Frequency of Cytotoxin Associated
Gene A(+) Helicobacter pylori in Peptic Ulcer Disease: Difference Between Gastric and Duodenal Ulcer Disease

Y. Rasm, M. Sadreddini, M. Jamali, T. Peirouvi and F. Khosravifar

In this study, we evaluated the frequency of cytotoxin associated gene A (cagA) positive strains of Helicobacter pylori in patients with PUD—either Gastric Ulcer (GU) or Duodenal Ulcer (DU) in Iran. Seventy-two patients (46 males and 26 females), who underwent endoscopy and diagnosed with PUD were considered for inclusion into present study. The presence of plasma IgG antibodies to H. pylori and cagA were determined by ELISA. Thirty-nine patients diagnosed with DU were available for analysis of which 32 of them were positive for H. pylori antibodies (32/39: 82%). Twenty cases from infected DU samples were positive for cagA antibodies (20/32: 62.5%). Thirty-three patients diagnosed with GU were available for analysis of which 19 were positive for H. pylori antibodies (19/33: 57.7%). Twelve cases from infected GU samples were positive for cagA antibodies (12/19: 63.2%). These results showed that duodenal ulcer has strong association with H. pylori infection than gastric ulcer (82 vs. 57.7%), but in the case of cagA positive strains of H. pylori, we found same association (62.5 vs. 63.2%, respectively). H. pylori and cagA (+) H. pylori prevalence in the GU and DU groups was found to be independent of age, sex and BMI (p>0.05).

Key words: Peptic ulcer disease, H. pylori, cagA, duodenal, gastric
INTRODUCTION

Helicobacter pylori is a Gram-negative bacterium that resides in a neutral microenvironment between the mucus and the superficial epithelium of the stomach, infects the stomach of the human. Helicobacter pylori is responsible for upper gastrointestinal diseases in humans, including gastritis, Gastric Ulcer (GU) and Duodenal Ulcer (DU), gastric adenocarcinoma and gastric B-cell lymphoma (Blaser and Atherton, 2004).

This microorganism’s prevalence is estimated at around 25% in developed countries and at more than 80% in developing countries (Godoy et al., 2003; Lin et al., 2004). However, only a part of the colonized population (<20%) develops reportable gastrointestinal diseases despite the relatively high rates of colonization (Khayat et al., 2006).

Helicobacter pylori strains are highly diverse (Go et al., 1996). A fundamental distinction among strains is the cagA pathogenicity island, a region of about 40 kb that is present or absent in the H. pylori chromosome (Censini et al., 1996). One gene, cagA, was the first discovered gene on the island and is a marker for cagA presence (Covacci et al., 1993). The H. pylori strains cag+ and cag- differ substantially in their biology, in that the former are more interactive with the host (Blaser, 2005), injecting the cagA protein into epithelial cells (Odenbreit and Haas, 2002) and inducing a more profound tissue response (Crabtree et al., 1991; Peek et al., 1995). Carriage of cag+ strains may be determined by detection of specific-serum Immunoglobulin G (IgG) antibodies to native or recombinant cagA (Cover et al., 1995). Despite the widespread spread of H. pylori, its prevalence and that of its virulence genes varies across countries and among ethnic groups. The variability of H. pylori strain genotypes, in addition to environmental and host modifying factors, appear to contribute to differing clinical outcomes in different geographic regions (Khayat et al., 2006). Reports from Europe have shown that 70% of H. pylori strains are cagA positive compared to 90% in Eastern Asian strains (Saribasak et al., 2004). Studies in the United States and Western Europe that have compared H. pylori positive patients with peptic ulcer disease with similar patients without ulcers have shown a significant association of cagA positivity and duodenal ulceration (Cover et al., 1995; Crabtree et al., 1991; Orsini et al., 1998; Peek et al., 1995). In contrast, among Asian populations in which cagA+ strains predominate, no clear-cut relation with ulcer disease has been found (Hoa et al., 2000). This different ascertainment of the significance of cagA positivity may reflect differences in the populations studied as well as the cross-sectional, rather than prospective, nature of the earlier investigations.

To date, only a few studies have attempted to characterize the prevalence and subtypes of H. pylori virulence genes and determine their correlation with PUD phenotype in Iran. The aim of this study was to assess the frequency of infection with H. pylori and virulence strain of cagA (+) H. pylori, in patients with PUD and its subgroups: GU and DU.

MATERIAL AND METHODS

Between May 2008 to August 2008, consecutive patients, who underwent endoscopy in one Endoscopy Unit at Urmia University of Medical Sciences, Urmia, Iran and diagnosed with PUD—either GU or DU were considered for inclusion into present study. The patients with gastric cancer or history of H. pylori eradication or consumption of proton pump inhibitors were excluded from present study. The study included a total of 72 Iranian subjects. After demographics were recorded and informed consent obtained, their medication history, smoking habit and other relevant clinical data were provided from related questionnaires. Peripheral heparinized blood samples were collected from each patient after endoscopy. The blood samples were centrifuged and the plasma was separated and frozen at -80°C until analysis. The presence of plasma IgG antibodies to H. pylori and cagA were determined by enzyme linked immunosorbent assay kits (Globe and DiaPro companies, respectively, Milan, Italy). Statistical analysis was performed using SPSS Version 12. Chi-square and Fisher exact tests for distribution and for trends were used at a 0.05 level of statistical significance.

RESULTS

Overall, 72 patients (46 males and 26 females) were eligible for the study during the 4 month period. The age of the participants ranged between 18 and 82 years (mean 49.3±18.3). Patients with DU (n = 39) or GU (n = 33) were considered as 2 separate groups of subjects.

Thirty nine patients with DU were available for analysis (mean 46.4±17.8; range 20-82; 26 male; 13 female). Also, thirty three patients were considered in GU group (mean 52.7±18.5; range 18-82; 20 male; 13 female) (Table 1).

From present analysis, the overall prevalence of H. pylori in PUD, GU and DU were 70.8% (51/72), 57.7% (19/33) and 82% (32/39) (Fig. 1).

Table 1: Demographic characterization of patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PUD</th>
<th>GU</th>
<th>DU</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>46/26</td>
<td>20/13</td>
<td>26/13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.3±18.3</td>
<td>52.7±18.5</td>
<td>46.4±17.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6±3.9</td>
<td>25.9±4.4</td>
<td>24.8±3.6</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
In infected cases, plasma IgG cagA antibodies were present in 63.2% (12/19) of patients with GU and 62.5% (20/32) of patients with DU (Fig. 2). In total, 62.7% (32/51) of PUD patients were cagA(+).

Statistical analysis revealed that there are significant associations between the presence of H. pylori infection and anti-cagA antibodies with the absence of H. pylori infection and anti-cagA antibodies, respectively (p<0.05). H. pylori and cagA(+) H. pylori prevalence in the GU and DU groups was found to be independent of age, sex and BMI (p>0.05).

DISCUSSION

Helicobacter pylori infection is very common, especially in developing countries; however, patients with this infection rarely develop clinically significant conditions, such as PUD. This situation has prompted researchers to investigate the possible roles of host and environmental factors and factors related to the bacterium itself in cases that show severe pathologies (Go, 1997). This infection is now accepted as being intimately related to PUD but the relation between H. pylori and PUD has been more difficult to establish and the reason for ulceration appearing in only a few people harboring the organism is not known, but possibility includes differences in H. pylori strains. Earlier studies identified associations between H. pylori strains that harbor cagA and significant gastroduodenal pathology (Blaser and Atherton, 2004; Crabtree et al., 1991; Peck et al., 1995). Individuals carrying cag positive strains have greater degrees of gastric inflammation and epithelial cell damage than those from whom cagA negative strains have been isolated. Both intensity of inflammation and epithelial damage may be involved in the pathogenesis of peptic ulceration (Go, 1997), however, the results of more studies are conflicting include the results of the recent studies in this aspect.

There are significant differences in H. pylori prevalence both within and between countries. Most studies from Asian countries have noted that there was no significant difference between these patient groups with respect to anti-cagA antibody positivity (Atherton, 1997; Yang et al., 1997)

In contrast, many studies, mostly from Western countries, have suggested that cagA+ strains of H. pylori are associated with severe gastrointestinal lesions, such as severe gastritis, peptic ulcer disease and gastric cancer (Kuipers et al., 1995; Rudi et al., 1997). Perhaps geographical differences in the prevalence of circulating H. pylori strains are responsible for the contradictory results reported. Only a few studies in Iran have assessed the presence of anti-cagA antibodies in PUD (Rudi et al., 1997; Salehi et al., 2008; Talebkhah et al., 2008). Salehi et al. (2008) reported that the H. pylori infection was positive in 95 and 86% of patients with DU and GU, respectively. Also, cagA genotype was present in 80% and 77% of DU and GU infected patients (Salehi et al., 2008). In addition, another study from Tehran, Iran reported that antibodies against the cagA protein were present in 100% of PUD patients (Talebkhah et al., 2008).

In contrast, it was shown that among 33 patients with PUD, 57.7% (19/33) and 37% (7/19) cases were H. pylori and cagA positive subjects (Jafari et al., 2008). Another study from Tehran reported that the prevalence of the cagA gene was only 44% (Siavoshi et al., 2005).

We evaluated two groups of patients with PUD diagnosed with DU or GU, all referred for upper endoscopy at the same center in a defined study period, in order to find the association between these two diseases and H. pylori infection, overall and specifically for cagA+ strains. A correct classification of H. pylori infection according to the presence or absence of specific strains may allow a better understanding of the causal associations with the outcome of interest, because the infecting organisms behave differently in terms of pathogenic potential. Present results showed that the risk of DU and GU obviously was high in all subjects infected by H. pylori.
The frequency of *H. pylori* infection in DU patients is greater than GU patients, but the same frequencies were found in cagA status in both of them. These results probably showing the important role of *H. pylori* in DU comparable to GU (82% vs. 57.7%). In contrast, cagA has similar responsibility in GU and DU pathogenesis. Although, outh cagA expressing *H. pylori* have been reported to elicit a more severe local inflammatory response. This was initially ascribed to the ability of *H. pylori* expressing this gene to stimulate robust interleukin-8 production by gastric epithelial cells in vitro. Because, the stomach may be infected with cagA positive and negative strains, it has been suggested that measurement of cagA- IgG antibody is the preferred technique to detect the presence of these potentially more virulent bacteria.

REFERENCES


