \pm s$. Measurement data in each group were compared with one-way ANOVA. Chi-square test was applied to compare the difference of gender, family medical history, smoking, diabetes, and hypertension in each group. A two-sided probability value of less than 0.05 was considered to indicate statistical significance.

Results

Baseline Characteristics of Subjects in Three Groups

Subjects did not differ in age, gender, TG, HDL, apolipoprotein A, fibrinogen, urine acid, family medical history, index weight, number with hypertension, number with diabetes, and number of those who had ever smoked. There was no significant difference in treatment with anti-platelet drugs, anti-ischemic, statin, antihypertension drugs, and so on, among the three groups. Data of age, gender, TG, HDL, apolipoprotein A, fibrinogen, urine acid, family medical history, index weight, number with hypertension, number with diabetes, and number of those who had ever smoked of the participants according to the study group are summarized in Table 1.

Helicobacter pylori Infection and Coronary Atherosclerosis

There were significant differences in coronary artery Gensini scores among the three groups. The coronary artery Gensini scores were not significantly higher in the

Table 1 Baseline characteristics of each group. Data are expressed as mean ± SD

	Gender (male)	Age (years)	Hypertension	Diabetes	FMH	Smokers	Urine acid (μmol/l)	Fibrinogen (g/l)	Index weight (kg/m^2)	TG (mmol/l)	HDL (mmol/l)	ApoA (mmol/l)
Uninfected ($n = 30$)	20	64.87 ± 9.33	20	10	8	6	325.86 ± 56.29	3.15 ± 1.43	23.40 ± 2.20	1.97 ± 0.59	0.84 ± 0.26	1.13 ± 0.27
Hp$^+$CagA$^-$ ($n = 69$)	51	63.84 ± 10.09	45	16	18	16	335.88 ± 114.41	3.16 ± 1.00	22.38 ± 2.46	1.60 ± 0.74	0.95 ± 0.25	1.29 ± 1.21
Hp$^+$CagA$^+$ ($n = 60$)	44	64.87 ± 9.33	44	18	15	13	385.79 ± 163.94	3.25 ± 0.98	22.30 ± 2.41	1.83 ± 1.00	0.98 ± 0.28	1.16 ± 0.28
Sig	0.743	0.517	0.288	0.511	0.983	0.937	0.052	0.066	0.206	0.091	0.059	0.563

FMH family medical history, *TG* triglyceride, *HDL* high-density lipoprotein, *ApoA* Apolipoprotein A

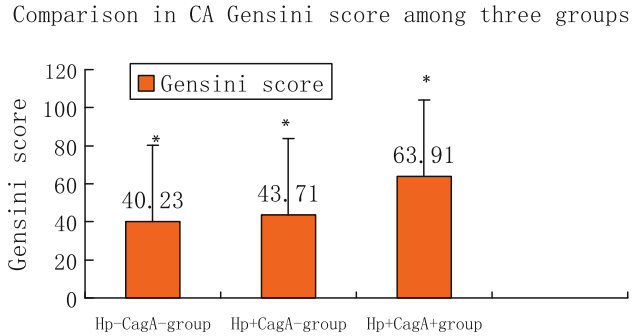

Fig. 1 Comparison of coronary artery Gensini scores among the three groups. The coronary artery Gensini scores in the uninfected, *H. pylori*$^+$CagA$^-$, and *H. pylori*$^+$CagA$^+$ groups were 40.23 ± 40.68, 43.71 ± 37.01, and 63.91 ± 43.48, respectively. * $p = 0.006$

H. pylori$^+$CagA$^-$ group compared to the uninfected group. However, in contrast to the *H. pylori*$^+$CagA$^-$ group and the uninfected group, the coronary artery Gensini scores significantly increased in the *H. pylori*$^+$CagA$^+$ group (Fig. 1).

Helicobacter pylori Infection and Serum Lipid, OxLDL, and HsCRP

Significant differences were noted among the three groups in the levels of serum hsCRP, TC, LDL, ApoB, and oxLDL. The levels of these parameters were significantly elevated in patients with *H. pylori* infection, and were highest in the *H. pylori*$^+$CagA$^+$ group (Table 2).

Discussion

The role of infection in the pathogenesis of atherosclerosis is still a matter of debate. In recent years, several studies have demonstrated that *H. pylori*-specific DNA had been detected in atheromatous plaque [18, 19]. The pathogen burden could increase the positively of vulnerable plaque [20]. The restenosis prevalence of coronary atherosclerosis would be reduced in vessels after *H. pylori* eradication [19]. However, some studies did not provide support for *H. pylori* for coronary atherosclerosis and evidence of *H. pylori* DNA in any plaque specimens. These studies supposed *H. pylori* would not be involved in the pathogenesis of aortoiliac atherosclerosis and there was no significant correlation between the *H. pylori*-IgG level and the Gensini score [5–7], but IgG antibodies against CagA protein of patients was not examined in these studies. The incomplete agreement existing on the role of *H. pylori* infection in CAD may in part be due to the fact in many studies.

In the present study, there were significant differences in the coronary artery Gensini scores among the three groups.

Table 2 Comparison of serum lipid, oxLDL, and hsCRP among the three groups ($\bar{x} \pm s$)

	TC (mmol/l)	LDL (mmol/ll)	ApoB (mmol/l)	OxLDL (mmol/l)	HsCRP (mmol/l)
Uninfected ($n = 30$)	4.05 ± 0.54	2.09 ± 0.54	0.97 ± 0.21	1.21 ± 0.42	2.65 ± 2.81
Hp^+CagA^- ($n = 69$)	4.45 ± 0.61	2.49 ± 0.64	1.00 ± 0.21	2.15 ± 1.06	5.73 ± 3.17
Hp^+CagA^+ ($n = 60$)	5.28 ± 1.32	2.89 ± 1.11	1.25 ± 0.44	2.60 ± 1.06	9.37 ± 6.39
Sig	<0.001	<0.001	<0.001	<0.001	<0.001

TC total cholesterol, *LDL* low-density lipoprotein, *ApoB* Apolipoprotein B, *OxLDL* oxidized low-density protein, *HsCRP* high-sensitivity C-reactive protein

The coronary artery Gensini scores were not significantly higher in the *H. pylori*$^+$CagA$^-$ group compared to the uninfected group. However, in contrast to the *H. pylori*$^+$CagA$^-$ group and the uninfected group, the coronary artery Gensini scores significantly increased in *H. pylori*$^+$CagA$^+$ group. We provided evidence that infections with CagA-positive but not CagA-negative *H. pylori* strains significantly increased the risk of advanced atherosclerosis in coronary arteries, suggesting that the association of *H. pylori* infections with coronary arterial atherosclerosis is restricted to the more virulent genotype. These findings are consistent with some recent studies demonstrating CagA-seropositive strains infection was significantly associated with susceptibility to ischemic strokes and coronary heart diseases [21–23]. Notably, the underlying processes that might explain the potential association between infectious agents and atherosclerotic disease also remain unclear. Various potential pathomechanisms have been postulated, e.g., increased production of cytokines and acute-phase reactants, local or systemic disturbance of fibrinolysis and blood coagulation, direct infection of the arterial wall via macrophages and alteration of vascular cell function, and an immunological response (cross-reaction) due to bacterial heat shock protein [24–26]. The virulence of pathogens may be a crucial determinant of its injurious and potential proatherogenic potencies. The most virulent *H. pylori* strains bear a high-molecular-weight toxin (VacA toxin). An immunodominant protein associated with VacA is the cytotoxin-associated gene A (CagA) [27]. Seropositivity to CagA is widely used to detect infections with virulent *H. pylori* strains. This VacA toxin has the potential to cause severe damage to the epithelium and is associated with an enhanced local or systemic inflammatory response.

The role of inflammation in the pathogenesis and progression of coronary artery disease (CAD) has been increasingly discussed. According to previous publications, *H. pylori* infection is linked to an increased systemic inflammatory response. *H. pylori*, especially CagA-positive *H. pylori* strains, could upregulate the levels of serum hsCRP [28–30]. Subjects with high CRP levels tend to have a higher risk of atherosclerosis if exposed to infectious agents [15, 31, 32]. HsCRP may directly contribute to a proinflammatory state in atheroma by inducing adhesion molecule expression on endothelial cells, stimulating cytokine release of monocytes and activating the complement cascade [33]. This capacity was recently shown to enhance the risk of atherosclerosis [34]. In this study, subjects significantly differed in the levels of serum hsCRP among the three groups. The levels of serum hsCRP significantly increased in patients with *H. pylori* infection, and were highest in the *H. pylori*$^+$CagA$^+$ group. These findings are consistent with some recent studies showing the correlation between CagA-seropositive strains infection and inflammatory responses.

In addition, it is well known that acute infections are able to modify serum lipids. Some authors have also suggested that alterations of the lipid metabolism due to chronic infections could represent an atherogenic link [8–10]. Hyperlipidemia, especially hypercholesterolemia, is an established risk factor of atherosclerosis. ApoB, the major protein of LDL, plays an important role in atherogenesis. There is consistent evidence that oxidized low-density lipoprotein (oxLDL) is a key factor in the initiation and progression of atherosclerosis [35–38]. It has been shown that advanced human atherosclerotic lesions have a highly pro-oxidant environment [39]. In particular, the progression of plaque inflammation in human coronary unstable plaques may induce a focal pro-oxidant/antioxidant imbalance and contribute to the enhancement of LDL oxidation [40–42]. In previous studies, Mayr et al. observed an association between human oxLDL markers and chronic infections [14], but Kayo et al. suggested that plasma oxLDL levels do not seem to be associated with *H. pylori* infection [13]. In this study, significant differences were noted among the three groups in the levels of serum TC, LDL, ApoB, and oxLDL. The levels of these parameters significantly increased in patients with *H. pylori* infection, and were highest in the *H. pylori*$^+$CagA$^+$ group. These findings provided evidence for *H. pylori*, especially CagA-seropositive strains, in contributing to coronary atherosclerosis.

In this study, the levels of triglycerides in uninfected patients was higher than the levels of triglycerides in infected patients, as well as in patients with CagA positive

higher than in patients with CagA negative. Our previous study investigating the effect of *Chlamydia pneumoniae* infection on aortic atherosclerosis in C57BL/6 J mice showed that the levels of triglycerides were lower in infected groups [43], which suggests that microorganism infection might decrease the levels of serum triglycerides. However, the mechanism by which this occurs is not yet clear.

Conclusions

We provide evidence that infections with CagA-positive (but not CagA-negative *H. pylori* strains) significantly increased the risk of advanced atherosclerosis in coronary arteries. CagA-positive *H. pylori* strains might be involved in coronary atherosclerosis by modifying serum lipids, enhancing LDL oxidation, and activating inflammatory responses, yet this remains to be confirmed by further studies.

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Conflicts of interest statement We have no conflicts of interest.

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