The Influence of *Helicobacter pylori* Eradication on Leptin, Soluble CD40 Ligand, Oxidative Stress and Body Composition in Patients with Peptic Ulcer Disease

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**Abstract**

**Objective** To determine the effect of *Helicobacter pylori* (*H. pylori*) eradication on blood levels of soluble CD40 ligand, leptin, oxidative stress and body composition in patients with dyspepsia infected with *H. pylori*.

**Methods** The infection of *H. pylori* was based on the presence of both ¹⁴C urea breath test (UBT) and histology. Patients were given triple eradication therapy for 14 days and at 3 months after the treatment, ¹⁴C UBT was reinstituted. Fasting glucose, leptin, body composition, soluble CD40 ligand, total oxidant status (TOS) were studied before and at 3 months after the treatment.

**Results** In 33 subjects, *H. pylori* infection was successfully eradicated. sCD40L, and TOS levels were significantly decreased after *H. pylori* eradication. The percentage of body fat and body fat mass significantly decreased whereas the fat free mass (FFM) increased after eradication. However, eradication of the organism yielded no differences in leptin levels.

**Conclusion** These findings suggest that *H. pylori* eradication reduces the sCD40L and oxidative stress, fat mass with a significant increase in fat free mass. Thus, eradication of *H. pylori* infection not only improves ulcer healing, but may also reduce the presumed atherosclerosis risk.

**Key words:** *Helicobacter pylori*, eradication, soluble CD40 ligand, total oxidant stress, leptin, fat mass

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**Introduction**

*Helicobacter pylori* (*H. pylori*) is a gram negative bacterium that affects individuals of all ages worldwide (1). A number of studies have suggested a relationship between *H. pylori* infection and coagulation (2-5). *H. pylori* infection could induce changes in coagulation parameters that has been investigated in a number of studies with a focus on fibrinogen (3), prothrombin fragments (4), and von Willebrand factor (vWF) (5). Recently, it has been suggested that activation of the CD40/CD40L pathway may enhance procoagulant activity and thrombus formation. sCD40 ligation on cells of the vascular wall promotes mononuclear cell recruitment and contributes to thrombosis in the setting of atherosclerosis (6). It is now generally accepted that the interaction between sCD40L and CD40 is an initial event in atherothrombosis, leading in turn to the activation of several proinflammatory and proatherosclerotic mediators (7). However, to the best of our knowledge, there is no study investi-
gating the role of modulation of the proinflammatory and atherogenic cytokine sCD40L levels in *H. pylori* positive individuals.

Several lines of evidence support a role for oxidative stress in atherogenesis. *H. pylori* infection causes inflammation, accumulation of reactive oxygen species (ROS), and oxidative DNA damage in gastric mucosa in animal and human models (8-12). ROS production of the blood samples from *H. pylori* infected subjects was significantly decreased 2 months after eradication (13). Such evidence included only *H. pylori*-infected patients with high levels of reactive oxygen species (ROS). Oxidative stress occurs through the overproduction of reactive oxygen and nitrogen species (14). Therefore, investigation of total oxidant status may be a more effective option for assessing oxidative stress in our study.

On the other hand, leptin is a protein product of the obese gene and is expressed primarily by adipocytes (15). Leptin concentrations are generally correlated with body fat stores, however, there is only one report on the effects of eradication of *H. pylori* on leptin with measurement of body composition and this report studied prepubertal children (16). However, to our knowledge, there are no data on the effect of *H. pylori* eradication on serum leptin with measurement of body composition in adults.

In this study, we aimed to determine the effect of *H. pylori* eradication on blood levels of leptin concentration, TOS, soluble CD40 ligand and body composition in patients with dyspepsia.

### Methods

#### Participants

Subjects with dyspeptic symptoms who visited the internal medicine outpatient clinic were included in the study. Subjects with hypertension (HT), diabetes mellitus (DM), known coronary artery disease, coagulation abnormalities, cerebrovascular disease, renal disease, smoking, cancer, systemic or local infection, prior history of gastric surgery, and pregnant or lactation women, usage of supplemental vitamins, statins, warfarin, nonsteroidal antiinflammatory drugs (NSAIDs) within the last 4 wk prior to study were excluded. Patients treated with antibiotic, bismuth salts, H2 receptor blocker or proton-pump inhibitors in the previous month were also not included.

*H. pylori* was considered to be present when the 14C-urea breath test (14C UBT) and histological examination were positive. Subjects who were positive for both 14C UBT and histology were given eradication therapy for 14 days. At 3 months after the treatment, 14C UBT was reinstituted and blood sampling and body composition measurements were repeated in patients who were negative for *H. pylori*. Statistical analysis was performed on the 33 subjects. This study was performed between July 2008 and March 2009. Written informed consent was obtained from each subject, and research protocols were approved by the Ethical Committee of our institution.

#### 14C urea breath test

After overnight fasting, patients swallowed 37 kBq (1 μCi) of an encapsulated form of 14C-urea/citric acid composition (Helicap™ Noster System, Stockholm, Sweden) in 25 mL water. Breath samples were collected with a special dry cartridge system (Heliprobe™ BreathCard™, Noster System) at 10 min. Patients exhaled gently into the cartridge mouth-piece until the indicator membrane changed color from orange to yellow. The BreathCard was inserted into a special small desktop Geiger-Muller counter (Heliprobe™ Analyzer; Noster System) and activity was counted for 250 s. Results were expressed both as counts per min (cpm) and as grade (0, not infected, <25 cpm; 1, equivocal, 25-50 cpm; 2, infected, >50 cpm), as suggested by the manufacturer according to the counts obtained from the cartridges.

#### Endoscopy and histology

After overnight fasting, esophago-gastro-duodenoscopy was performed with an Olympus videoscope. Two biopsy specimens were obtained from both the corpus and the antrum of the stomach. Biopsies were processed separately for histological examination according to standard procedure and Hematoxylin and Eosin staining and Giemsa staining was performed on these samples. A pathologist examined samples for the presence of active and chronic *H. pylori* infection.

#### Eradication regimen

Lansoprazole 30 mg twice daily, amoxicillin 1 g twice daily, and clarithromycin 500 mg twice daily given for 14 days were to all infected patients.

#### Laboratory evaluation

Following an overnight fasting, venous blood samples were collected for laboratory measurements in the morning (8:00-9:00 A.M.). Blood samples for the measurement of soluble sCD40L, leptin, TOS, and body composition were evaluated in patients before and at 3 months after eradication.

Blood samples were immediately centrifuged at 4°C, and stored at -70°C until analysis.

Serum leptin concentrations were determined by radioimmunoassay (RIA) (Human Leptin RIA Kit, Linco Research, Inc., St. Charles, MO, USA). Plasma sCD40L levels were determined using a commercially available ELISA kit (BioSource International, Nivelles, Belgium) according to the manufacturer’s instructions. The detection limit was 0.062 ng/mL. The overall interassay and intra-assay CVs were 6.8% and 4.0%, respectively. Serum TOS levels were determined using a novel automated measurement method, developed by Erel (17). In this method, oxidants present in the sample oxidize the ferrous ion-odanisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol.
Table 1. Clinical and Laboratory Characteristics of the Patients before and after H. pylori Eradication. Results are Presented as Mean±SD.

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After eradication</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8±4.3</td>
<td>26.1±4.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.1±10</td>
<td>89.0±11</td>
<td>0.7</td>
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<tr>
<td>Soluble CD40L levels (ng/mL)</td>
<td>8.1±6.7</td>
<td>4.1±2.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Leptin levels (ng/mL)</td>
<td>4.6±4.0</td>
<td>4.0±2.7</td>
<td>0.47</td>
</tr>
<tr>
<td>Serum TOS levels</td>
<td>13.1±9.0</td>
<td>7.0±3.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>28.5±10.5</td>
<td>25.1±9.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>21.0±9.0</td>
<td>18.4±8.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>51.3±10.8</td>
<td>53.1±10.1</td>
<td>0.009</td>
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molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylene orange in an acidic medium. The color intensity, which can be measured spectrophotometrically at 530 nm, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H₂O₂ equiv./L). All anthropometric measurements were made by the same observer using the same equipment for each subject. Height, weight, and waist circumference (WC) were measured. Foot-to-foot body fat analyzer (Tanita TBF 300, Tanita, Tokyo, Japan) was used for bioelectrical impedance analysis. Total body water (TBW), FFM, fat mass (FM), and fat percent were recorded before and after eradication.

Statistical analysis

The results are presented as mean±SD. The Wilcoxon signed rank test was used for the comparison of treatment effects on variables. The statistical analysis was carried out by using Statistical Package of Social Science (SPSS), version 13.0 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered as statistically significant.

Results

Study group was consisted of 33 H. pylori infected patients (32.5±9 years; 19 males [M]/14 females [F]). Clinical and laboratory characteristics of the patients before and after H. pylori eradication are summarized in Table 1. Plasma soluble CD40 ligand concentrations decreased significantly after eradication (8.1±6.7 vs. 4.1±2.7 ng/mL; p<0.007). However, in the comparison, the leptin levels were not different after eradication (4.6±4.0 vs. 4.0±2.7 ng/mL; p>0.05). BMI was not statistically significant decreased after H. pylori eradication but fat percent and fat mass assessed by bioelectrical impedance analysis (BIA) were significantly decreased (28.5±10.5 vs 25.1±9.2, p=0.03 for fat percentage; 21.0±9.0 vs 18.4±8.1, p=0.03 for fat mass) whereas FFM increased after eradication (51.3±10.8 vs 53.1±10.1 for FFM, p=0.009). H. pylori eradication was associated with a progressive decrease in serum TOS concentrations over baseline, achieving statistical significance at both 3 months (13.1±9.0 vs 7.0±3.8 umolEq/L, p=0.012). Before eradication, Spearman’s correlation analysis showed that leptin was found to be significantly correlated with BMI (r=0.75, p=0.008), fat mass (r=0.767, p<0.001), and percentage of fat mass (r=0.874, p<0.001). Spearman’s correlation analysis before eradication showed that plasma sCD40L and TOS levels were not correlated with variables in the group. After eradication, Spearman’s correlation analysis showed that leptin was found to be significantly correlated with BMI (r=0.549, p=0.012), fat mass (r=0.676, p<0.001), and percentage of fat mass (r=0.745, p<0.001). Spearman’s correlation analysis after eradication revealed that plasma sCD40L and TOS levels were not correlated with variables in the group.

Discussion

In this study, we demonstrated that eradication of H. pylori was associated with significantly reduced total oxidant status (TOS) and soluble CD40 ligand levels and fat mass in patients who were free of classical cardiovascular (CV) risk factors. On the other hand, eradication of the microorganism did not change the leptin concentrations.

Oxidative stress could well play a role in the altered epithelial proliferation, increased apoptosis (18), and increased oxidative DNA damage (19, 20) and atherosclerosis (21) as-
associated with H. pylori infection. The high levels of initial serum TOS may reflect free radical induced damage which may increase the risk of both atherosclerosis and cancer. The present study showed that TOS production was significantly reduced after the eradication, implying a beneficial effect in decreasing the oxidative stress by H. pylori infection. These results indicate that oxidative stress occurs in asymptomatic patients and can be modulated by H. pylori eradication. The relationship between before and after eradication of H. pylori on leptin levels has been studied in a few reports (22, 23). However, conflicting results have been reported concerning the evaluation of leptin levels in H. pylori eradicated subjects in these reports. Though some authors have reported no variation in plasma leptin levels in H. pylori-infected patients even after H. pylori eradication (22), in the study by Konturek et al (23) a significant decrease in plasma level of leptin was observed after successful H. pylori eradication. Our findings were corroborate with the report of Azuma et al (22). There were no differences in serum leptin levels before and after eradication.

On the other hand, serum leptin levels are inversely correlated with serum ghrelin levels. Serum ghrelin levels decrease with the progression of gastric atrophy associated with H. pylori (24, 25). Serum ghrelin levels are variable in H. pylori infected-patients because such patients have varying degrees of atrophic gastritis, leading to diverse levels of serum leptin levels. Therefore, our data may not be sufficient to evaluate serum leptin levels before and after eradication in only 33 cases.

Otherwise, we have found that the body composition is altered in H. pylori infected patients. The fat mass and fat percentage were significantly decreased and FFM increased 3 months after H. pylori eradication. Pacifco et al reported that H. pylori eradication is associated with the long-term effect of a significant increase in BMI, FFM and fat mass in children (16). These discrepancies could be explained by successful eradication of H. pylori which may lead to improved appetite followed by increased body weight, and that one of the possible mechanisms to explain these changes could be supported previously by a decrease in gastric ghrelin and a compensatory increase in plasma ghrelin levels after food deprivation in infected with H. pylori (26).

To our knowledge, there is no report in the literature with respect to the treatment effect on body composition in H. pylori infected patients. In the present study, we demonstrated that the eradication of the offending microorganism was associated with significantly decreased levels of fat mass and fat percentage. It has been demonstrated that fat accumulation correlates with the markers of systemic oxidative stress in humans and mice (27). A recent population-based study among adults confirmed that abdominal fat accumulation is associated with oxidative stress (28). Based on these observations, H. pylori eradication has a beneficial effect in decreasing the fat accumulation which may be induced by this infection.

Several reports in Western and Asian countries suggested that H. pylori infection could cause coronary artery diseases (3, 29). Clinical studies have shown that higher levels of sCD40L are associated with cardiovascular disease (6, 7). Nevertheless, the effects of H. pylori eradication on soluble CD40L levels have not been completely confirmed. In the present study, we demonstrated that the eradication of the microorganism was associated with significantly decreased levels of sCD40L levels. This finding is important because current literature suggests an increased risk of atherosclerosis in H. pylori infected patients and H. pylori eradication might improve the coagulation and atherosclerosis in these patients.

Atherosclerosis is also influenced by Chlamydia pneumonia as well as H. pylori. Infectious agents may trigger a cascade of biological and biochemical reactions leading to inflammation, atherogenesis, and vascular thrombosis (29-31). Chlamydia pneumonia and H. pylori are both thought to exert their atherogenic effects by up-regulating the inflammatory process and directly infecting endothelial tissue. The relationship between H. pylori and Chlamydia pneumonia and atherosclerosis may be due to confounding or co-linearity with socioeconomic status (29-31). The high level infection burden could predict vulnerable plaque (30). Therefore, these infection agents should be investigated together to determine which may cause direct vascular injury and enhanced atherosclerosis risk. The other limitations of the current study are the absence of H. pylori negative controls and the small sample size of H. pylori eradicated patients. However, treatment of H. pylori infection may not only improve ulcer healing, but it also reduces the presumed atherosclerosis risk by decreasing traditional risk factors such as oxidative stress, fat accumulation and soluble CD40L levels in these patients.

References