

Helicobacter Pylori and CagA: Relationships With Esophageal and Gastroduodenal Disorders in Iranian Patients

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Background: The severity of *Helicobacter pylori* infection is associated with virulence factors of the bacteria and host immune response. *H. pylori* has several virulence factors which a number of them are essential to emerge clinical outcomes. Cytotoxin-associated gene A (CagA) is the most important *H. pylori* virulence factor.

Objectives: The aim of our study was to assess a significant relationship between presence of *cagA* and severity of clinical manifestation in esophageal and gastroduodenal disorders.

Patients and Methods: A total of 240 gastric biopsies were collected between March 2012 and August 2013 from Tehran's hospitals. Three sets of biopsy specimens were obtained from the antrum and rapid urease tests, histological examination, Polymerase Chain Reaction (PCR) assay were performed on the biopsy specimens.

Results: One hundred and eight (45%) of biopsy specimens were positive with rapid urease test and *ureC* gene PCR. Moreover, thirty eight (35.1%) of positive specimens had *cagA* gene. The rate of gastric and duodenum inflammation was more in patients who carried CagA positive *H. pylori* strains. Whereas less inflammation and sever lesions in esophagus were found in CagA negative *H. pylori* strains.

Conclusions: Our study demonstrates a strong relationship between CagA and esophageal and gastroduodenal disorders. The number of CagA negative *H. pylori* was larger than CagA positive in esophagus lesion grade A, C, and D. Therefore, *cagA* may have a protective effect on some esophageal diseases. In addition, the number of CagA positives was larger than CagA negative *H. pylori* in gastric antrum and duodenum ulcer. Thus, CagA play a role to emerge peptic and duodenal ulcers.

Keywords: *Helicobacter Pylori*; Esophageal Disorders; CagA

1. Background

After discovering the *H. pylori* pathogenesis by Barry and Marshal, many research groups have focused on the *H. pylori* virulence factors and co-relation between its virulence factors and pathological or clinical outcomes (1-3).

It has been proved that the bacterium is the main cause of gastritis, gastric and duodenal ulcer and gastric cancer (4, 5). WHO has declared that *H. pylori* should be recognized as type I carcinogen (6).

The pathogenesis of *H. pylori* is complicated, and many infected people are asymptomatic (7, 8). Moreover, *H. pylori* virulence factors play an important role in clinical outcomes, and CagA is the most important virulence factor of the bacteria (9). Additionally, it has been proved that 60% of *H. pylori* expresses this 128 kDa protein, and many investigators have reported that there is a co-relation between *H. pylori* CagA positive and severity of stomach disorder (2). These strains are leading cause of IL-8 production, actins rearrangement, cell cycle interference, inducing of oncogenes expression and eventually damage to cells (10-12).

Some research groups have reported that *H. pylori* can interfere to signal transduction and cell cycle via CagA. Thus, injection of this virulence factor into host cells causes activation of NF- κ B and turning cells into cancer (13). The rate of *H. pylori* carriers in Iran is very high, and there are increasing reports of gastric cancer and stomach disorder in this region as well (14, 15).

2. Objectives

The main aim of present study was to assess a significant relationship between presence of CagA and severity of clinical manifestations in esophageal and gastroduodenal disorders.

3. Patients and Methods

3.1. Patients

The present study was conducted from March 2012 to August 2013. A total of 240 gastric biopsies were collected from patients who were referred to general hospitals in Tehran (Iran).

3.2. Biopsy Sampling

Three sets of biopsy specimens were obtained from the antrum, and each set of three specimens was divided as follows: One each for rapid urease tests; one for histological examination; One for Polymerase Chain Reaction (PCR) assay.

3.3. Rapid-Urease Test

Antrum biopsy specimens were put into a semisolid 2% urea agar and the results were observed 0.5 to 4 hours incubation at room temperature.

3.4. Histological Examination

The specimens were fixed by formalin, embedded in paraffin and stained by H and E to find out the severity of gastritis.

3.5. Preparation of Samples for Polymerase Chain Reaction

Genomic DNAs were extracted from gastric biopsy specimens using the extraction kit (Sinagen, Iran) according

to the company instructions. The presence of *H. pylori* DNA in biopsy samples were detected by PCR amplification of *H. pylori ureC* gene (Table 1). In addition, *cagA* gene was detected by PCR amplification of a 297-bp region in the *cagA* gene as previously described (Table 1) (16, 17). *H. pylori* ATCC49503 was used as positive control.

3.6. PCR Conditions

Total volume of each reaction was 50 μ L including 5 μ L 10 \times buffer, 1 μ L dNTP (100 mM), 2.5 μ L MgCl₂ (250 mM), 1 μ L Primers (25 pmol), 3 unit Taq DNA polymerase, 10 μ L DNA template (100 - 450 ng), 28 μ L DDW. Primary denaturation (94°C, 5 minutes), secondary denaturation (93°C, 1 minute), annealing (*cagA* 55°C, *ureC* 50°C, 1 minute), elongation (72°C, 1 minute), 38 cycles, final extension (72°C, 10 minutes).

3.7. Statistical Analysis

The SPSS software (Chicago, Ill., USA) was used for data analysis and for discovering a significant different between the groups we used the chi-square test. A P < 0.05% was accepted as statistically significant.

Table 1. Primer Sequences, Annealing and Expected Lengths of Amplified DNA Products (16, 17)

Primer	Sequences	Annealing, °C	Product Length, bp
<i>ureC</i> Gene		50	294
Forward	5'-AAGCITTTAGGGGTGTAGGGGTTT-3'		
Reverse	5'-AAGCTTACTTTCTAACACTAACGC-3'		
<i>cagA</i> Gene		55	297
Forward	5'-ATAATGCTAAATTAGACAACITGAGCGA-3'		
Reverse	5'-TTAGAATAATCAACAAACATCAGCCAT-3'		

Table 2. Prevalence of *cagA H. pylori* in Different Esophageal and Gastroduodenal Disorders

Variables	<i>cagA</i> Positive	<i>cagA</i> Negative	P value
Age average	22	24	
Female (n = 62)	16	46	0.00 ^a
Male (n = 46)	42	36	0.337
Esophagitis (n = 22)	10	12	0.546
Esophagus lesion grade A (n = 18)	6	12	0.046 ^a
Esophagus lesion grade B (n = 9)	4	5	0.637
Esophagus lesion grade C (n = 2)	0	2	0.046 ^a
Esophagus lesion grade D (n = 2)	0	2	0.046 ^a
Gastric mucosa atrophy (n = 6)	4	2	0.248
Gastric antral ulcer (n = 2)	2	0	0.046 ^a
Duodenal ulcer (n = 14)	10	4	0.023 ^a
Deformation of duodenum (n = 18)	10	8	0.505

^a Statistically significant differences between CagA positive and CagA negative groups.

4. Results

The rapid-urease test was positive for 108 (45%) of biopsy specimens and the color change of semi-solid media indicated that the specimens had urease activity of *H. pylori*.

4.1. PCR Based ureC Gene

The presence of *H. pylori* was detected in 45% (n = 108) biopsy samples. The *ureC* (*glmM*) gene was amplified in all of urease positive samples 35.5% (n = 108) and PCR method did not miss any urease positive biopsy specimen.

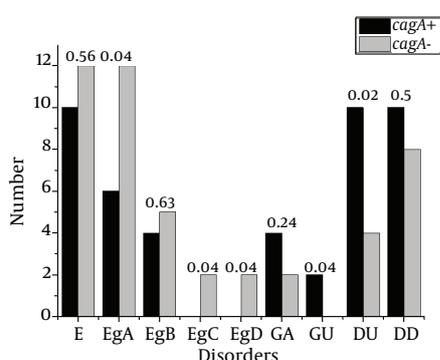
4.2. PCR Based cagA Gene

35.1% (n = 38) of *H. pylori* strains harbored virulence gene (*cagA*).

4.3. Endoscopic Findings

Demographic information and endoscopic finding were shown in Table 2. According to the endoscopic findings the rate of lesion and duodenum inflammation was more in patients who carried CagA positive *H. pylori* strains ($P < 0.05$). Whereas there is less inflammation and sever lesions in esophagus were found in CagA negative *H. pylori* strains (Table 2).

Figure 1. Prevalence of cagA *H. pylori* in Different Esophageal and Gastro-duodenal, a $P < 0.05\%$ Was Accepted as Statistically Significant



E: Esophagitis, EgA: Esophagus lesion Grade A, EgB: Esophagus lesion Grade B, EgC: Esophagus lesion Grade C, GA: Gastric Atrophy, GU: Gastric Ulcer, Du: Duodenal Ulcer, DD: Deformation of Duodenum.

5. Discussion

Our study demonstrates a strong relationship between CagA and esophageal and gastro-duodenal disorders in Iran. Moreover, our investigation has interestingly revealed that the number of CagA negative in females is considerably larger than CagA positive *H. pylori*, while there was no statistically significant difference between CagA positive and negative among males.

Although presence of *H. pylori* in stomach may result in gastritis and gastric cancer, all infected individuals don't express clinical manifestations. Therefore, a number of people remain as healthy carriers (18). There are several factors which contribute to emerge clinical outcomes including bacterial virulence factors, host genetics and unhealthy lifestyle (19).

The bacterial virulence factors play a main role in the pathogenesis of *H. pylori*. Furthermore, there is much concrete evidence which *H. pylori* virulence factors are essential to express clinical symptoms (20). In addition, it has been proved that CagA is the most important virulence factor in the bacteria, and individuals infected by CagA positive *H. pylori* are at high risk of developing gastric adenocarcinoma and duodenal ulcer disease (21).

Even though *H. pylori* contributes to gastro-duodenal diseases, there are a number of reports that the bacterium may play a protective role against esophageal adenocarcinoma (22). Moreover, some investigators have claimed that CagA positive *H. pylori* have an inverse association with esophageal adenocarcinoma (23). The present study has revealed that there was a significant difference between CagA positive and negative *H. pylori* in patients with some esophageal disorders. To further expand, while we did not find significant difference between CagA positive and negative in esophagitis and esophagus lesion grade B, we discovered that the number of CagA negative *H. pylori* was larger than CagA positive in esophagus lesion grade A, C, and D ($P > 0.05$). Therefore, our results have shown that cagA may have a protective effect on some esophageal diseases.

However, CagA positive *H. pylori* presents in 60 - 70% all isolated strains, and some epidemiological studies have shown that this virulence factor contributes to developing gastritis, peptic ulcer and gastric cancer (24, 25). Our finding confirmed the previous investigations which are performed in Iran (26, 27). Although, there was no significant difference between CagA positive and negative *H. pylori* in gastric mucosa atrophy and deformation of duodenum, we have figured out that the number of CagA positives was larger than CagA negative *H. pylori* in gastric antrum and duodenal ulcer. Our results showed that the percentage of CagA positive *H. pylori* is lower than other countries.

In conclusion, our findings have confirmed a number of previous reports which had claimed that CagA positive *H. pylori* probably benefits for preventing from esophageal disorders and this virulence factor has protective effects by unclear mechanism. On the contrary, our results have shown that CagA positive *H. pylori* was leading cause of gastric and duodenal ulcers. Moreover, while there was no significant difference between CagA positive and negative in men we have found that the number of CagA negatives was remarkably larger than CagA positives in women.

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Authors' Contributions

Study concept and design: Leili shokoohizadeh and Ashraf mohabati Mobarez; Acquisition of data Omid Teymournejad, Leili Shokoohizadeh, and Mohsen Amini; Analysis and interpretation of data: Omid Teymournejad; Drafting of the manuscript: Omid Teymournejad and Leili Shokoohizadeh; Critical revision of the manuscript for important intellectual content: Leili Shokoohizadeh; Statistical analysis: Omid Teymournejad.

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