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

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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

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INTRODUCTION

Helicobacter pylori a Gram-negative bacterium that resides in a neutral micro environment 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 gastroduodenal 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 much 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 worldwide 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 (Hua *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 were 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

Parameters	PUD	GU	DU	p-value
Sex (male/female)	46/26	20/13	26/13	>0.05
Age (years)	49.3±18.3	52.7±18.5	46.4±17.8	>0.05
BMI (kg m^{-2})	26.6±3.9	25.0±4.4	24.3±3.6	>0.05

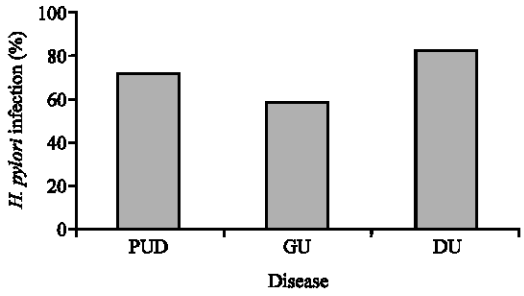

Fig. 1: The frequency of *H. pylori* infection in PUD, GU and DU patients

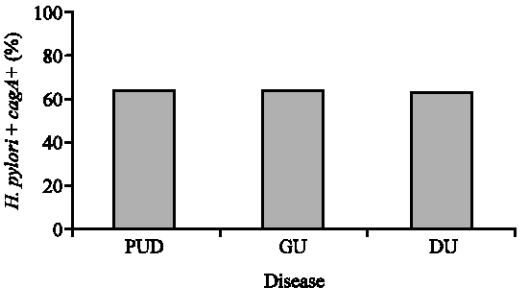

Fig. 2: *CagA* status in PUD, GU and DU patients with *H. pylori* infection

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; Peek *et al*., 1995). Individuals carrying *cag* positive strains have greater degrees of gastric inflammation and epithelial cell damage than do 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; Talebkhan *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 (Talebkhan *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 compatible 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.

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