Helicobacter

Helicobacter ISSN 1523-5378

Association of Inflammation and Cytotoxin-Associated Gene A Positive Strains of *Helicobacter Pylori* in Cardiac Syndrome X

Yousef Rasmi,* Sina Raeisi* and Mir H. Seyyed Mohammadzad†

Departments of *Biochemistry, †Cardiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

Keywords
Cardiac syndrome X, *Helicobacter pylori*, inflammation, interleukin-6, tumor necrosis factor-alpha, cytotoxin-associated gene A.

Reprint requests to: Yousef Rasmi, Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.
E-mail: rasmiy@umsu.ac.ir

Abstract

Background: Cardiac syndrome X (CSX) is a condition in which patients have the pain of angina despite normal coronary angiogram. *Helicobacter pylori* (*H. pylori*) infection causes chronic inflammation which may play a pathogenic role in CSX. We surveyed the association of inflammation with *H. pylori* and its virulent strain (cytotoxin-associated gene A positive; CagA+) infections with CSX.

Material and Methods: Sixty patients with CSX (38 women/22 men; mean age: 51.8 ± 12.3) and 60 age- and gender-matched healthy controls (39 women/21 men; mean age: 48.9 ± 6.3) were enrolled.

Plasma samples were tested for the presence of IgG antibody to *H. pylori* using enzyme linked immunosorbent assay (ELISA) method. IgG- positive patients were determined by the presence of IgG antibody to CagA, also by ELISA method. Also, plasma levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were measured by ELISA method.

Results: Patients with CSX were detected to have significantly higher plasma IL-6 and TNF-α level in comparison with normal controls (33.6 ± 3.5 vs 3.2 ± 0.4 and 24.2 ± 2.3 vs 3.1 ± 0.4, respectively; $p < 0.01$). The plasma levels of these inflammatory factors in CgA+ were significantly higher than those in CagA− (CSX: IL-6: 43.05 ± 5.04 vs 23.97 ± 4.58 and TNF-α: 31.43 ± 3.13 vs 16.47 ± 2.93, Controls: IL-6: 3.52 ± 1.39 vs 2.90 ± 0.67 and TNF-α: 5.39 ± 1.17 vs 2.22 ± 0.43, respectively; $p < 0.05$).

Conclusion: The CagA+ strain of *H. pylori*, can not only be a trigger, and may also have a role via chronic inflammation in the pathogenesis of CSX.

Cardiac syndrome X (CSX) is a condition characterized by the presence of angina pectoris and a positive response to stress or radionuclide tests (thallium scan) with a normal coronary arteriogram [1]. It is found in up to 20% of angina patients undergoing angiography [2]. Despite the extensive studies, the pathogenesis of this syndrome, however, is not well known but coronary endothelial dysfunction has been proposed as the most important pathogenetic mechanism in CSX [3,4]. Previous studies have shown an association between viral and bacterial infections such as *Helicobacter pylori* (*H. pylori*) infection with vascular diseases such as CSX [5]. *H. pylori* is a gram-negative bacterium which infects the human stomach [6]. It may cause extra-intestinal manifestations some of which are functional ischemic heart disease and respiratory system disease [7,8] and has recently been associated with CSX [2]. Chronic infection of *H. pylori* is most probably the cause of increased production of various inflammatory metabolites, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) which also affect blood vessel motility and induce endothelial dysfunction [9]. IL-6 is one of the most important mediators of fever and of the acute phase response [10,11]. IL-6 has been described as ''hepatocyte stimulating factor'' and strongly stimulates hepatocytes to make acute phase proteins in response to inflammation [12]. This cytokine is always found in increased levels in sites of inflammation and is likely very important in a number of undescribed ways in inflammatory regulation [13,14].

The TNF-α is a cytokine involved in systemic inflammation [15,16]. Most organs of the body appear to be affected by TNF-α and the cytokine serves a variety of functions, many of which are not yet fully understood. In the liver, it stimulates the acute phase response [17].

On the other hand, the virulent cytotoxin-associated gene A positive (CagA+) strain of *H. pylori* may evoke a more consistent release of these cytokines and it has been identified as a possible marker of virulence of *H. pylori* [18,19]. In this study, we survived our hypothesis that *H. pylori* and its CagA+ strain, by increasing inflammation, might be involved in the pathogenesis of CSX.

Methods

Study Population

Patients with CSX and apparently healthy controls were studied. The case group consisted of 60 consecutive patients. Entry criteria were typical anginal chest pain, normal 12-lead ECGs at rest, a positive exercise ECG stress test response and normal coronary angiogram. Non-cardiac causes of chest pain, such as gastrointestinal and musculoskeletal disorders were also investigated and ruled out as appropriate. Patients with diabetes mellitus were not included, since confirmed inflammatory markers increase in diabetes mellitus. The control group consisted of 60 (39 women and 21 men) apparently healthy individuals. None of the controls had a previous history of chest pain or acute/chronic diseases. Also, none of these subjects were taking cardiac or non-cardiac medications. The study was approved by the local research ethics committee and all subjects gave written informed consent.

Study Protocol

A 5-ml tri-sodium-citrated blood sample was obtained from an antecubital vein in each subject and centrifuged at $2000 \times g$ for 15 minutes. Plasma was aliquoted and stored at −80 °C until analysis. Specific anti-*H. pylori* immunoglobulin-G (IgG) concentration was measured with a commercial enzyme-linked immunosorbent assay (ELISA; Glob anti-HP/IgG, Milan, Italy) according to the manufacturer's instructions (sensitivity 96.5% and specificity 98.6%). Plasma positivity to the specific virulence-associated *H. pylori* antigen CagA was also assessed by ELISA (Dia.Pro, Milan, Italy; sensitivity and specificity >98%) in IgG- *H. pylori* positive samples. Also plasma levels of IL-6 and TNF-α were determined by using ELISA kits from Bender MedSystems Inc. (Vienna, Austria).

Statistical Analysis

The data were analyzed by SPSS 16.0 software. Age, systolic blood pressure, diastolic blood pressure, and BMI were shown as mean ± standard deviation (SD). The levels of inflammatory factors (IL-6 and TNF-α) were shown as mean ± standard error of mean (SEM).

The differences between the groups and subgroups were interpreted on the basis of independent-samples *T*-test and for qualitative data on the basis of chi-square test. A *p*-value of <0.05 was considered statistically significant.

Results

Sixty patients (38 women and 22 men) with a mean age of 51.8 ± 12.3 years were compared with 60 healthy controls (39 women and 21 men) with a mean age of 48.9 ± 6.3 years. Demographic and clinical characteristics of both groups are depicted in Table 1. Data are showed as mean ± SD.

The levels of inflammatory markers were measured and were compared between CSX and control groups. Data are shown as mean ± SEM. As presented in Table 2, patients with CSX were detected to have significantly ($p < 0.01$) higher plasma IL-6 and TNF-α levels in comparison with healthy controls.

Anti-*H. pylori* antibody was diagnosed in 57 (95.0%) CSX patients and 24 (40.0%) individuals in control group ($p < 0.01$). Also, among 31 CSX patients, were CagA+ (54.4% of anti-*H. pylori*+), whereas only 13 individuals in control group were CagA+ (54.2% of anti-*H. pylori*+, $p > 0.05$).

The levels of IL-6 and TNF-α were compared between anti-*H. pylori*+ with anti-*H. pylori*− and anti-CagA+ with

Table 1 Demographic and clinical characteristics of CSX patients and control group

Risk factors	CSX	Control	*p*-Value
Mean age (years)	51.8 ± 12.3	48.9 ± 6.3	0.096
Gender (female/male)	38/22	39/21	0.849
Systolic BP (mmHg)	122.5 ± 9.3	120.2 ± 7.0	0.127
Diastolic BP (mmHg)	77.6 ± 8.5	75.8 ± 6.9	0.201
BMI (kg/m^2)	27.0 ± 4.2	26.2 ± 3.4	0.301

BP, blood pressure; BMI, body mass index; CSX, Cardiac syndrome X.

Table 2 The levels of inflammatory factors in CSX and control groups

Inflammatory factors	CSX	Control	*p*-Value
IL-6 (pg/mL)	33.6 ± 3.5	3.2 ± 0.4	0.0001
TNF-α (pg/mL)	24.2 ± 2.3	3.1 ± 0.4	0.0001

IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; CSX, Cardiac syndrome X.

anti-CagA− subgroups. As presented in Table 3, in both groups the plasma levels of IL-6 and TNF-α in anti-*H. pylori*+ subgroups were significantly higher than those in anti-*H. pylori*− subgroups. Limitation in our analysis in this step was the low number of anti-*H. pylori*− patients in CSX group (5%: three of the samples were anti-*H. pylori*−). Regarding this limitation, *p*-value stated was not available (Table 3) for anti-*H. pylori* status in CSX patients.

In addition, as shown in Table 4, in both groups the plasma levels of IL-6 and TNF-α in anti-CagA+ subgroups were significantly higher than those in anti-CagA− subgroups.

Discussion

Cardiac Syndrome X encompasses several possible causal mechanisms. Cardiac and non-cardiac mechanisms have been proposed, among which endothelial dysfunction of the coronary microcirculation features prominently [20].

In addition, *H. pylori* recently has been associated with CSX. Eskandarian et al. [2], showed that 95% of CSX patients were *H. pylori*+, while only 47.5% of healthy control group were infected. *H. pylori* may cause chronic inflammation and immune response with the release of some cytotoxic substances which are mainly responsible for the systemic manifestations of *H. pylori* [21].

Recent findings have suggested that chronic inflammation may contribute to endothelial dysfunction in CSX. Lanza et al. [22], reported that two indexes of systemic inflammation, C-reactive protein and interleukin-1 receptor antagonist, increased in patients with CSX compared with those in well-matched healthy control subjects, suggesting that low-grade inflammation may play a pathogenetic role in CSX patients. The possibility that inflammatory mechanisms might contribute to endothelial activation and dysfunction in CSX was first suggested by Tousoulis et al. [23], who found increased blood levels of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, adhesion molecules that are synthesized by activated endothelial cells in response to inflammatory stimuli, in patients with CSX. Previously, due to the factors that occur secondary to *H. pylori* infection, we speculated that *H. pylori* may also cause endothelial dysfunction directly by affecting the structure and function of vascular endothelial cells via inflammation [24].

Helicobacter Pylori leads to mucosal increases in many proinflammatory and immunoregulatory cytokines, and also increases in members of the chemokine group of peptides [25]. As a most probable target in *H. pylori* infection, and a major interface between the host and pathogen, the epithelial cell initiates acute mucosal inflammation and interacts with the other mucosal cell proliferation via a cytokine network [26].These two responses may be regulated differentially following induction of cytokines in the inflammatory cascade, including TNF-α and IL-6 [27,28]. Extensive research since then has revealed that TNF-α is a major mediator of inflammation, viral replication, tumor metastasis, transplant rejection, rheumatoid arthritis, and septic shock [29]. Dysregulation of TNF-α has been implicated in a wide variety of inflammatory diseases including rheumatoid arthritis, Crohn's disease, multiple sclerosis,

Table 3 The levels of inflammatory factors in anti-*H. pylori*+ and anti-*H. pylori*− subgroups

	CSX			Control		
Inflammatory factors	Anti-*H. pylori*+	Anti-*H. pylori*−	*p*	Anti-*H. pylori*+	Anti-*H. pylori*−	*p*
IL-6 (pg/mL)	34.35 ± 3.64	20.21 ± 3.49	NA	4.87 ± 0.88	2.11 ± 0.32	0.001
TNF-α (pg/mL)	24.61 ± 2.36	15.75 ± 2.26	NA	3.94 ± 0.73	2.49 ± 0.28	0.039

IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; NA, not available; CSX, Cardiac syndrome X.

Table 4 The levels of inflammatory factors anti-CagA+ and anti-CagA− subgroups

	CSX			Control		
Inflammatory factors	Anti-CagA+	Anti-CagA−	*p*	Anti-CagA+	Anti-CagA−	*p*
IL-6 (pg/mL)	43.05 ± 5.04	23.97 ± 4.58	0.008	6.52 ± 1.39	2.90 ± 0.67	0.037
TNF-α (pg/mL)	31.43 ± 3.13	16.47 ± 2.93	0.001	5.39 ± 1.17	2.22 ± 0.43	0.027

CagA, cytotoxin-associated gene A; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; CSX, Cardiac syndrome X.

psoriasis, scleroderma, atopic dermatitis, systemic lupus erythematosus, type II diabetes, atherosclerosis, myocardial infarction, osteoporosis, and autoimmune deficiency disease [30].

Interleukin (IL)-6 is produced at the site of inflammation and plays a key role in the acute phase response as defined by a variety of clinical and biological features such as the production of acute phase proteins [31].

In this study, patients with CSX were detected to have significantly higher plasma IL-6 and TNF-α levels in comparison with healthy controls. Also in both groups, the plasma levels of these inflammatory factors in anti-CagA+ subjects were significantly higher than those in anti-CagA− subjects. There are no data in literature concerning the relation between CagA status and inflammation in CSX patients.

Regarding our results, we speculate that *H. pylori* and prominently its CagA+ strain infection causes chronic inflammation and increases the generation of various inflammatory metabolites such as cytokines. Increase in these factors may affect vessel motility and can elicit inflammatory and proliferative changes in the vessel walls leading to endothelial dysfunction which is the most prominent cause of the CSX.

Conclusion

In conclusion, we hypothesize that *H. pylori* infection can be a trigger for the probable mechanism of endothelial dysfunction via chronic inflammation in the pathogenesis of CSX. Since endothelial dysfunction is the trigger point for many diseases, we must pay more attention to the diagnosis and treatment of *H. pylori*. *H. pylori* may be the cause of, or at least one of the leading factors in many other diseases. To address this hypothesis, further prospective studies are warranted to evaluate the role of *H. pylori* eradication on inflammation status and endothelial function.

Acknowledgements and Disclosures

Competing interests: the authors have no competing interests.

References

1 Hurst T, Olson TH, Olson LE, Appleton CP. Cardiac syndrome X and endothelial dysfunction: new concepts in prognosis and treatment. *Am J Med* 2006;119(7):560–6.
2 Eskandarian R, Malek M, Mousavi SH, Babaei M. Association of *Helicobacter pylori* infection with cardiac syndrome X. *Singapore Med J* 2006;47(8):704–6.
3 Alroy S, Preis M, Barzilai M, Cassel A, Lavie L, Halon DA, Amir O, Lewis BS, Flugelman MY. Endothelial cell dysfunction in women with cardiac syndrome X and MTHFR C677T mutation. *Isr Med Assoc J* 2007;9(4):321–5.
4 Quyyumi AA, Cannon RO III, Panza JA, Diodati JG, Epstein SE. Endothelial dysfunction in patients with chest pain and normal coronary arteries. *Circulation* 1992;86(6):1864–71.
5 Nocente R, Gentiloni N, Cremonini F, Giorgi A, Serricchio M, Santoliquido A, Gasbarrini G, Gasbarrini A. Resolution of syndrome X after eradication of virulent CagA-positive *Helicobacter pylori*. *South Med J* 2000;93(10):1022–3.
6 Beier D, Frank R. Molecular characterization of two-component systems of *Helicobacter pylori*. *J Bacteriol* 2000;182(8):2068–76.
7 Oshima T, Ozono R, Yano Y, Oishi Y, Teragawa H, Higashi Y, Yoshizumi M, Kambe M. Association of *Helicobacter pylori* infection with systemic inflammation and endothelial dysfunction in healthy male subjects. *J Am Coll Cardiol* 2005;45(8):1219–22.
8 Kanbay M, Kanbay A, Boyacioglu S. *Helicobacter pylori* infection as a possible risk factor for respiratory system disease: a review of the literature. *Respir Med* 2007;101(2):203–9.
9 De Meyer GR, Herman AG. Vascular endothelial dysfunction. *Prog Cardiovasc Dis* 1997;39(4):325–42.
10 Sun R, Jaruga B, Kulkarni S, Sun H, Gao B. IL-6 modulates hepatocyte proliferation via induction of HGF/p21cip1: regulation by SOCS3. *Biochem Biophys Res Commun* 2005;338(4):1943–9.
11 Messina GA, Panini NV, Martinez NA, Raba J. Microfluidic immunosensor design for the quantification of interleukin-6 in human serum samples. *Anal Biochem* 2008;380(2):262–7.
12 Fielding CA, McLoughlin RM, McLeod L, Colmont CS, Najdovska M, Grail D, et al. IL-6 regulates neutrophil trafficking during acute inflammation via STAT3. *J Immunol* 2008;181(3):2189–95.
13 Mukaino M, Nakamura M, Okada S, Toyama Y, Liu M, Okano H. Role of IL-6 in regulation of inflammation and stem cell differentiation in CNS trauma. *Nihon Rinsho Meneki Gakkai Kaishi* 2008;31(2):93–8.
14 Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001;104(4):487–501.
15 Sbarsi I, Falcone C, Boiocchi C, Campo I, Zorzetto M, De Silvestri A, Cuccia M. Inflammation and atherosclerosis: the role of TNF and TNF receptors polymorphisms in coronary artery disease. *Int J Immunopathol Pharmacol* 2007;20(1):145–54.
16 Old LJ. Tumor necrosis factor (TNF). *Science* 1985;230(4726):630–2.
17 On YK, Park R, Hyon MS, Kim SK, Kwon YJ. Are low total serum antioxidant status and elevated levels of C-reactive protein and monocyte chemotactic protein-1 associated with cardiac syndrome X? *Circ J* 2005;69(10):1212–7.
18 Mine T. Heterogeneity in the cagA gene of *Helicobacter pylori* and its clinical role. *J Gastroenterol* 2000;35(12):945–6.
19 Kato S, Sugiyama T, Kudo M, Ohnuma K, Ozawa K, Iinuma K, Asaka M, Blaser MJ. CagA antibodies in Japanese children with nodular gastritis or peptic ulcer disease. *J Clin Microbiol* 2000;38(1):68–70.
20 Arroyo-Espliguero R, Kaski JC. Microvascular dysfunction in cardiac syndrome X: the role of inflammation. *CMAJ* 2006;174(13):1833.
21 Karatas OF, Bayrak O, Cimentepe E, Unal D. An occult risk factor for chronic prostatitis: *Helicobacter pylori*. *Med Hypotheses* 2007;69(4):963–4.

22 Lanza GA, Sestito A, Cammarota G, Grillo RL, Vecile E, Cianci R, Speziale D, Dobrina A, Maseri A, Crea F. Assessment of systemic inflammation and infective pathogen burden in patients with cardiac syndrome X. *Am J Cardiol* 2004;94(1):40–4.
23 Tousoulis D, Davies GJ, Asimakopoulos G, Homaei H, Zouridakis E, Ahmed N, Kaski JC. Vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 serum level in patients with chest pain and normal coronary arteries (syndrome X). *Clin Cardiol* 2001;24(4):301–4.
24 Rasmi Y, Raeisi S. Possible role of *Helicobacter pylori* infection via microvascular dysfunction in cardiac syndrome X. *Cardiol J* 2009;16(6):585–7.
25 Crabtree JE. Role of cytokines in pathogenesis of *Helicobacter pylori*-induced mucosal damage. *Dig Dis Sci* 1998;43(9 Suppl.): 46S–55S.
26 Stadnyk AW. Intestinal epithelial cells as a source of inflammatory cytokines and chemokines. *Can J Gastroenterol* 2002;16(4):241–6.
27 Crabtree JE, Peichl P, Wyatt JI, Stachl U, Lindley IJ. Gastric interleukin-8 and IgA IL-8 autoantibodies in *Helicobacter pylori* infection. *Scand J Immunol* 1993;37(1):65–70.
28 Gionchetti P, Vaira D, Campieri M, Holton J, Menegatti M, Belluzzi A, Bertinelli E, Ferretti M, Brignola C, Miglioli M. Enhanced mucosal interleukin-6 and -8 in *Helicobacter pylori*-positive dyspeptic patients. *Am J Gastroenterol* 1994;89(6): 883–7.
29 Aggarwal BB, Shishodia S, Ashikawa K, Bharti AC. The role of TNF and its family members in inflammation and cancer: lessons from gene deletion. *Curr Drug Targets Inflamm Allergy* 2002;1(4):327–41.
30 Aggarwal R, Shishoida S, Takada Y, Jackson-Bernitsas D, Ahn K, Sethi G, Ichikawa H. TNF blockadae: an inflammatory issue. *Ernst Schering Res Found Workshop* 2006;56:161–86.
31 Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 2006;8(Suppl. 2):S3.