

Frequency of CagA in *Helicobacter Pylori* Isolates of Patients with Peptic Ulcer Diseases (PUD) and Non-ulcer Dyspepsia (NUD) at Namazi Hospital, Shiraz, Iran

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ABSTRACT

Background

Data concerning the information on the prevalence and association of the *Helicobacter pylori* cytotoxin-associated gene A (CagA) with disease is still controversial. The aim of this study was to isolate and identify *H. pylori* by culture method from biopsy specimens and its relationship with associated diseases by molecular techniques (PCR).

Materials and Methods

Gastric biopsy specimens obtained from 220 patients (aged 18 to 68 years) were evaluated for presence of *H. pylori* using PCR assay on isolates for CagA gene.

Results

From 220 patients that included in this study, 120 patients, 51 from PUD (38 duodenal ulcer and 13 gastric ulcer) and 69 from NUD patients (35 gastritis, 18 reflux disease without and 16 with esophagitis) yielded positive for *H. pylori* culture. Frequency of CagA gene in *H. pylori* isolated from patients with peptic ulcer diseases (PUD) and non-ulcer dyspepsia (NUD) was 82.3% and 59.4%, respectively.

Conclusions

Our data confirmed that CagA gene in *H. pylori* is a virulence factor with high frequency in PUD. Therefore, we suggest that detection of *H. pylori* gene expression may contribute in improving the diagnosis and understanding the pathogenesis of *H. pylori* infections.

Keywords: *Helicobacter pylori*, Peptic ulcer diseases, Non-ulcer dyspepsia, Cytotoxin associated gene A (CagA)

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BACKGROUND

H. pylori is now accepted as the most important cause of gastritis in human being, as an essential factor in the etiopathogenesis of peptic ulcer diseases (an important cause of morbidity), and as a risk factor for gastric cancer, a very common malignancy all over the world.(1), *H. pylori* is one of the most genetically diverse bacteria, as demonstrated by DNA fingerprinting and other

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genotyping and DNA sequencing techniques.(2-3), There is also evidence of important geographic differences among *H. pylori* strains.(4-5), Attempt to identify virulent strain of *H. pylori* which are more likely to result in clinically important outcomes, have focused on two groups of potential bacterial virulence factors, the cytotoxin associated gene pathogenicity island (CagPAI), and vacuolation cytotoxin, VacA.(6), The CagPAI contains 31 genes, including six genes named Cag. CagA gene represents a putative virulence marker and present in 60-70% of *H. pylori* strains, which codes for 96 to 138 kDa protein that is associated with the production of toxin. In vitro studies has

shown that CagPAI-positive strains induce gastric damage and the presence of this gene is associated with peptic ulcers diseases.(7-9), Several studies reported that CagPAI-positive strains is high in Asian countries.(10)

For these reasons, we undertook this study in order to investigate isolation and identification of *H. pylori* from biopsy specimens by culture techniques and to detect the virulence marker, CagA gene by PCR*.

MATERIALS AND METHODS

Gastric biopsy and culture

The specimens were collected in sterile micro-centrifuge tubes containing 2 ml of transfer media (thioglycolate broth) and stored at 4°C until transfer to laboratory, then used for bacteriological assessment. Before culture, the specimens were grounded and homogenized with a scalpel on the sterile slide, and then inoculated in a commercial selective medium (Campylobacter selective media with antibiotics and other supplements) for a maximum 7 days at 37°C. *H. pylori* isolates were defined as Gram-negative spiral-shaped bacilli that were oxidase, catalase and rapid urease tests positive.(11)

The primary culture of biopsy specimens were collected in 2 ml of 8% glycerol peptone and the suspensions were stored at -70°C until DNA** extraction.

Preparation of samples for PCR amplification

Genomic DNA was initially isolated from the *H. pylori* suspension by phenol-chloroform-isoamyl alcohol extraction and ethanol precipitation according to the standard protocols.(12), At the same time genomic DNA from all samples were used for detection of ureC gene (confirmation of *H. pylori*) and CagA gene (Multiplex PCR technique). Synthetic oligonucleotide primers and methodology reported by Andrey *et al.* 1995, were

used for the amplification of a 294 bp fragment of ureC (5' AAG CTT TTA GGG GTG TTA GGG GTT 3', 5' AAG CTT ACT TTC TAA CAC TAA CGC 3'). *H. pylori* ATCC 53726 was used as a positive control. PCR amplification of CagA was performed with a 400bp synthetic oligonucleotide primers (5' AAT ACA CCA ACG CCT CCA AG 3', 5'TTG TTG CCG CTT TTG CTC TC 3') by using the methodology reported by Andrey *et al.*, 1995. *Helicobacter pylori* ATCC 53726 was used as a positive control.

PCR condition

PCR was carried out in a 25µL volume of Techne thermocycler. For both amplification, cycling condition were as followed: initial denaturation at 95°C for 5 minutes, followed by 34 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 94°C for 1 minute. A final extension cycle was added, increasing the 72°C incubation to 5 minutes.

Detection of amplified DNA products

Eight micro liters amounts of each PCR mixture was subjected to gel electrophoresis (2% agarose) and ethidium bromide staining for detection of amplified DNA products.

Statistical analysis

Data were analyzed statistically by SPSS 10.

RESULTS

Two hundred and twenty patients undergoing upper gasteroduodenal endoscopy were included in this study (116 females and 104 males, age range 18-68 years). Two biopsy specimens were collected from each patient to isolate *H. pylori*. Finally 120 patients, 51 from PUD (38 duodenal ulcer and 13 gastric ulcer) and 69 from NUD (35 gastritis, 18 reflux disease without and 16 with esophagitis) yielded positive culture for *H. pylori* culture. All of 120 specimens were confirmed by PCR assay.

* Polymerase Chain Reaction

** Deoxyribonucleic Acid

The distribution of CagA gene among these isolates in PUD and NUD was 82.3% (42 CagA⁺ from 51), 59.4% (41 CagA⁺ from 69) P? respectively. This gene was more frequently found in PUD patients.

Amplicons with size of 294 bp and 400 bp that represent the UreC and CagA gene are shown in figure 1.

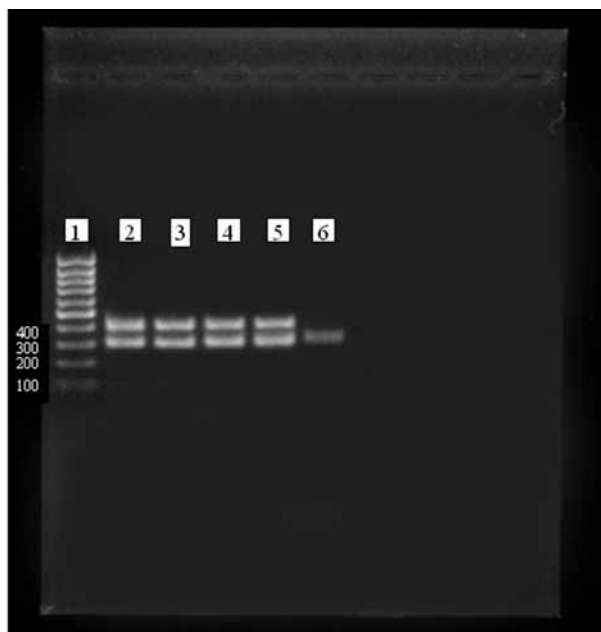


Figure 1. Gel electrophoresis after PCR amplification of the *H. pylori* CagA and ureC. Amplicons with size of 294 bp represent the ureC, amplicons with size of 400 bp represent CagA gene.

Lanes: 1. DNA marker, 2. *Helicobacter pylori* CagA⁺(control), 3-5 *Helicobacter pylori* CagA⁺(isolated from patients with PUD), 6. *Helicobacter pylori* CagA⁻ (control)

DISCUSSION

H. pylori infection is now recognized as an important risk factor for peptic ulcer disease, gastric adenocarcinoma, and mucosa associated lymphoid tissue lymphoma.(13-14)

Despite being one of the most common bacterial infections all over the world, most infected individuals are asymptomatic throughout their lives and only a minority of them develops such severe diseases. In this light, accurate diagnosis of

infection with *H. pylori* is important in these patients. We examined the clinical usefulness of both culture technique and PCR assay to detect *H. pylori* in gastric biopsy specimens.

We could isolate 120 *H. pylori* from 220 biopsy specimens of gastric patients. The isolates were identified based on colony morphology, biochemical tests and PCR assay, which indicates that culture and PCR are the gold standard method for identification of *H. pylori*. Our assessment of 120 individual isolates from 220 PUD and NUD patients showed that frequency of CagA was more in PUD patients as compared to NUD. This study is very similar to what has been reported in Brazil and other Asian countries. In Brazil, Queiroz DM *et al.* found that CagA⁺ strains *H. pylori* in children with PUD was 95% as compared with NUD patients.(15)

In Japan, Yamazaki S *et al.* showed that the molecular genetics of CagPAI is 90% associated with peptic ulcer diseases.(16), Similarly in India, Tiwari SK *et al.* reported that CagPAI were more in PUD (92.5%) as compared to NUD (77.5%).(17), Our result correlates with their finding. Although our findings are similar to that of obtained by Saffari *et al.*(18) but in contrast to our results Mohammadi *et al.* reported that prevalence of CagA in Iranian *H. pylori* strains is not associated with peptic ulcer diseases.(19)

CONCLUSION

Studies on the prevalence and diversity of *H. pylori* genes may be important not only for predicting the clinical outcomes of the infection but also to understand better the worldwide distribution of the microorganism and its evolutionary origin.

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