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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 (*cag*PAI) 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; Odenbreit *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 Censini *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; Occhialini *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 (Odenbreit *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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Table 1: The primers set used in the study

Region detected	Primer designation	Sequence of primers	Size of PCR product (bp)
cagA	cagA-F	5-GATCTCGGTGGGTCTTTCC	506
	cagA-R	5-TCTTTTACGGCATTGTTCA	
cagE	cagE-F	5-TTGAAAACTTCAAGGATAGGATAGAGC	508
	cagE-R	5-GCCTAGCGTAATATCACCATTACCC	

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% O_2, 5% CO_2, 90% N_2). *Helicobacter pylori* strains was 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 (Qia gene, Hilden, Germany) according to manufacturer's instructions. PCR was used 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 mmol of KCl, 100 mmol of Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 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; Tomasini *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 2: Prevalence of *cagA* and *cagE* genes in different patient groups

Patient groups*	*cagA* gene (%)	*cagE* gene (%)
NUD	22 (64.7)	25 (73.5)
DU	28 (100)	27 (96.4)
GU	18 (90)	19 (95)
GC	10 (100)	7 (70)

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

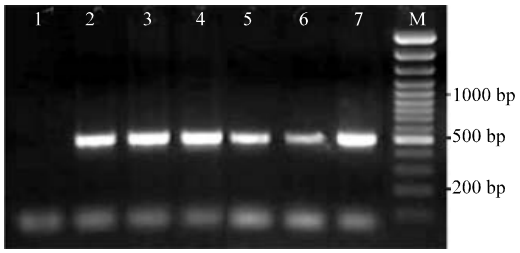

Fig. 1: Detection of PCR products of *cagA* gene by agarose gel electrophoresis. Lane 1: Negative control, Lanes 2-6: Clinical representative samples, Lane 7: Positive control (ATCC 43504 strain), M: Molecular weight marker (100 bp)

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 *cage* 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 DQ991147).

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

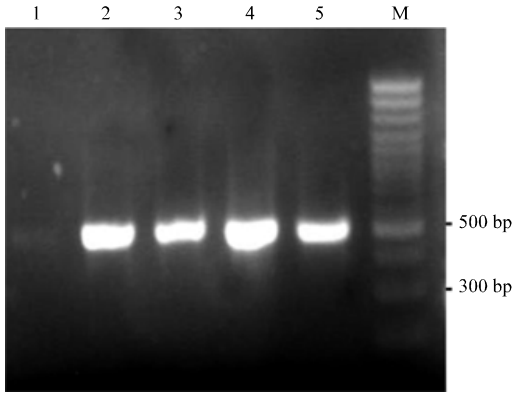

Fig. 2: Identification of the *cagE* by Polymerase Chain Reaction (PCR). Lanes 1: Negative control sample (without DNA); Lanes 2-4, Clinical representative samples, Lane 5: Positive control *H. pylori* ATCC43504, M: Molecular weight marker (100 bp)

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 Módena *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 *cag*E 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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