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Original Article

Association of endothelial dysfunction and cytotoxin-associated gene A-positive *Helicobacter pylori* in patients with cardiac syndrome X

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ABSTRACT

Background: Existence of coronary endothelial dysfunction has been demonstrated in patients with cardiac syndrome X (CSX). In addition, *Helicobacter pylorus* (*H. pylori*) has been associated with CSX. We aimed to assess the possible association of endothelial dysfunction and cytotoxin-associated gene A-positive *H. pylori* (CagA+) infection in CSX patients.

Methods: Fifty-six patients with CSX (23 male/33 female; age: 51.25 ± 8.86 years) who were anti-*H. pylori* IgG-positive [*H. pylori*(+)] and 24 CSX patients (7 male/17 female; age: 52.79 ± 9.88 years) who were *H. pylori*(−) were included. Also, anti-*H. pylori* IgG-positive patients were determined by the presence of IgG antibody to CagA. Levels of endothelin-1 (ET-1), E-selectin and intercellular adhesion molecule-1 (ICAM-1) were measured.

Results: Endothelial dysfunction biomarkers were higher in *H. pylori*(+) than in *H. pylori*(−) patients (ET-1: 54.60 ± 25.39 vs. 42.59 ± 18.37 pg/ml, $p = 0.04$; E-selectin: 42.68 ± 14.26 vs. 31.72 ± 8.26 ng/ml, $p = 0.001$; ICAM-1: 339.68 ± 135.8 vs. 266.51 ± 125.1 ng/ml, $p = 0.02$). Among *H. pylori*(+) subjects, 28 cases were CagA(+) and 28 cases were CagA(−). There were significant differences in measured levels of E-selectin between CagA(+) and CagA(−) groups (48.00 ± 16.37 vs. 37.37 ± 9.37 ng/ml, $p = 0.004$). For ET-1 and ICAM-1 levels, the difference between CagA(+) and CagA(−) was insignificant ($p = 0.174$ and $p = 0.07$, respectively).

Conclusion: High levels of endothelial dysfunction biomarkers are found in CSX patients with anti-CagA(+). These findings suggest the infection with CagA(+) *H. pylori* strain may play a role as a risk factor in development of CSX through provocation of endothelial dysfunction. Therefore, a long term follow up to investigate the outcomes of these patients is proposed.

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At a glance commentary

Scientific background on the subject

Cardiac syndrome X (CSX) describes patients with angina-like chest pain, a positive exercise stress test result, and angiographically normal epicardial coronary arteries. Endothelial dysfunction is among the most commonly suggested pathogenic mechanisms responsible for CSX. Previous studies have revealed an association between *Helicobacter pylori* (*H. pylori*) infection with vascular discomforts. Specifically, strains bearing the cytotoxin-associated gene A [CagA(+)] exacerbate a heightened inflammatory response *in vivo*. In this study we would like to see whether infection with CagA(+) bearing strain of *H. pylori* is associated with higher endothelial dysfunction.

What this study adds to the field

Study results show the infection with CagA(+) *H. pylori* weather play a role as a risk factor in development of CSX through provocation of endothelial dysfunction.

Cardiac syndrome X (CSX) describes patients with angina-like chest pain, a positive exercise stress test result, and angiographically normal epicardial coronary arteries [1]. More than 40 years after the first description of the disease, the debate continues to the CSX mechanisms. Inflammation and microvascular dysfunction are among the most commonly suggested pathogenic mechanisms responsible for CSX [1–3]. The endothelial function has been studied mainly by invasive methods and by measuring humoral factors [4]. Increased levels of plasma adhesion molecules like soluble intercellular adhesion molecule-1 (sICAM-1) and soluble E-selectin (sE-selectin) have been considered as markers of endothelial injury [4,5]. For example, Senen et al. [6] found increased plasma concentrations of ICAM-1 and sE-selectin in CSX patients. Moreover, it is known that damaged or activated endothelial cells can secrete vasoconstrictor factors such as endothelin-1(ET-1). Kaski et al. [7] suggested the association between high plasma concentrations of ET-1 and genesis of chest pain in patients with CSX.

On the other hand, previous studies have revealed an association between *Helicobacter pylori* (*H. pylori*) infection with vascular discomforts [8,9]. *H. pylori* is a microaerophilic spiral shaped gram negative bacterium that colonizes the gastric lumen of humans and other primates [10]. It may cause extra-intestinal expressions such as functional ischemic heart disease [11,12] and it has recently been associated with CSX [9,13]. There is genetic diversity between *H. pylori* strains that affects virulence [14]. Specifically, strains bearing the cytotoxin-associated gene A [CagA(+)] exacerbate a heightened inflammatory response *in vivo* [15]. The virulent CagA(+) may induce a more consistent release of cytokines with vasoactive properties, which might be the basis of systemic extradigestive effects that led to cardiac microvascular dysfunction [8]. We aimed to evaluate the possible association of chronic CagA(+) infection and endothelial dysfunction in CSX patients.

Methods

Patient characteristics

The present study included 80 patients (30 male/50 female; mean age: 51.71 ± 9.2 years) who had been diagnosed as CSX. The entry criteria of CSX were recurrent typical angina chest pain at rest and on effort, a normal 12-lead electrocardiogram at rest, positive exercise ECG stress test response and normal coronary angiogram. Patients with evidence of myocardial infarction, valvular heart disease, left and right ventricular dysfunction, concomitant acute and chronic disease were excluded from the study. Also, patients with diabetes mellitus were not included, as endothelial dysfunction markers increase in diabetes mellitus. Non-cardiac causes of chest pain such as gastrointestinal and musculoskeletal disorders were also investigated and ruled out as appropriate. All subjects gave their informed consent prior to their inclusion in the study. The study protocol approved by the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran by our university of Medical Research Ethics committee (approved number: 481). A questionnaire was administrated to obtain general information regarding age, sex, body mass index (BMI), systolic and diastolic blood pressures.

Measurement of parameters

EDTA-anticoagulated peripheral blood sample was taken from each subject in resting on the same day that clinical data were recorded and the plasma was obtained after a centrifugation of 3000 rpm for 10 min. Collected plasma for determination of biomarkers of endothelial function (sICAM-1 and sE-selectin and ET-1) were stored at −80 °C before laboratory testing.

Specific anti-*H. pylori* immunoglobulin-G (IgG) positivity was determined with a commercial enzyme-linked immunosorbent assay (ELISA) kit (*H. pylori*-IgG and CagA–IgG, Enzyme Immunoassay; Dia pro, Italy) according to the manufacturer's instructions.

Also, plasma positivity to the antigen CagA was assessed by ELISA (Dia.Pro, Milan, Italy; sensitivity and specificity >98%) in anti-*H. pylori* positive (anti-*H. pylori*+) samples.

Endothelial function markers were measured using commercially available kits for measurement of ICAM-1 and sE-selectin levels (Platinum ELISA, Bender Med Systems, Austria). In addition, plasma levels of ET-1 were measured using immunosorbent assay method (Human ET-1, USCN LIFE, USA).

Laboratory glucose and lipid profile results used for comparison of baseline characteristics. These routine tests are performed for any patient admitted to hospital.

Table 1 – Demographic and baseline clinical characteristics of patients with cardiac syndrome X.

Variable	*H. pylori*(–) (n = 24)	*H. pylori*(+) (n = 56)	*p*-value	*H. pylori* status		*p*-value
				CagA(–) (n = 28)	CagA(+) (n = 28)	
Age (years)	52.79 ± 9.88	51.25 ± 8.86	$p = 0.493$	52.18 ± 11.11	50.32 ± 5.91	$p = 0.438$
Sex (M/F)	7/17	23/33	$p = 0.450$	9/19	14/14	$p = 0.139$
(BMI) (Kg/m^2)	26.48 ± 2.51	26.56 ± 5.90	$p = 0.949$	27.82 ± 6.04	25.31 ± 5.60	$p = 0.113$
Smokers, n(%)	2 (8.3%)	11 (19.6%)	$p = 0.324$	5 (17.9%)	6 (21.4%)	$p = 0.500$
SBP (mmHg)	113.75 ± 11.35	115.18 ± 10.44	$p = 0.586$	117.50 ± 13.23	112.86 ± 6.00	$p = 0.197$
DBP (mmHg)	71.04 ± 4.42	74.20 ± 8.67	$p = 0.096$	72.86 ± 10.50	75.54 ± 6.29	$p = 0.252$
FBS (mg/dl)	91.24 ± 6.83	94.52 ± 5.72	$p = 0.454$	92.24 ± 7.33	96.01 ± 8.02	$p = 0.383$
TC (mg/dl)	163.50 ± 11.43	159.33 ± 14.02	$p = 0.332$	158 ± 13.56	164.44 ± 11.66	$p = 0.298$
LDL (mg/dl)	86.4 ± 8.9	92.06 ± 83	$p = 0.139$	94.1 ± 6.22	89.62 ± 5.7	$p = 0.212$
HDL (mg/dl)	44.52 ± 10.85	46.22 ± 10.93	$p = 0.789$	46.5 ± 11.8	39.12 ± 12.86	$p = 0.286$
TG (mg/dl)	148.14 ± 22.8	154.30 ± 18.54	$p = 0.632$	158.32 ± 20.22	144.30 ± 24.45	$p = 0.294$

All values are means ± SD. Abbreviations: *H. pylori*: *Helicobacter pylori*; CagA: cytotoxin-associated gene A; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglyceride.

Statistical analysis

Data analysis was conducted using Statistical Package of the Social Sciences (IBM SPSS Statistics 19) software.

Chi square and independent sample T tests were carried out for statistic analysis. Age, systolic blood pressure, diastolic blood pressure, glucose, lipid profile and body mass index (BMI) were shown as mean ± standard deviation (SD). One-Way ANOVA with Tukey HSD test used to compare amount of endothelial function markers among three groups. Statistical significance was defined as a p-value <0.05.

Results

All individuals diagnosed as CSX and there were no significant differences in the medication status. Baseline characteristics are not different among the groups in respect to age, sex, BMI, smoking, systolic and diastolic blood pressure, glucose, lipids and medications [$p > 0.05$, Table 1].

At first, we divided CSX patients into two groups according to the presence (23 male/33 female, mean age: 51.25 ± 8.86) or absence (7 male/17 female, mean age: 52.79 ± 9.88) of anti-*H. pylori* IgG antibody (*H. pylori*(+) and *H. pylori*(–), respectively). The measured plasma concentrations of ET-1 were significantly greater in *H. pylori*(+) than in *H. pylori*(–) patients (54.60 ± 25.39 vs. 42.59 ± 18.37 pg/ml, $p = 0.040$). Also the plasma E-selectin levels were higher in *H. pylori*(+) than in *H. pylori*(–) patients (42.68 ± 14.26 vs. 31.72 ± 8.26 ng/ml, $p = 0.001$). These patterns also were seen in plasma levels of ICAM-1. The levels of ICAM-1 concentration in *H. pylori*(+) were higher than *H. pylori*(–) patients (339.68 ± 135.8 vs. 266.51 ± 125.1 ng/ml, $p = 0.02$).

In the second step, the *H. pylori*(+) group divided into two sub-groups according to presence (14 male/14 female, mean age: 50.32 ± 5.91 years) or absence (9 male/19 female, mean age: 52.18 ± 11.11 years) of anti-CagA IgG antibody (CagA(+) and CagA(–), respectively). Differences in plasma levels of ET-1 in CagA(+) and CagA(–) groups was insignificant (60.21 ± 25.12 vs. 49.00 ± 24.83 pg/ml, $p = 0.174$). Furthermore, levels of plasma E-selectin levels in CagA(+) patients were much greater than CagA(–) patients (48.00 ± 16.37 vs. 37.37 ± 9.37 ng/ml, $p = 0.004$; [Table 2]). Greater concentrations of ICAM-1 are also seen in CagA(+) patients than CagA(–) patients (388.34 ± 149.38 vs. 308.87 ± 120.11 ng/ml, $p = 0.070$).

According to multiple comparison procedures, the increased levels of plasma ET-1 concentrations in CagA(+) group than *H. pylori*(–), were significant ($p = 0.021$). The mean of plasma E-selectin in CagA(+) group were higher than *H. pylori*(–) group ($p < 0.0001$). This pattern also seen for ICAM-1; the mean in CagA(+) group was greater than in *H. pylori*(–) group ($p = 0.004$), [Fig. 1].

Discussion

CSX is a heterogeneous condition that encompasses several possible causal mechanisms. Cardiac and non-cardiac mechanisms have been proposed, among which endothelial dysfunction of the coronary microcirculation features prominently [3]. Current research suggests that coronary endothelial dysfunction and subsequent microvascular ischemia is the likely pathophysiologic mechanism for patients with CSX, which results in their angina-like chest pain [1]. *H. pylori* recently has been associated with CSX. In a previous case-control study we showed the high frequency of *H. pylori* in CSX [16]. Eskandarian et al. [17], showed that 95% of CSX patients were *H. pylori*(+), while only 47.5% of healthy control group were infected. Recent findings suggest that there are relations between chronic infection of *H. pylori* and endothelial dysfunction. Innocenti et al. [18] showed that *H. pylori* induced activation of human endothelial cells. Also, Oshima et al. [19] studied the association of *H. pylori* infection with systemic inflammation and endothelial dysfunction in healthy male subjects. They reported that chronic infection of *H. pylori* involved in the development of the atherosclerosis via endothelial dysfunction. These studies are consistent with our findings which all three endothelial dysfunction markers were significantly higher in *H. pylori*(+) than *H. pylori*(–) groups. We speculated that *H. pylori* may also cause endothelial

Table 2 – Multiple comparisons.

Dependent variable		(I) HP_CagA	(J) HP_CagA	Mean difference (I–J)	Std. error	Sig.	95% Confidence interval	
							Lower bound	Upper bound
Endothelin	Tukey HSD	HPpCAGp	HPpCAGn	11.20567	6.20094	0.174	−3.6137	26.0251
			HPnCAGn	17.61515[a]	6.45415	0.021	2.1906	33.0397
		HPpCAGn	HPpCAGp	−11.20567	6.20094	0.174	−26.0251	3.6137
			HPnCAGn	6.40948	6.45415	0.583	−9.0150	21.8340
		HPnCAGn	HPpCAGp	−17.61515[a]	6.45415	0.021	−33.0397	−2.1906
			HPpCAGn	−6.40948	6.45415	0.583	−21.8340	9.0150
	LSD	HPpCAGp	HPpCAGn	11.20567	6.20094	0.075	−1.1420	23.5533
			HPnCAGn	17.61515[a]	6.45415	0.008	4.7633	30.4670
		HPpCAGn	HPpCAGp	−11.20567	6.20094	0.075	−23.5533	1.1420
			HPnCAGn	6.40948	6.45415	0.324	−6.4424	19.2613
		HPnCAGn	HPpCAGp	−17.61515[a]	6.45415	0.008	−30.4670	−4.7633
			HPpCAGn	−6.40948	6.45415	0.324	−19.2613	6.4424
sEselectin	Tukey HSD	HPpCAGp	HPpCAGn	10.62429[a]	3.21998	0.004	2.9290	18.3196
			HPnCAGn	16.27393[a]	3.35146	0.000	8.2644	24.2835
		HPpCAGn	HPpCAGp	−10.62429[a]	3.21998	0.004	−18.3196	−2.9290
			HPnCAGn	5.64964	3.35146	0.217	−2.3599	13.6592
		HPnCAGn	HPpCAGp	−16.27393[a]	3.35146	0.000	−24.2835	−8.2644
			HPpCAGn	−5.64964	3.35146	0.217	−13.6592	2.3599
	LSD	HPpCAGp	HPpCAGn	10.62429[a]	3.21998	0.001	4.2125	17.0361
			HPnCAGn	16.27393[a]	3.35146	0.000	9.6003	22.9475
		HPpCAGn	HPpCAGp	−10.62429[a]	3.21998	0.001	−17.0361	−4.2125
			HPnCAGn	5.64964	3.35146	0.096	−1.0240	12.3233
		HPnCAGn	HPpCAGp	−16.27393[a]	3.35146	0.000	−22.9475	−9.6003
			HPpCAGn	−5.64964	3.35146	0.096	−12.3233	1.0240
ICAM1	Tukey HSD	HPpCAGp	HPpCAGn	79.47500	35.41326	0.070	−5.1578	164.1078
			HPnCAGn	121.83464[a]	36.85929	0.004	33.7460	209.9233
		HPpCAGn	HPpCAGp	−79.47500	35.41326	0.070	−164.1078	5.1578
			HPnCAGn	42.35964	36.85929	0.487	−45.7290	130.4483
		HPnCAGn	HPpCAGp	−121.83464[a]	36.85929	0.004	−209.9233	−33.7460
			HPpCAGn	−42.35964	36.85929	0.487	−130.4483	45.7290
	LSD	HPpCAGp	HPpCAGn	79.47500[a]	35.41326	0.028	8.9582	149.9918
			HPnCAGn	121.83464[a]	36.85929	0.001	48.4384	195.2309
		HPpCAGn	HPpCAGp	−79.47500[a]	35.41326	0.028	−149.9918	−8.9582
			HPnCAGn	42.35964	36.85929	0.254	−31.0366	115.7559
		HPnCAGn	HPpCAGp	−121.83464[a]	36.85929	0.001	−195.2309	−48.4384
			HPpCAGn	−42.35964	36.85929	0.254	−115.7559	31.0366

[a] The mean difference is significant at the 0.05 level.

dysfunction directly by affecting the structure and function of vascular endothelial cells via inflammation in CSX [20].

On the other hand, a number of virulence factors of *H. pylori* are associated with disease outcome, including the CagA [21]. CagA is a 128-kDa *H. pylori* antigen, associated with enhanced virulence and cytotoxin production [22]. Recently researchers have revealed an association between CagA(+) strains and rigorous forms of gastrointestinal diseases including peptic ulcer and gastric cancer [15,23,24]. CagA recently has been associated with CSX [17,25].

Although prevalence of CagA has been studied widely in gastrointestinal diseases, but no previous study has investigated the possible association of these more virulent *H. pylori* strains in CSX patients with involvement of endothelial dysfunction. In this study, we compared the levels of three plasma endothelial dysfunction markers, ICAM-1, sE-selectin and ET-1 among three CSX patient groups including CagA(+) *H. pylori*, CagA(−) *H. pylori* and *H. pylori*(−) that were statistically similar for age, sex, BMI, blood pressure, lipids, glucose, smoking and medications. We showed that the CSX patients with CagA bearing strain of *H. pylori* chronic infection have significantly higher levels of plasma soluble endothelial dysfunction markers when compared to CagA(−), or even *H. pylori*(−) groups. Therefore, finding the "High" levels of plasma soluble adhesion molecules; ICAM-1 and sE-selectin in CagA(+) group than CagA(−) and *H. pylori*(−) groups in the present study suggests that CagA(+) strain of this bacterium may be more associated with endothelial activation. Also, in this study, the measured levels of ET-1 in CagA(+) were higher than *H. pylori*(−) group and tended to be greater than CagA(−) patients. This also may indicate that the main association between *H. pylori* infection and endothelial dysfunction is due to CagA(+) *H. pylori* infection.

Recent researches suggest a role of inflammation in the pathogenesis of endothelial dysfunction and correlation of CRP-concentration (C-Reactive Protein, a sensitive marker of inflammation) with severity of symptoms in patients with CSX [26,27]. Chronic inflammation leads to an increase in the generation of pro-inflammatory cytokines, cell adhesion molecules and growth factors that can elicit inflammatory

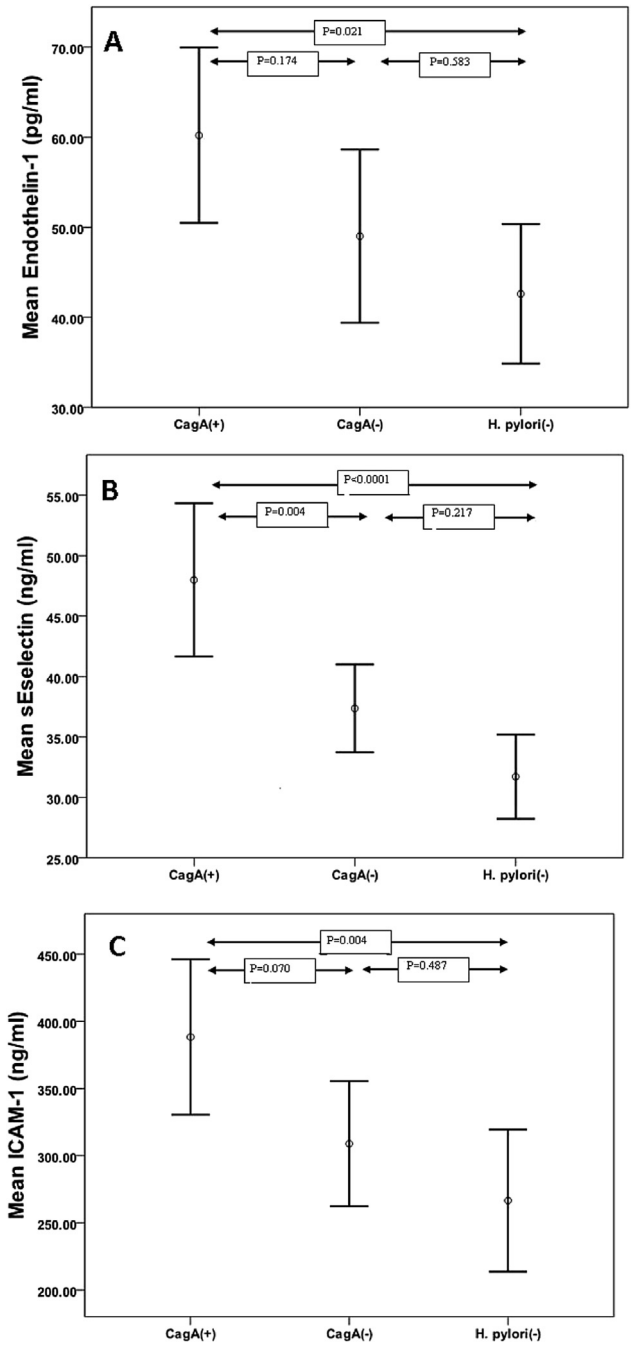

Fig. 1 – Plasma levels of measured endothelial dysfunction markers in patients with CSX. The means of plasma levels of ET-1, E-selectin and ICAM-1 compared among three groups. The groups consisted of CagA(+) [CagA IgG(+) *H. pylori* IgG(+)], CagA(–) [CagA IgG(–) *H. pylori* IgG(+)] and *H. pylori*(–) [*H. pylori* IgG(–)]. Abbreviations used: *H. pylori*: *Helicobacter pylori*; CagA: cytotoxin-associated gene A; ICAM-1: intercellular adhesion molecule-1.

and proliferative changes in the vessel walls, resulting in endothelial dysfunction [25]. In a previous case-control study, we investigated the association of inflammation and CagA(+) strains of *H. pylori* in CSX using inflammation markers such as Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). We found that the CagA positive strain of *H. pylori* can not only be a trigger, and may also have a role via chronic inflammation in the pathogenesis of CSX [28]. Previous studies showed the contribution of endothelium to the inflammatory response with regard to production of cytokine and chemokine after infection with pathogenic bacteria like *H. pylori* [29]. During inflammatory responses, the transcription factor nuclear factor kappa B (NF-κB) plays a key role in the regulation of participating genes [30]. The expression of ICAM-1 and E-Selectin is under NF-κB control in endothelial cells [31] and NF-κB activation has been shown during infection with *H. pylori* [32]. Giving these information together, it is possible that CagA(+) may induce upregulation of special expression factors that led to higher levels of endothelial dysfunction markers in plasma of CagA(+) group in this study.

In this study, we had some limitations. Our criteria for being diagnosed as infection were IgG positivity to *H. pylori*. Because positivity of IgG to *H. pylori* can provide evidence of chronic infection even following eradication of *H. pylori* [33,34], it is possible some of our patients not to have current active infection and probably they have been infected in the past. Therefore, it should be considered that infection to *H. pylori* in this research means patients who their plasma is currently positive for anti-*H. pylori* IgG antibody. The mean age of the CSX patients in this study were 51 which nearly consist of 2/3 female individuals. As CAD prevalence in middle age is low, resulting in high pseudopositive exercise stress test results and this was another limitation in our study.

After all, it should be added that well designed clinical trial studies might to be needed to further confirm these results. Although all patients in this study were CSX patients, studies with adjoining normal controls by regarding *H. pylori* and CagA status and by using non-humoral endothelial dysfunction evaluation methods like FMD is proposed. In addition, a study with *H. pylori* eradication and following up the angina symptoms and endothelial function quality propose to future investigation. Currently we can not prove the eradication of *H. pylori* infection can either improve angina symptom or progression of atherosclerosis.

Conclusion

The conclusion drawn from the results is that high levels of markers of endothelial dysfunction are related with CagA(+) *H. pylori* infection in patients with CSX, given contributors to endothelial dysfunction including age, sex, obesity, blood pressure, lipid, glucose, smoking and medications are statistically controlled. Consequently, the possible role of CagA(+) infection in the pathogenesis of CSX with involvement of endothelial dysfunction is suggested. This study revealed that patients with chronic CagA(+) *H. pylori* infection have high degree of endothelial dysfunction.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding this manuscript.

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