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

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

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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 CagA+ 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.
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 \(-80^\circ C\) 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-\(\alpha\) 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 \pm standard deviation (SD). The levels of inflammatory factors (IL-6 and TNF-\(\alpha\)) were shown as mean \pm 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 \pm 12.3 years were compared with 60 healthy controls (39 women and 21 men) with a mean age of 48.9 \pm 6.3 years. Demographic and clinical characteristics of both groups are depicted in Table 1. Data are showed as mean \pm SD.

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

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

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

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

<table>
<thead>
<tr>
<th>Inflammatory factors</th>
<th>CSX</th>
<th>Control</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>33.6 \pm 3.5</td>
<td>3.2 \pm 0.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/mL)</td>
<td>24.2 \pm 2.3</td>
<td>3.1 \pm 0.4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

IL-6, interleukin-6; TNF-\(\alpha\), 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-
\textit{H. pylori}+ subgroups were significantly higher than those in anti-\textit{H. pylori}− subgroups. Limitation in our
analysis in this step was the low number of anti-\textit{H. pylori}− patients in CSX group (5%: three of the
samples were anti-\textit{H. pylori}−). Regarding this limitation, \textit{p}-value stated was not available (Table 3) for anti-
\textit{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, \textit{H. pylori} recently has been associated with CSX. Eskandarian et al. [2], showed that 95% of CSX patients were \textit{H. pylori}+, while only 47.5% of healthy control group were infected. \textit{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 \textit{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 Tousoils 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 \textit{H. pylori} infection, we speculated that \textit{H. pylori} may also cause endothelial dysfunction directly by affecting the structure and function of vascular endothelial cells via inflammation [24].

\textit{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 \textit{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,
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**


