Carotid intima-media thickness and serum paraoxonase-1 activity in patients with Helicobacter pylori

R. METE, M. ORAN1, S. ALPSOY2, H. GUNES3, F. TULUBAS4, C. TURAN5, B. TOPCU6, M. AYDIN4, O. YILDIRIM

Department of Gastroenterology, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey
1Department of Internal Medicine, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey
2Department of Cardiology, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey
3Department of Microbiology, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey
4Department of Biochemistry, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey
5Department of Anesthesiology and Reanimation, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey
6Department of Biostatistics, Namik Kemal University Faculty of Medicine, Tekirdag, Turkey

Abstract. – AIM: To evaluate serum paraoxonase(PON)-1 activity and carotid intima-media thickness (CIMT) in patients with Cytotoxin-associated antigen(CagA)-positive and negative Helicobacter pylori strains.

PATIENTS AND METHODS: The study group included a total of 134 individuals, of whom 103 were H. pylori positive, and 31 were H. pylori negative. Five biopsies were collected from each patient for histological examination: two from the antrum, two from the corpus, and one from the incisura angularis. The presence of H. pylori was determined using a modified Gram staining protocol. Peripheral blood was collected from each patient to determine levels of triglyceride, TC, HDL-cholesterol and LDL-cholesterol. IgG antibodies against CagA protein were analyzed by enzyme immunoassays. PON-1 activity was measured by colorimetric method. Carotid intima-media thickness and atherogenic plaques were measured using a grey scale color Doppler ultrasound. Data were analyzed by descriptive and inferential statistics.

RESULTS: The right, the left and the mean CIMT were significantly higher in H. pylori (+) group versus H. pylori (-) group (p < 0.001 for all). However, the mean PON-1 concentration was significantly lower in H. pylori (+) group versus H. pylori (-) group (p < 0.001). The right, the left and the mean CIMT of CagA (+) group were significantly higher than that of CagA (-) group and controls, while PON-1 concentrations of CagA (+) group were significantly lower than that of CagA (-) group and controls (for all p = 0.0001). The right, the left and the mean CIMT of CagA (-) group were significantly higher than that of the control group, while the mean PON-1 concentration were significantly lower (for all p = 0.0001).

CONCLUSIONS: Decreased PON-1 activity may be an etiopathogenetic factor in increased atherosclerosis in patients with H. pylori infection, especially in those infected with the CagA positive strain.

Key Words: Cytotoxin-associated antigen, Atherosclerosis, Carotid intima-media thickness, Paraoxonase-1, Helicobacter pylori strains.

Introduction

Accumulating evidence indicates that some atherogenic cardiovascular risk factors (e.g., homeostatic factors and lipids) are liable to be altered by inflammation and infection by certain microbial agents including Helicobacter pylori1-3. H. pylori, a Gram-negative microaerophilic spiral bacterium that is frequently found in the human stomach, causes chronic and active gastritis, peptic ulcer disease and is also associated with gastric adenocarcinoma4,5. H. pylori synthesizes numerous virulence factors like urease, arginase, blood group antigen binding adhesin (BabA), vacuolating toxin (VacA) or cytotoxin-associated antigen (CagA). CagA was originally discovered in the early 90s by the pioneering works of Cover et al6, Jean Crabtree et al7 and Covacci et al8; thereafter, it was recognized as a major disease-associated virulans factor. CagA-positive H. pylori strains were shown to be related with increased levels of C-reactive protein (CRP), total
cholesterol (TC), low-density lipoprotein (LDL), oxidized LDL (oxLDL), and apolipoprotein B.

Several studies have shown that the seroprevalence of CagA-positive strains is significantly higher in patients with coronary atherosclerosis.

Atherosclerosis is increasingly recognized as an inflammatory disease. The inflammatory process begins with the oxidation of LDL in the artery wall. The ability of high-density lipoprotein (HDL) to inhibit the oxidation of LDL (and cell membranes) plays a pivotal role in prevention of atherosclerosis. The anti-inflammatory and antioxidant activity of HDL is largely due to the paraoxonase (PON) located on it. PON1 circulates in the blood, binds to HDL and prevents LDL oxidation by hydrolyzing lipid peroxides; therefore, it may protect against atherogenesis.

Carotid intima-media thickness (CIMT) is known to be a surrogate marker of clinical and subclinical atherosclerosis and CagA-positive strains are argued to be related with increased CIMT. However, the relation between PON-1 activity and CIMT is not well elucidated and there is a lack of data about CagA-bearing strains and PON-1 activity.

The aim of our study was to evaluate serum PON-1 activity and CIMT in patients infected with CagA positive and CagA negative H. pylori strains, to determine the etiological relation of PON-1 with CIMT in CagA positive H. pylori infection.

**Patients and Methods**

**Patients**

Between January 2012 and August 2012, we evaluated 134 patients with upper gastrointestinal diseases referred for endoscopy in the Gastroenterology Clinics of Namk Kemal University Faculty of Medicine. Blood samples and gastric mucosal biopsy specimens of each patient were obtained. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m²). Written informed consent was obtained from each patient prior to enrolling in the study. Patients with known diabetes mellitus, hypertension, infectious, rheumatologic and cardiovascular diseases, those who had smoking habit and those who had received previous H. pylori eradication therapy were excluded.

**Endoscopic Evaluation**

Endoscopy was done with Olympus Evis Exera 160 videoendoscopes (Olympus America, Inc., Center Valley, PA, USA). Five biopsies were collected from each patient for histological examination: two from the antrum, two from the corpus, and one from the incisura angularis.

**Histological Assessment**

The biopsy samples were fixed in 10% formalin, then sliced into 4- to 6 mm pieces, dehydrated in ethanol, embedded in paraffin wax, sectioned (5 µm thick), and stained with hematoxylin and eosin (H&E). The presence of H. pylori in the sections was determined using a modified Gram staining protocol and taking into consideration its morphological characteristics, a curved and spiral form and intense purple coloring. Pathological diagnosis was made by a blinded pathologist, and cases were diagnosed as gastritis (active chronic gastritis or closed-type atrophic gastritis), gastric ulcer and gastric cancer.

**Laboratory Methods**

Peripheral blood was collected from each patient. Sera obtained by centrifugation were stored at −20°C and analyzed simultaneously by technicians who were blinded to group allocation. The levels of triglyceride (TG), TC, HDL-cholesterol and LDL-cholesterol were determined using commercially available assay kits (Abbott®) with an autoanalyzer (Aeroset®, Abbott®, Wiesbaden, Germany). IgG antibodies against CagA protein were analyzed by enzyme immunoassays (DIA.PRO Diagnostic, Bioprobes S.r.l, Milan, Italy). CagA antibody titers (≥8 U/mL) were classified as positive, according to the instructions of the manufacturer. PON-1 activity was measured by colorimetric method. The results are expressed as U/L.

**Determination of Carotid Intima-Media Thickness**

Carotid intima-media thickness and atherogenic plaques were measured using a grey scale high-resolution color Doppler ultrasound Esaote MyLab 50 (Genoa, Italy) equipped with a 5-12-MHz linear transducer. All the ultrasound scans and measurements were performed by one observer. The measurements were done between 7:00a.m. and 10:00a.m., with the patient in supine position, and the head tilted backwards at an angle of 45°. All procedures were performed on both sides of two longitudinal images of each common carotid.
artery. CIMT was measured in the distal 10 mm of the common carotid artery. The viewable distance between the lumen-intima interface and the media-adventitia interface was defined as CIMT. Three CIMT measurements were taken from each side to calculate the mean CIMT: antero-lateral, lateral and postero-lateral aspects. CIMT measurements taken from both sides were averaged to obtain the mean CIMT of each patient.

**Statistical Analysis**

Data were analyzed using Predictive Analytics SoftWare (PASW) statistics version 18 for Windows (SPSS Inc., Chicago, IL, USA). Shapiro Wilk test was used to test for normality. Parametric continuous variables were expressed as mean±standard deviation, nonparametric continuous variables as median (minimum-maximum) and categorical variables were presented as numbers and percentages. Categorical variables were analyzed with the χ² test. Paired group comparisons were done with Mann Whitney U test for variables with non-normal distribution, with independent sample t test for variables with normal distribution. A p-value < 0.05 was considered to be statistically significant.

**Results**

The study group included 134 individuals. Of the individuals, 103 were *H. pylori* positive, while 31 were *H. pylori* negative. At first, *H. pylori* (+) and *H. pylori* (-) groups were compared in terms of CIMT and PON-1 concentrations. The right, the left and the mean carotid IMT were significantly higher in the *H. pylori*(+) group versus the *H. pylori*(-) group (0.72 [34-1.2] mm versus 0.57 [0.4-0.85] mm); (0.74 [34-1.6] mm versus 0.55 [0.44-0.67] mm), and (0.73 [34-1.35] mm versus 0.57 [0.44-0.70] mm), respectively (for all p < 0.001; Table I). However, the mean PON-1 concentration of the *H. pylori*(+) group (0.56 [7.3-263] mm) was significantly lower than that of the *H. pylori*(-) group (225 [82-249] mm) (for all p < 0.001; Table I). TC, HDL, LDL and TG levels were similar between the two groups (for all p > 0.05; Table I).

In order to determine the difference between *CagA*(+) and *CagA*(-) groups, *CagA*(+) *H. pylori*(+) group was compared with *CagA*(-) *H. pylori*(-) group. The right, the left and the mean CIMT measurements of *CagA*(+) group were significantly higher than that of *CagA*(-) group and controls, while PON-1 concentrations of *CagA*(+) group were significantly lower than that of *CagA*(-) group and controls (for all p = 0.0001; Table II). The right, the left and the mean CIMT of *CagA*(-) group were significantly higher than that of the control group, while the mean PON-1 concentration was significantly lower (for all p = 0.0001; Table II). TC, HDL, LDL and TG values were similar between the groups (for all p < 0.05; Table II).

**Discussion**

Major risk factors of atherosclerosis may explain only 50% of its etiology. It has been in-

| Variable                  |  
|----------------------------|---------------------------------------------------------------|-------------------|
| Age, years                | 49.8 ± 8.7                                                    | 50.20 ± 9.33      |
| Gender, male              | 44 (42.7)                                                     | 12 (37)           |
| BMI, kg/m²                | 28.9 ± 3.6                                                   | 28.7 ± 3.8        |
| Systolic BP, mmHg         | 131 (110-140)                                                | 130 (115-140)     |
| Diastolic BP, mmHg        | 79 (70-90)                                                   | 78 (70-90)        |
| PON-1, U/L                | 56 (7.3-263)                                                 | 225 (82-249)      |
| Right carotid IMT, mm     | 0.72 (34-1.2)                                                | 0.57 (0.4-0.85)   |
| Left carotid IMT, mm      | 0.74 (34-1.6)                                                | 0.55 (0.44-0.67)  |
| Mean carotid IMT, mm      | 0.73 (0.34-1.35)                                             | 0.57 (0.44-0.70)  |
| Total cholesterol, mg/dL  | 205 (180-218)                                                | 200 (180-215)     |
| HDL, mg/dL                | 48 (34-96)                                                   | 47 (35-76)        |
| LDL, mg/dL                | 134 (55-203)                                                 | 128 (49-195)      |
| Triglyceride, mg/dL       | 122 (90-319)                                                 | 118 (90-266)      |

Data are expressed as mean ± standard deviation or n (%) or median (min-max), where appropriate. BMI = Body mass index; BP = Blood pressure; HDL = High-density lipoprotein; IMT = Intima media thickness; LDL = Low-density lipoprotein; PON-1 = Paraoxonase-1.
Carotid intima-media thickness and serum paraoxonase-1

Increasingly recognized as an inflammatory disease. Accumulating data suggest that many infections such as *H. pylori* may play an important role in the development of atherosclerosis. Izadi et al. showed that infection/replication of Helicobacter species in the coronary artery wall was associated with atherosclerotic plaque formation. Akbas et al. found that *H. pylori* positivity was related with increased carotid-intima-media thickness, which is one of the surrogate markers of atherosclerosis, and argued that *H. pylori* might have a role in atherosclerotic processes. Similarly, in this work patients with *H. pylori* infection were demonstrated to have increased right, left and mean CIMT when compared with the control group.

There is conflicting data about the relation of CagA positivity with increased risk of atherosclerosis. Rožanković et al. provided evidence of molecular mimicry between the CagA antigen of *H. pylori* and vascular wall antigens of atherosclerotic carotid arteries. They also demonstrated that anti-CagA antibody titer was higher in patients with symptomatic carotid artery disease. Conversely, Schöttker et al. found no relation between CagA positivity and atherosclerosis. However, in a recent meta-analysis, Wang et al. indicated that anti-CagA IgG positivity was significantly associated with increased risk of ischemic stroke caused by atherothrombosis, which support atherosclerosis caused by CagA positive *H. pylori* infection. Consistent with this data, in this paper, anti-CagA IgG positivity was found to be related with increased CIMT that support the increased atherosclerosis in CagA positive subjects. CagA negative group had less increased right, left and mean CIMT when compared with CagA positive group. Additionally, both CagA positive and CagA negative group had more increased right, left and mean CIMT than control group.

The exact mechanism of augmented atherosclerosis in *H. pylori* infected individuals, especially in those infected with CagA positive strains, is still not well elucidated. It has been hypothesized that CagA bearing *H. pylori* strains may play a role in the pathogenesis of atherosclerotic process due to autoimmune mechanisms. However, Ott et al. suggested that bacterial agents could have secondarily colonized atheromatous lesions and could act as an additional factor accelerating disease progression. Recently, decrease in the activity of PON-1, an antioxidant enzyme, was shown to be related with increased atherosclerosis by some investigators. PON-1 is a HDL-bound ester hydrolase enzyme that is deemed responsible for many antiatherogenic and cardioprotective characteristics of HDL. PON-1 has been found to be responsible for HDL-mediated cholesterol efflux from macrophages, and protection of LDL from oxidative modification; thus, prevents atherosclerosis. Besides inhibiting LDL oxidation, PON-1 may also directly suppress pro-inflammatory responses of macrophages, decrease sustained pro-inflammatory reactions, which subsequently

Table II. Comparison of cytotoxin-associated antigen groups and control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CagA (+) Group</th>
<th>CagA (-) Group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>45.63 ± 8.03</td>
<td>50.28 ± 9.94</td>
<td>50.20 ± 9.33</td>
</tr>
<tr>
<td>Gender, male</td>
<td>24 (37.5)</td>
<td>20 (51.28)</td>
<td>12 (38.71)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.8 ± 3.7</td>
<td>29 ± 3.5</td>
<td>28.7 ± 3.8</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>130 (120-150)</td>
<td>132 (110-145)</td>
<td>130 (115-150)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>80 (70-90)</td>
<td>78 (70-90)</td>
<td>78 (70-90)</td>
</tr>
<tr>
<td>PON-1, U/L</td>
<td>30.4 (7.3-263.22)</td>
<td>132 (7.6-253)</td>
<td>225 (82-249)</td>
</tr>
<tr>
<td>Right carotid IMT, mm</td>
<td>0.75 (0.44-1.2)</td>
<td>0.6 (0.34-0.85)</td>
<td>0.57 (0.4-0.85)</td>
</tr>
<tr>
<td>Left carotid IMT, mm</td>
<td>0.78 (0.40-1.60)</td>
<td>0.65 (0.34-0.98)</td>
<td>0.55 (0.43-1.50)</td>
</tr>
<tr>
<td>Mean carotid IMT, mm</td>
<td>0.77 (0.43-1.5)</td>
<td>0.64 (0.34-0.95)</td>
<td>0.56 (0.42-1.35)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>203 (175-215)</td>
<td>208 (185-218)</td>
<td>210 (180-215)</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>48 (37-96)</td>
<td>48 (34-76)</td>
<td>46.5 (35-76)</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>139.88 ± 38.73</td>
<td>136.28 ± 44.50</td>
<td>125.65 ± 36.96</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>125.5 (69-255)</td>
<td>125.5 (75-266)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean±standard deviation, or n (%), or median (min-max), where appropriate. BMI = Body mass index; BP = Blood pressure; CagA = Cytotoxin-associated antigen; IMT = Intima media thickness; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; PON-1 = Paraoxonase-1. *Independent sample t-test and Mann Whitney U test were used for paired group comparisons. a CagA(+) group and Cag A(-) group comparison; p = 0.0001. b CagA(+) group and control group comparison; p = 0.0001. c CagA(-) group and control group comparison; p = 0.0001.
can attenuate plaque progression\textsuperscript{28}. Many factors including infections may affect serum PON-1 activity. Aslan et al\textsuperscript{29} showed that PON and arylesterase activities decrease significantly in \textit{H. pylori} infected subjects. Consistent with this data, in this report, \textit{H. pylori} infected subjects had more decreased levels of PON-1 when compared with non-infected ones. Not surprisingly, patients infected with a more virulent strain, the CagA positive group, had the most decreased activity of PON-1 when compared to CagA negative and control group.

CIMT is a well-known marker of early atherosclerosis. Although the relation of decreased PON-1 activity with atherosclerosis was well elucidated, there is controversial data about the relation between increased CIMT and decreased PON-1 activity in different patient groups. Wang et al\textsuperscript{30} showed that decreased PON-1 activity was related to increased CIMT and arterial stiffness in hypertensive patients. Similarly, Cece et al\textsuperscript{31} (2011) suggested that decreased PON-1 activity was related to increased CIMT in patients with ankylosing spondylitis. Nevertheless, Akbas et al\textsuperscript{32} found no relation between PON-1 activity and CIMT in \textit{H. pylori} positive subjects. In the present study, decreased PON-1 activity was found to be related with increased CIMT in patients infected with \textit{H. pylori}. Furthermore, CagA positive patients had the lowest concentrations of PON-1 and the highest CIMT measurements when compared with CagA negative patients and controls. Additionally, CagA negative patients had more decreased PON-1 activity and increased right, left and mean CIMT than the control group.

Conclusions

This study provides evidence of increased CIMT and decreased serum PON-1 activity in patients infected with \textit{H. pylori}. Furthermore, the influence of CagA antigen on increased atherosclerosis in \textit{H. pylori} infection was confirmed. These findings may imply that the decrease in PON-1 activity may be an etiopathogenetic factor in increased atherosclerosis associated with \textit{H. pylori} infection, especially in those infected with a more virulent strain, the CagA positive strain.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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