Original Article

Correlation of cagA positive Helicobacter pylori Infection with clinical outcomes in Alzahra hospital, Isfahan, Iran


Abstract

BACKGROUND: Helicobacter pylori causes chronic active gastritis, peptic ulcer, non-cardia gastric cancer and mucosal–associated lymphoid tissue (MALT) lymphoma. Different genotypes of Helicobacter pylori are confirmed from disease geographical areas. Its association with clinical disease remained controversial. The aim of the present study was to investigate the relationship of the cagA genotype of Helicobacter pylori isolates with clinical manifestations and its relation to age and sex of patients.

METHODS: A total of 100 patients (60 male and 40 female) biopsy specimens were obtained from 3 groups of patients (40 chronic active gastritis, 40 duodenal ulcers and 20 non-gastric gastric cancers). Biopsies were cultured on specific medium and after growth colonies were confirmed as Helicobacter pylori. DNA extraction and polymerase chain reaction (PCR) were used to detect the presence or absence of cagA gene.

RESULTS: From a total of 100 positive samples of H. pylori, cagA genes were detected in 68% of patients and 32% of samples were negative. Mean age of normal gastritis, duodenal ulcer and gastric adenocarcinoma was 44.94, 44.97 and 67.5 years, respectively.

CONCLUSIONS: The present study showed no significant relationship between cagA genotype of H. pylori and chronic active gastritis, duodenal ulcer and non-cardia gastric cancer as well as sex of patients. But, in gastric adenocarcinoma, there was significant discrepancy between ages of patients in comparison with the other two groups.

KEY WORDS: Helicobacter pylori, cagA, virulence factors.

Helicobacter pylori are a major etiological agent in a range of gastroduodenal diseases including chronic active gastritis, peptic ulcer, gastric cancer, and lymphoma.1-3 Although H. pylori infection always results in histological gastritis, only minorities of infected subjects develop an associated clinical disease.14,5 H. pylori infection has world wide distribution, and its prevalence ranges from 25% in developed countries to more than 90% in developing areas.4 It has heterogeneous genotypes and phenotypes. The clinical outcome of H. pylori infection is proposed to be linked to certain strains such as the vacuolating cytotoxin (vacA) and the cytotoxin–associated gene (cagA).6,7 cagA is considered...
as a marker for the presence of the cluster of genes (pathogenicity island) of about 35 kilo base pairs,8-10 and present in more than 50% of the H. pylori strains and encodes the 120–140 KDa. It is now appreciated that the cagA gene product is not itself a virulence factor, but the gene is a member of a pathogenicity island, which do contribute to bacterial pathogenicity.2 Several genes of this cag island, such as picB, encode proteins that enhance the virulence of the strain by increasing interleukin 8 in gastric epithelial cells and mucosal inflammation.11,12 cagA is related to virulence of the H. pylori strain and is associated with peptic ulcer, and gastric malignancy in some populations.13 Although H. pylori infection is very common, geographical distribution of different subtypes exists.5,12,14 The association between genotypes of H. pylori and clinical diseases remained controversial,9 but study on genes variation of H. pylori not only is important for predicting the clinical outcome, also, for better understanding of microorganism distribution in all of the world and its evolutionary regions. We do not have enough information about distribution of cagA genotypes of H. pylori strains isolated from patients with different clinical symptoms in IRAN. So, the aim of the present study was to determine the cagA genotypes of H. pylori isolated from patients and its association with chronic active gastritis, duodenal ulcer and non-cardia gastric cancer.

Methods

Patients and Helicobacter pylori isolates

Between May 2004 and December 2005, from 164 patients referred to Alzahra hospital in Isfahan, Iran, attending an open access endoscopy service after obtaining informed consent for the investigation of gastritis (diagnosed as histological gastritis without peptic ulcer, gastric cancer or any esophageal disease such as gastroesophageal reflux and esophageal cancer), non-ulcer dyspepsia, duodenal ulcer (detected by endoscopy) and non-cardia gastric cancer (detected by endoscopic view and pathologic confirmation) had mucosal biopsies taken from the prepyloric and incisura before receiving anti H. pylori treatment. We took one specimen for a rapid urease test (RUT), and one specimen for culture that were sent to microbiology laboratory in normal saline. Among 164 samples, 64 samples were excluded from our study, because 18 samples were contaminated with molds after culture in medium (including 8 samples that were RUT negative and culture negative and 10 samples that were RUT positive and culture negative), and 29 samples were RUT positive and culture negative and 17 samples were RUT negative and culture negative. So, we had 100 biopsy samples (60 males and 40 females), from which 40 samples were from gastritis, 40 samples from duodenal ulcer and 20 samples were from gastric adenocarcinoma. After each endoscopic examination, endoscopes were cleaned and washed with Cidex solution for 30 minutes. A new autoclaved biopsy forceps was used for each patient.

Isolation of H. pylori from biopsy samples

Biopsy samples that were sent with sterile normal saline to microbiology laboratory, cultured directly on Columbia agar medium supplemented with 10% fetal calf serum, 5% blood and 10 μg/ml trimethoprim, 6 μg/ml cefsulodin and 5 μg/ml vancomycin and followed by incubation for 3-5 days at 37ºC under microaerophilic conditions. After observing the colonies, they were identified as H. pylori if they were catalase, oxidase, and urease positive, with the appearance of Gram negative curved bacilli. Isolates were harvested for storage in Brucella broth containing 20% glycerol and stored at -80ºC.

DNA extraction from H. pylori isolates

DNA was extracted from the fresh isolates before storage at -80ºC using DNA extraction kit (Roche Co., Germany) according to the manufacturer. After extraction, DNA density was assessed by optic densitometry.

Detection of cagA gene using polymerase chain reaction

All extracted genomic DNA were amplified for cagA gene by PCR using automatic thermocy-
PCR reactions were performed in a final volume of 25 µL containing 2.5 µL 10x buffer + Mg²⁺, 0.625 mM/L dNTP (Cinnagene Co., Iran), 2 unit Taq DNA polymerase (Cinnagene Co., Iran), 100 ng from genomic DNA as a template, and 50 Pico mole from each primers of D008 and R008 as described by Covacci et al.15 These primers (table 1) were used to amplify a fragment of 297 bp from the middle conservative region of the cagA gene (cagA).10 For cagA, amplification was performed with 30 cycles of pre-incubation (95°C, 3 minutes), denaturation (94°C, for 1 minutes), annealing (60°C, 2 minutes), extension (72°C, 3 minutes), and a final extension (72°C, 5 minutes). PCR yields were electrophoresed in 1.5% agarose gel (Roche, Germany) containing ethidium bromide. DNA ladder (Roche Co, Germany) was used to detect the molecular weights of observed bands under UV lamp (figure 1).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’ → 3’)</th>
<th>Product (size, bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IcagA D008</td>
<td>ATAATGCTAAATTAGACAACCTTGAGCGA</td>
<td>IcagA (297)</td>
</tr>
<tr>
<td>IcagA R008</td>
<td>TTAGAATAATCAACAAACATCACGCCAT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. DNA PCR results of H. pylori. Lanes 1-4 and 7 were cag positive samples and lanes 5 and 6 were cag negative H. pylori (100 bp DNA ladder).

Statistics
Results were analyzed using Pearson correlation for assessing the relationship between cagA genotype and sex with duodenal ulcer, chronic active gastritis and gastric adenocarcinoma. A one-way analysis of variance (ANOVA) was used to evaluate the difference among the groups. To determine the prediction of education, sex, residence, age and diseases of patients for being positive or negative for cagA gene, logistic regression was used.

Results
CagA genotype was obtained using PCR method on 100 clinical samples of H. pylori isolated from patients referred to Alzahra hospital in Isfahan, Iran, with chronic active gastritis, duodenal ulcer and gastric adenocarcinoma. Among these samples, 40 samples were from chronic active gastritis (40%), 40 samples were from duodenal ulcer (40%), and 20 samples were from gastric adenocarcinoma (20%). In the present study, we had 68 positive samples for cagA (68%) and 32 samples were negative (32%). Relative frequency of cagA genotype of H. pylori isolated from gastric biopsies of patients with duodenal ulcer, chronic active gastritis and gastric adenocarcinoma were 72.5%, 65% and 60%, respectively (table 2).
Table 2. Frequency of cagA genotype of Helicobacter pylori in 100 patients biopsy specimens.

<table>
<thead>
<tr>
<th>Group</th>
<th>Positive</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent (%)</td>
<td>Number</td>
<td>Percent (%)</td>
<td>Number</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>26</td>
<td>65%</td>
<td>14</td>
<td>35%</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>29</td>
<td>72.5%</td>
<td>11</td>
<td>27.5%</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Non-cardia gastric cancer</td>
<td>12</td>
<td>60%</td>
<td>8</td>
<td>40%</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

From 100 positive samples of *H. pylori* isolates, 60 samples were from men (60%) and 40 samples were from women (40%) (table 3). Mean age for chronic active gastritis, duodenal ulcer and non-cardia gastric cancer was 44.94, 44.97 and 67.5, respectively. Also, in our study, the sensitivity of RUT (rapid urease test) was 86%.

Table 3. Frequency of chronic active gastritis, duodenal ulcer and non-cardia gastric cancer according to sex.

<table>
<thead>
<tr>
<th>Group</th>
<th>Men</th>
<th>Percent (%)</th>
<th>Women</th>
<th>Percent (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis</td>
<td>20</td>
<td>50%</td>
<td>20</td>
<td>50%</td>
<td>40</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>28</td>
<td>70%</td>
<td>12</td>
<td>30%</td>
<td>40</td>
</tr>
<tr>
<td>Non-cardia gastric cancer</td>
<td>12</td>
<td>60%</td>
<td>8</td>
<td>40%</td>
<td>20</td>
</tr>
</tbody>
</table>

Discussion
The presence of *H. pylori* in the gastric mucosa leads to chronic active gastritis and eventually atrophic gastritis and is associated with diseases such as peptic ulcer, non-cardia gastric cancer, and MALT lymphoma. There are several factors, which is effective on developing gastric illness in all individuals infected with *H. pylori* such as, environmental condition, host genetic factors and bacterial virulent ability. Certain genotypes (e.g., cagA, vacA s1a) have been closely related to severe clinical outcome and response to anti-*H. pylori* therapy, whereas other variants appeared less pathogenic. The present study detected the cagA, one of the virulence factors of *H. pylori* and association between cagA and chronic active gastritis, duodenal ulcer and non-cardia gastric cancer of 100 biopsy specimens from patients referred to Alzahra hospital for endoscopy using PCR. Also, we described the association between presence of cagA gene and age and sex of patients. In this study, cagA gene was detected in 68% of our patients. The results showed no statistically significant association between cagA gene presence with chronic active gastritis, duodenal ulcer and non-cardia gastric carcinoma. Several recent studies were agreed with our findings that there is no relationship between cagA status and clinical symptoms in different patient populations. Kamali-Sarvestani and their colleague reported that cagA statuses are not significantly different among different disease groups in Shiraz, Iran (a different province). However, other studies have shown that individuals who were colonized with cagA positive strains of *H. pylori* are at more risk of gastric ulcer. The discrepancy between these reports and the results of the present study may have several causes. First, patient selection is extremely important, and the study group should be sufficiently large and diverse with respect to genotypes and clinical symptoms. Second, the geographic origin of the patients may also play an important role. Recent studies suggested the existence of separate bacterial lineage in different parts of the world. Third, some studies have investigated the association between cagA genes with only one disease such as gastritis, gastric ulcer or duodenal ulcer. We found no significant relationship of cagA gene and sex of patients (P > 0.05, figure 2). Mean age of patients with chronic...
active gastritis, duodenal ulcer and non-cardia gastric carcinoma were 44.94, 44.97 and 67.5, respectively. Only in cancer group compared to the other two groups, there were significant differences on frequency of cag positive genotypes among different age groups (P < 0.001). Data analysis using logistic regression showed no statistically significant relationship between the presence of the cagA gene and education, sex, residence, age and diseases of patients (P > 0.05, figure 3) and none of these factors were predictor for being positive or negative. In summary, the present study showed no significant relationship between cagA genotype of H. pylori and chronic active gastritis, duodenal ulcer and non-cardia gastric carcinoma. Prediction of clinical outcome according to cagA genotype isn’t helpful. We need further investigations to determine other genes effects like vacA and iceA in our local area.

**Figure 2.** Frequency of cag positive genotype in men and women in chronic active gastritis, duodenal ulcer and non-cardia gastric cancer.

**Figure 3.** Frequency of cag positive genotype according to age in chronic active gastritis, duodenal ulcer and non-cardia gastric cancer.
Acknowledgements
This study was supported by vice chancellor for research affairs of Isfahan University of Medical Sciences.

References