The Prevalence of CagA and CagE Genes in Helicobacter pylori Strains Isolated from Different Patient Groups by Polymerase Chain Reaction

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Abstract: The aim of this study was to investigate the prevalence of cagA and cagE genes in H. pylori strains isolated from different patient groups with Non-Ulcer Dyspepsia (NUD), Duodenal Ulcer (DU), Gastric Ulcer (GU) and Gastric Cancer (GC). The patients admitted to the gastroenterology unit at Sharyati hospital in Tehran in 2006 were included in this study. Gastric biopsy specimens were obtained from the antrum of the stomach from each patient then cultured for detection of H. pylori. Identification of H. pylori was performed according to the standard bacteriological methods. Genomic DNA was extracted using a commercially available Qia gene kit. PCR was done using primers cagA-F, cagA-R and cagE-F, cagE-R to detect the target genes cagA and cagE, respectively. Amplified products of target genes were confirmed by sequencing. The cagA and cagE were detected among 85 and 86% of H. pylori isolates, respectively. Prevalence of cagA and cagE genes in the patients with NUD, DU, GU and GC were 22 (64.7%), 28 (100%), 18 (90%), 10 (100%) and 25 (73.5%), 27 (96.4%), 19 (95%), 7 (70%), respectively. The current study demonstrated a significant correlation between peptic ulceration and the presence of H. pylori isolates carrying cagE and cagA genes in Iranian patients.

Key words: Dyspepsia, duodenal ulcer, gastric ulcer, gastric cancer, molecular detection

INTRODUCTION

Gastroenteritis is a common and important public health problem (Ranjbar et al., 2007a). Colonization of the stomach mucosa by H. pylori is a major cause of acute and chronic gastric pathologies in humans. Several virulence genes of H. pylori have been identified. The most important determinants are cagE and cagA genes in the cag pathogenicity island (cagPAI) genes and vacA (Tan et al., 2006). The cytotoxin associated gene A (cagA) provides a key marker for the cag-PAI present in type I strains and its product, the cagA protein, has been shown to be delivered into cultured gastric epithelial cells (AGS cells) and immediately phosphorylated close to the site of attachment of the bacteria (Argent et al., 2008; Ondenbreit et al., 2001; Stein et al., 2000; Asahi et al., 2000; Backert et al., 2000). The type I isolates may differ in structure of the cag-PAI as proposed by Censi et al. (1996), but evidence from investigation of multiple loci suggest that most isolates contain an uninterrupted and intact cag-PAI (Jenks et al., 1998; Slater et al., 1999; Oechslins et al., 2001; Owen et al., 2001; Maeda et al., 1999). Cytotoxin associated gene E (cagE) is also one of the marker genes in cagI of the cag-PAI. It is essential for cagA translocation and phosphorylation (Ondenbreit et al., 2000). The presence of the cagE gene has been associated with a bad clinical outcome, especially in developed countries (Yamazaki et al., 2005). The vacuolating cytotoxin induces cytoplasmic vacuolation in a variety of mammalian cell lines in vitro and produces epithelial cell damage and mucosal ulceration when administered intragastrically in mice (Lin et al., 2000). However, there seems to be no functional link between cagA and vacA and it is likely that cagA is a genotypic marker for the presence and/or expression of other ulcer- or cancer-related virulence genes (Evans et al., 1998).

Molecular approach has provided powerful tools for diagnosis, epidemiological surveillance and tracking of key genes among the microbial pathogens (Ranjbar et al., 2007b). The aims of the present study were to determine the prevalence of cagA and cagE genes in Iranian patients with non-ulcer dyspepsia, duodenal ulcer, gastric ulcer and gastric cancer by PCR.

MATERIALS AND METHODS

In the present study, a total of 150 Iranian patients (78 male and 72 female; mean age 40.9 years; ranged from...

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16 to 79 years) admitted to the gastroenterology unit in Sharyati Hospital, Tehran, in the years 2005 and 2006 were enrolled for upper endoscopy. An informed consent was obtained from all patients who were included in the study according to the protocol approved by the local ethics committee.

During endoscopic examination, gastric biopsy specimens were obtained from the antrum of the stomach. Gastric biopsy specimens from each patient were inoculated onto Brucella agar base medium containing sheep blood (10%) (Merck, Germany) and antibiotic supplement (Merck, Germany) and cultured for 3 to 5 days at 37°C under microaerobic conditions (5% O₂, 5% CO₂, 90% N₂). *Helicobacter pylori* strains were identified by typical gram stain, colony morphology, and by positive biochemical tests for urease, catalase and oxidase (Smith *et al.*, 2002).

The patients who infected with *H. pylori* were clustered into four groups according to their clinical and endoscopic presentation: non-ulcer dyspepsia (NUD; n = 34), duodenal ulcer (DU; n = 28), gastric ulcer (GU; n = 20) and gastric cancer (GC; n = 10).

Bacterial genomic DNA was extracted using a commercially available kit (Qiagen, Hilden, Germany) according to manufacturer’s instructions. PCR was carried out to detect the *cagA*, *cagE* genes. All primer sets used were selected from the published literature as shown in Table 1.

The master mixes used for PCR consisted of 5 mM of 10X PCR buffer, 500 mM of KCl, 100 mM of Tris-HCl (pH 8.8), 2 mM MgCl₂, 250 μM each of the four deoxynucleoside triphosphates, 0.5 mM of each primer and 0.3 mM of Taq DNA polymerase (BioEngland). Five microliters from each *H. pylori* diluted extract, positive control DNA, or sterile water (extraction blank and negative control) was added to the mixture to obtain a final volume of 50 μL. PCR amplification was performed according to earlier reports (Stone *et al.*, 1997; Tomasin *et al.*, 2003). For *cagA* and *cagE*, PCR conditions were as follows: 3 min at 95°C and then 50 cycles of 94°C for 1 min, 48°C (*cagA*) and 53°C (*cagE*) for 45 sec and 72°C for 45 sec. PCRs were performed using a Robocycler Gradient 40 temperature cycler (Stratagene).

## RESULTS AND DISCUSSION

A total of 92 *H. pylori* strains were isolated from different groups of patients: NUD (n = 34), DU (n = 28), GU (n = 20) and GC (n = 10).

### Table 1: The primers set used in the study

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer designation</th>
<th>Sequence of primers</th>
<th>Size of PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA</td>
<td>cagA-F</td>
<td>5-GATCTCGGTTGGGTGGTTCC</td>
<td>506</td>
</tr>
<tr>
<td></td>
<td>cagA-R</td>
<td>5-GTCCTACGGCATATTGCA</td>
<td></td>
</tr>
<tr>
<td>cagE</td>
<td>cagE-F</td>
<td>5-CCGAATACTGAGTGTGAGTC</td>
<td>508</td>
</tr>
<tr>
<td></td>
<td>cagE-R</td>
<td>5-GGCTAGCGTAATATCCATTACC</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Prevalence of *cagA* and *cagE* genes in different patient groups

<table>
<thead>
<tr>
<th>Patient groups</th>
<th><em>cagA</em> gene (%)</th>
<th><em>cagE</em> gene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUD</td>
<td>22 (64.7)</td>
<td>25 (73.5)</td>
</tr>
<tr>
<td>DU</td>
<td>28 (100)</td>
<td>27 (96.4)</td>
</tr>
<tr>
<td>GU</td>
<td>18 (90)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>GC</td>
<td>10 (100)</td>
<td>7 (70)</td>
</tr>
</tbody>
</table>

*NUD: Non-Ulcer Dyspepsia, DU: Duodenal Ulcer, GU: Gastric Ulcer, GC: Gastric Cancer*

Fig. 1: Detection of PCR products of *cagA* gene by agarose gel electrophoresis. Lane 1: Negative control, Lanes 2-6: Clinical representative samples, Lane 7: Positive control (ATCC 43504 strain), M: Molecular weight marker (100 bp)

Overall, *cagA* was detected in 78 (85%) of the isolates. The carriage of *cagA*-positive strains of *H. pylori* in the patients with DU was 100% (28/28), with GU, 90% (18/20), with GC, 100% (10/10) and with NUD, 64.7% (22/34). A significant difference was observed in carriage of the *cagA*-positive strains of *H. pylori* in those with PUD and Gastric cancer compared to NUD (p<0.05) (Table 2).

The *cagE* positive *H. pylori* strains were isolated from 79 patients (86%). The frequency of *cagE*-positive strains in patients with PUD was 92% (46/48). Seventy percent and 73.5% of strains isolated from patients with GC and NUD were *cagE* and *cagA* positive, respectively (Table 2). Figure 1 and 2 show the expected amplified fragments of *cagA* and *cagE* genes, respectively in PCR reaction. Amplified products of target genes were confirmed by sequencing. The confirmed sequences have been submitted to GenBank (accession numbers DQ512724 and DQ999147).

*Helicobacter pylori* infection is extremely common worldwide with a prevalence ranging from 25% in developed countries to more than 80% in the developing world (Parsonnet, 2003; Pounder and Ng, 1995). Various factors such as the environment, host genetic factors and
bacterial virulent ability contribute to infection outcome caused by H. pylori (Campbell et al., 1997; Malaty and Graham, 1994).

The different molecular methods could provide sensitive interpreting keys suitable for microbiological studies (Ranjbar et al., 2008a, b). In present study we applied PCR to investigate the prevalence of cagA and cagE genes in Iranian patients with non-ulcer dyspepsia, duodenal ulcer, gastric ulcer and gastric cancer. We also studied the relationships between the presence of H. pylori strains carrying cagE and cagA genes and the clinical outcome in the patients studied.

A higher prevalence of the cagA gene was observed in the patients with DU (100%) and gastric cancer when compared to the NUD group (64.3%) (p<0.05).

Subsequent studies have shown more inconsistent results (Kim et al., 2004; Proença Ódena et al., 2007). The current study demonstrated that the majority (85%) of H. pylori strains isolated from Iranian patients were cagA positive. This finding is similar to the pattern usually described in Asian populations (Hirata et al., 2004; Zhou et al., 2004). Oliveira et al. (2003) demonstrated that more than 79% of subjects with ulcer disease in Brazil were infected with H. pylori strains carrying cagA. They also showed that the prevalence of cagA in the patients with gastritis, duodenal ulcer and gastric carcinoma were 59.21, 90 and 94.23%, respectively. These data are similar to present findings in this study. In contrast, Aydin et al. (2004) reported that only 59.2% of Turkish strains carried cagA gene and prevalence of cagA in patients with PUD and NUD were 72.3 and 47%, respectively. In China and Japan, cagA-positive strains are nearly universally present and are not associated with disease complications (Hirata et al., 2004; Zhou et al., 2004).

It has been reported that infection with a cagE positive H. pylori strain is associated with the presence of duodenal ulcer. In addition, Day et al. (2000) reported that infection of gastric cells in tissue culture by cagE positive H. pylori resulted in greater increments in IL-8 levels compared with cagE-negative strains and concluded that enhanced chemokine production after infection with cagE-positive H. pylori could affect disease outcome for duodenal ulcer.

In this study, 96.4% (27/28) of strains isolated from patients with duodenal ulcer carried the cagE gene.

This study also demonstrated that infection with a cagE-positive H. pylori strain was associated with peptic ulcer disease 95.7% (46/48). Similarly, preliminary data from this study also show that the presence of the cagE gene in strains of H. pylori is associated with duodenal ulceration. For instance, in a study by Fallone et al. (2000) 31 (37%) of 84 patients with gastroduodenal disease (including both peptic ulceration and gastric cancer) were infected with cagE-positive strains, compared to only 20.7% of 92 patients with gastritis alone. However, association of cagE in patients with GC and NUD is equal in present study and it was consistent with other studies where no difference was found in the frequency of cagE positive isolates among patients with gastritis, duodenal ulcer or gastric cancer (Hsu et al., 2002; Tan et al., 2006).

In this study, 69 H. pylori strains carried both cagA and cagE genes and only 4 strains did not carry each of them. Seventy-four percent stains associated with PUD, carried both of cagA and cagE genes.

The current study demonstrated a significant correlation between peptic ulceration and the presence of H. pylori isolates carrying cagE and cagA genes in Iranian patients studied.

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