Lack of significant association between *Helicobacter pylori* infection and homocysteine levels in patients with cardiac syndrome X

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**Abstract**

**Background:** Helicobacter pylori (H. pylori) has been implicated in the pathogenesis of several diseases such as cardiac syndrome X (CSX), which includes chest pain, positive exercise stress test and normal angiography. Also, elevation of homocysteine (Hcy) level is associated with CSX, as it can severely disturb vascular endothelial function. We aimed to elucidate whether the infection of H. pylori affect the level of Hcy in CSX.

**Methods:** Eighty-eight patients with CSX (32 men, 56 women; mean age: 53.8 ± 11.9) and 97 healthy controls (36 men, 61 women; mean age: 45.7 ± 7.3) were enrolled. Plasma samples were tested for the presence of IgG antibody to H. pylori using enzyme linked immunosorbent assay method. Hcy levels were measured enzymatically.

**Results:** Plasma Hcy concentration in CSX patients is higher than control group (13.1 ± 2.6 vs. 11.8 ± 2.5 \(\mu\)mol/L; \(p = 0.002\)). There was no significant difference between Hcy in H. pylori\(^+\) and H. pylori\(^-\) individuals in CSX group (13.1 ± 2.7 vs. 12.2 ± 0.6 \(\mu\)mol/L; \(p = 0.554\)) and between two groups in controls, respectively (12.1 ± 2.2 vs. 11.4 ± 2.9 \(\mu\)mol/L; \(p = 0.148\)).

**Conclusions:** Although there is Hcy level increase in H. pylori\(^+\) CSX patients and controls comparing to H. pylori\(^-\) subjects, but other factors may affect on Hcy level, too. (Cardiol J 2012; 19, 5: 466–469)

**Key words:** cardiac syndrome X, *Helicobacter pylori*, homocysteine

**Introduction**

Cardiac syndrome X (CSX) is a clinical definition, characterized by three main features: angina like chest pain; ST segment depression on treadmill exercise testing and normal coronary arteriography [1, 2]. In about 10 to 30% patients who are referred for coronary angiography, does not show vessel stenosis or coronary spasm [3]. The pathogenesis of CSX is still uncertain but microvascular and endothelial dysfun-
tion has been invoked [4]. On the other hand, plasma homocysteine (Hcy) level, which is known to cause endothelial dysfunction and microvascular ischemia were higher in CSX patients [5]. Hcy is a sulphur amino acid formed from methionine during transmethylation, and is either salvaged to methionine by a folate and co-balamin-dependent re-methylation reaction or directed toward degradation by vitamin B6-dependent enzyme cystathionine beta-synthase [6, 7]. Also, previous studies has shown an association between viral and bacterial infections including Helicobacter pylori (H. pylori) and some vascular disease such as CSX [8]. H. pylori is a spiral shaped positive gram that cause the most common chronic bacterial infection in the world [9].

Recent data indicate a possible correlation between H. pylori infection and elevated Hcy levels [10]. This study was aimed to examine and reveal the possible relation between H. pylori infection and Hcy levels in CSX.

Methods

Study population

CSX patients and apparently healthy controls were enrolled. The CSX group consisted of 88 patients (32 men, 56 women). Entry criteria were typical angina 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 Hcy increase in diabetes mellitus. The control group consisted of 97 (36 men, 61 women) apparently healthy individuals. None of the controls had a previous history of chest pain or acute/chronic diseases. Also, none of controls were taking cardiac or non-cardiac medication. 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 each subject and centrifuged at 2000 × g for 15 min. Plasma was aliquoted and stored at –80°C until analysis. 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 instruction (sensitivity 96.5% and specificity 98.6%). The plasma Hcy levels were measured enzymatically (Diazyme, USA). Briefly, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosyl methionine (SAM), catalyzed by a Hcy S-methyltransferase. The co-substrate conversion product is amplified by coupled enzymatic cycling reactions. The total Hcy level in the sample is indirectly proportional to the amount of NADH conversion to NAD+.

Statistical analysis

The data were analyzed by SPSS 16.0 software. Age and BMI were shown as mean ± standard deviation (SD). The levels of homocysteine were shown as mean ± standard error of mean (SEM) where 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 χ² test. A p-value less than 0.05 was considered statistically significant.

Results

The main demographic characteristics of the two groups are presented in Table 1. The mean body mass index was 27.2 ± 4.4 kg/m² in CSX group and 26.1 ± 3.2 kg/m² in control group (p > 0.05).

Eighty two (93.2%) of CSX patients and 56 (57.7%) of controls were seropositive for H. pylori (p = 0.001). In this study we divided the patients in 3 groups according to the age including 25–40 years, 40–55 years and over 55 years (Table 2). In 25–40 years and 40–55 years group there were

Table 1. The main demographic characteristics of cardiac syndrome X (CSX) and control groups.

<table>
<thead>
<tr>
<th></th>
<th>CSX</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>32/56</td>
<td>36/61</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Age [years]</td>
<td>53.8 ± 11.9</td>
<td>45.7 ± 7.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Body mass index [kg/m²]</td>
<td>27.2 ± 4.4</td>
<td>26.1 ± 3.2</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. The prevalence of H. pylori in cardiac syndrome X (CSX) and control groups.

<table>
<thead>
<tr>
<th>Age groups [years]</th>
<th>CSX</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25–40</td>
<td>15 (100%)</td>
<td>0 (0%)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>19 (73.1%)</td>
<td>7 (26.9%)</td>
<td></td>
</tr>
<tr>
<td>40–55</td>
<td>31 (96.9%)</td>
<td>1 (3.1%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>31 (50.8%)</td>
<td>30 (49.2%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 55</td>
<td>36 (87.8%)</td>
<td>5 (12.2%)</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>6 (60%)</td>
<td>4 (40%)</td>
<td></td>
</tr>
</tbody>
</table>
a significant difference between prevalence of anti-
*H. pylori* positive (*H. pylori*⁺) and anti-*H. pylori* nega-
tive (*H. pylori*⁻) among CSX and controls (p = 0.03
and p < 0.001, respectively). But there was no sig-
nificant difference in > 55 group (p = 0.061).

In CSX and control groups the mean level of Hcy was 13.1 ± 2.6 and 11.8 ± 2.5 μmol/L, respec-
tively (p = 0.002). On the other hand, the mean Hcy
levels in *H. pylori*⁺ and *H. pylori*⁻ individuals of CSX
group was 13.1 ± 2.7 and 12.2 ± 0.6 μmol/L, respec-
tively (p = 0.554). In control group, the mean
Hcy levels of *H. pylori*⁺ and *H. pylori*⁻ individuals, was
12.1 ± 2.2 and 11.4 ± 2.9 μmol/L, respectively
(p = 0.148; Table 3). Hcy level in *H. pylori*⁺ individu-
als tend to increase but it is not significant in com-
parison with *H. pylori*⁻ individuals.

### Discussion

Many studies showed that the elevated Hcy is
associated with endothelial dysfunction and contrib-
utes to increased risk of cardiovascular diseases
[11]. These effects are mediated by its cytotoxic ef-
fects on endothelial cells, stimulation of platelet
adhesion and/or its promotion of pro-coagulant ac-
tivity [12]. There is some evidence that thiols re-
act in the presence of NO to form S-nitrosothiols,
compounds with vasodilatory and antiplatelet ef-
fects [13]. Timurkaynak et al. [5] reported that plas-
ma Hcy level, which is known to cause endothelial
dysfunction and microvascular ischemia were high-
er in CSX patients.

In addition, Hcy metabolism involves a com-
plex interaction between folate and vitamin B₁₂. So
chronic *H. pylori* infection causes decreased absorp-
tion of both folate and vitamin B₁₂ and this condi-
tion is associated with hyperhomocysteinemia [7].
In this study because of some limitation we could
not measure folate and B₁₂ levels in plasma.

Multiple pathophysiologic abnormalities have
been reported in patients with CSX. The most con-
vincing evidence includes generalized endothelial
dysfunction and inflammation [14]. In addition
*H. pylori* recently has been associated with CSX [15].
Mendall et al. in 1994, for the first time showed that
*H. pylori* seropositivity was twice as common in co-
ronary arteries disease patients as in control subjects
[9]. Since then, many studies have been demon-
strated an association between *H. pylori* infections
and some vascular diseases, such as ischemic heart
disease [16]. Chronic infection may be accompanied
by persistently increased production of inflamma-
tory metabolites [8]. *H. pylori* produces some anti-
genic substances, including heat shock protein, ure-
ase, and lipopolysaccharide, all of which can be
taken up and processed by lamina properia mac-
rophages and active T-cells and cause increased
production of inflammatory cytokines such as inter-
leukine-1 (IL-1), IL-6, tumor necrosis factor alpha
(TNFα), and most important IL-8. These agents
may affect vessel motility and induce endothelial
dysfunction and microvascular hyperconstriction
[15]. On the other hand, Elizade et al. in 1997
showed that *H. pylori* infection induces an increase
in the flux of leukocytes and in the appearance of
platelet and leukocyte-platelet aggregates in gas-
tric venules in an animal model, so platelet activa-
tion and aggregation contribute to the associated
microvascular dysfunction and inflammatory cell
recruitment [17].

The main finding of the present study is that
patients with CSX showed increased Hcy levels as
compared to normal controls. The frequency of
*H. pylori* infection was also increased in patients with
CSX, and the presence of *H. pylori* infection did not
affect the Hcy level significantly.

Because there is no consistent evidence, the
hypothesis that *H. pylori* causes increased Hcy le-
vel in patients with CSX was still controversial. Re-
cent studies reported no significant difference in
blood Hcy level in *H. pylori*⁺ and *H. pylori*⁻ individu-
als [18]. Our analysis showed the similar results
indicating that there is no link from *H. pylori*
fection to increased Hcy levels in CSX patients. We
suggest that the postulated link between *H. pylori*
and CSX [19], if it is actually exists, is unlikely to
be mediated through elevated Hcy levels.

### Conclusions

Although there is an increase in Hcy level in
*H. pylori*⁺ in CSX and controls comparing to *H. pylori*⁻
subjects, but other factors may affect on Hcy level.

**Conflict of interest:** none declared
Y. Rasmi et al. Lack of association between H. pylori infection and Hcy in CSX

References