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Prevalence of *cagA* and *babA2* genes in *Helicobacter Pylori* strains Isolated from Iranian gastrointestinal disorder patients and their gastritis classification



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ABSTRACT

Helicobacter pylori is a spiral gram negative flagellate bacteria and localize in the stomach. H.p infection is a worldwide health problem and identified as an important cause of gastritis and gastric cancer and its ability to develop such disorders is related to its virulence factors and environment. *cagA* is the most important Hp virulence factor that directly penetrate into gastric epithelial cells by bacterial secretion system (T4SS) from pathogenicity island (PAI) and disrupts cell homeostasis. Adherence factors are significant for bacterial colonization and suitable function of other virulence factor. Blood group antigen binding adhesion (*babA*) is an outer membrane protein (OMP) that binds to ABO blood group antigen and can stimulate inflammatory response in gastric cells. Our main target was to determine the roles and prevalence of *cagA* and *babA₂* virulence factor in gastrointestinal disorders in Iranian patients. Existences of These factors were determined by PCR in 218 patients with gastrointestinal disorders. Semi-quantitative methods of scoring according to the Updated Sydney classification system were used for detection of H.pylori density, neutrophil and monocyte cells infiltration. A high prevalence of *cagA* positive (81.4%) and *babA₂* positive (35%) were found. The most combined genotype (*cagA*&*babA₂*) prevalence was found in gastritis & ulcer (100%) ($P < 0.001$). High prevalence of *cagA* positive observed in active inflammation phase 76.9% and high prevalence of *babA₂* positive was in active phase 61.1% of H.pylori gastritis ($P=0.001$). Results of this study showed information about the high prevalence of *cagA* genes in H.pylori infected patients and their rolls in active gastrointestinal disorders.

Key words: *Helicobacter pylori*, *cagA*, *babA2*, Gastrointestinal Diseases

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1. INTRODUCTION

Helicobacter pylori (H.pylori) is a micro aerophilic gram negative spiral shaped flagellated bacteria that localized in stomach mucosa for almost the entire lifetime of the host (1, 2). H.pylori infection is an endemic worldwide health problem that infects more than half of the world population and accepted as the main cause of gastrointestinal disorders. The infection causes variety of gastrointestinal symptoms such as chronic gastritis, peptic ulcer, and adenocarcinoma (3). H.pylori associated inflammation is observed by mucosal infiltration of polymorphonuclear leukocytes (PMN), macrophages and T cells (1). The ability of H.pylori to cause disease is related to differences in the virulence of H.pylori strains, host immune responses, environment and nutrition (4). This bacteria can adheres to gastric epithelial

cells and release pathogen molecules to damage to gastric epithelial cell and their viability (5). In 1994, The WHO concluded that H.pylori is a class I carcinogen in human (6). H.pylori is responsible for about 75% of all non cardia gastric cancer and 63.4% of all stomach cancer worldwide (7). Several H.pylori virulence factors such as ureA, *cagA*, VacA, dupA, *babA₂* and SabA have been observed to be associated with gastrointestinal dysfunctions. CagA is the most considerable studied H.pylori virulence factor (8). Which encodes a highly immunogenic protein (9). In West, it has been reported that individuals infected with *cagA*-positive H.pylori is a high risk for gastrointestinal disorders (10). The CagA gene is located at one end of the cag pathogenicity island (PAI), that insert into H.pylori genome from an unknown source (11). *cagA* molecules are directly translocated into gastric epithelial cells by a bacterial type-IV secretion system (T4SS). *cagA* has been

interact with some intracellular components of signal transduction pathways and clearly disrupts gastric epithelial cells homeostasis, additional pro-inflammatory factors is required for complete pathogenic potential (12). Adherence factors is important for H.pylori virulence that facilitate bacteria colonization and efficient delivery of virulence factors such as cagA and vacA into stomach cells (2). Blood group antigen binding adhesion (BabA) is another virulence factor that encoded by the babA2 gene. BabA is an outer membrane protein (OMP) that binds to lewis b antigens and ABO antigen. Exist 2 babA alleles (babA1 and babA2) and one closer homology gene, babB, but only the babA2 allele has active function. the difference between babA1 and babA2 is in the 10-bp insertion, encoding a signal peptide, that babA₁ has not this sequence also babA1 gene being silent (13). Expression of the babA gene can be modulated (switched from on to off or off to on) through a gene conversion between babA and babB. The adherence of h.pylori to stomach cells is related to babA and stimulate mucosal inflammation. The presence of babA and cagA is associated with duodenal ulcer and gastric adenocarcinoma in west countries (14-16).

2. MATERIALS AND METHODS

2.1. Patients

218 samples were collected with dyspepsia symptoms and gastrointestinal disorders. Gastric biopsy specimens were taken from the antrum (pyloric gland area). Gastritis was investigated by H.pylori gastritis in absence of peptic ulcer (PU), DU and GU were identified by endoscopy (17). All the patients were attending the internal medicine ward in Hajar University hospital, Shahr-e-kord, Iran, after informed and data was recorded and process approved by the medical ethics committee. All of them had not received nonsteroidal anti-inflammatory drugs (NSAIDs) for 1 month before specimens collection and none of them had treatment for H.pylori infection.

2.2. Methods

Methods that utilized for H.pylori detection were rapid urease test (RUT), polymerase chain reaction (PCR) and histopathological exam. Patients were classified as H.pylori infected only if all three tests were positive. One

Fresh biopsy sample was evaluated by RUT color change kit and second specimen was kept in physiologic serum for DNA extraction and another sample was conserved in paraffin for histological exam.

2.3. PCR Amplification for detection of 16sRNA, CagA, babA₂ genes

Total DNA was extracted from biopsy specimens by Biospin Tissue Genomic DNA Extraction kit (Bioflux, Japan) according to the manufacturer's guides. The polymerase chain reaction followed the methods described by Bagheri N (1, 18) with some modification. PCR products were analyzed by PAGE gel electrophoresis.

2.4. Gastric histopathology of H.pylori infected specimens

Endoscopic biopsies were fixed in 10% formalin than embedded in paraffin, so Tissue section were obtained and stained with haematoxylin and eosin (H&E) stain and silver stain to detect H.pylori. The specimens were analyzed to assess the histopathological aspects of gastritis. Semi-quantitative methods of scoring according to the Updated Sydney classification system were used. The histopathological variables such as H.pylori density, neutrophil and mononuclear cell infiltration, atrophy, intestinal dysplasia were scored on a scale of 3 (mild, moderate and severe) (19).

2.5. Statistical analysis

The association between the prevalence of cagA and babA2 with clinical outcome is analyzed by the chi-square test and fisher exact probability test.

3. RESULTS AND DISCUSSION

A total of 238 specimens collected for H.pylori detection in Iranian patient that confirmed by RUT, PCR and histological exam in this study. Twenty patients by diseases other than gastritis, GU, DU, were excluded. Overall, a total of 140 H.pylori positive patients [86 males and 54 females; mean age 50 years, age range: 18-80 years] were included in our analysis. In this numbers, 91 patients had gastritis, 25 had gastric ulcer (GU), 14 had duodenum ulcer (DU), and 10 had gastritis and ulcer (Table 1).

Table 1. Prevalence of cagA, babA₂ genes and clinical outcomes

Description	Gastritis	GU	DU	Gastritis & ulcer	Total	P value
	No (%)	No (%)	No (%)	No (%)	No (%)	-
All samples	91 (64.9)	25 (18)	14 (10)	10 (7.1)	140 (100)	-
16sRNA & glmM	+	+	+	+	+	-
cagA positive	79 (86.8)	16 (64)	9 (64.3)	10 (100)	114 (81.4)	=0.008
babA2 positive	19 (21)	13 (52)	7 (50)	10 (100)	49 (35)	<0.001
cagA, babA2 positive	7 (7.8)	4 (16)	2 (14.3)	10 (100)	23 (16.4)	<0.001

Mean age	48.6	53.2	50	60	54	<0.01
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The average age was significantly higher in gastritis and ulcer patients than GU, DU and gastritis patients were. The male/female patients ratio was significantly higher for the gastritis ($P < 0.01$).

3.1. *cagA* and *babA2* status in south west of Iran

The distribution of *cagA* and *babA2* genotypes in south

west of Iran is shown in Table 2. Prevalence of *cagA* was 81.4% and *babA2* genotype prevalence was 35%. For the combination of the *cagA* and *babA2* genotypes 23 specimens were both positive 16.4%. In table 2 we can observe numbers and percent of The *cagA* and *babA2* genes were found in of *H.pylori* isolates.

Table 2. Frequency of virulence factors in Hp isolates

Genotype		Number	(%)
<i>cagA</i>	<i>babA2</i>		
+	-	91	79.8
+	+	23	20.2
-	+	26	100
-	-	0	0

3.2. Association between virulence factors and clinical outcomes

The prevalence of *cagA* was significantly higher in specimens in gastritis & ulcer than those from GU, DU and gastritis. ($p=0.008$). The *babA2* genotype has more detect in gastritis & ulcer (100%) than GU(52%), DU(50%) and gastritis(21%) ($p < 0.001$). The prevalence of *cagA/babA2*

genotype was significantly higher in gastritis and ulcer specimen (100%) ($p < 0.001$) than others (Table1).

3.3. Genotype

Table 3 gives an overview of the frequency distribution of the *cagA* and *babA2* gene status.

Table 3. Frequency distribution of the *cagA* and *babA2* status for the total of study patients analyzed

Genotype	Totale	
	No.	%
<i>cagA</i>		
positive	114	81.4
Negative	26	18.6
<i>babA2</i>		
Posetive	49	35
Negative	91	65

During this study, semi-quantitative methods of scoring according to the updated Sydney system were used. The degree of active inflammation was evaluated and scored as below: 19.8% mild, 24.8% moderate and 19.8% sever, more than 64% gastritis patients in an active stage of *H.pylori* gastritis. In addition, the degree of chronic

inflammation was assessed and graded as follow: 11.9% mild, 13.9% moderate, 9.9% sever, more than 35% gastritis patients in a chronic phase of *H.pylori* gastritis (Table 4)

Table 4. Updated Sydney classification on *H.pylori* gastritis and gastritis&ulcer

Description	Mild	Moderate	Sever	Total
	N (%)	N (%)	N (%)	N (%)
Active	20 (19.8)	25 (24.8)	20 (19.8)	65 (64.4)
Chronic (no activity)	12 (11.9)	14 (13.9)	10 (9.9)	36 (35.6)
<i>H.pylori</i> positive	all	all	all	all
Total	32 (31.7)	39 (38.6)	30 (29.7)	101 (100)

3.4. Correlation between *H.pylori* virulence factors and gastric inflammation classification

High prevalence of cagA positive observed in active inflammation phase 76.9% (p= .01) and high prevalence of babA2 positive was in active phase 61.1% (p= .001) of

H.pylori gastritis. So for cagA/babA2 positive specimens high prevalence was in active phase by 50.8% (p<.001) (Table 5).

Table 5. Relation between pathological statuses in gastritis biopsy specimens by virulence factors

Description	Active gastritis		Chronic gastritis		P value
	No.	%	No.	%	
cagA					=0.01
positive	50	76.9	19	52.8	
negative	15	23.1	17	47.2	
babA2					=0.001
positive	40	61.5	10	27.8	
negative	25	38.5	26	72.2	
cagA babA2					<0.001
positive	33	50.8	10	27.8	
negative	8	12.3	17	47.2	
Total		65		36	-

The Gram-negative bacterium helicobacter pylori is clear to have a incredibly high level of genetic diversity and is involved in human disease after decades of persistence in the stomach (20, 21). Our study included 140 *H.pylori* positive patients to obtain the distribution of cagA and babA2 genes and their association with clinical outcomes such as gastritis, GU and DU in southwest of Iran. The cagA gene was observed in (78.6%) patients that in agreement with other article concluded in Europe and East Asia where a higher prevalence (67% or more) of cagA gene (22). The prevalence of cagA was (86.8%) in gastritis, (64.3%) in DU, (64%) in GU and (100%) in gastritis & ulcer. Our results showed that the prevalence of cagA was significantly higher in specimens in gastritis & ulcer than GU, DU and gastritis that has a statistically significant in this area. (The number of patients maybe too small for this concludes). This result is agreement with some previous study (23, 24). Some genotype of *H.pylori* have been observed for the induce of the other form of the disease (25). A important virulence factor babA, encoded by the babA2 gene, make possible colonization for *H.pylori* in the stomach (26). The babA2 gene was observed in (35%) patients that not agreement with other studies in western countries. The prevalence of babA was significantly higher in specimens in gastritis & ulcer (100%) than GU (52%), DU (50%) and gastritis (21%). This prevalence of babA2 gene is not similar to same prevalence in western countries(27). It is crucial to note that babA2 positive *H.pylori* strains have been related by severe infection and disorders in stomach, while babA2 negative strains have not important association with gastritis (28).

4. CONCLUSION

In conclusion, *H.pylori* infection is an endemic problem in Iranian patients, so the genotype detection may be helpful to identify patients who are at high risk for gastrointestinal disorders. Our results indicate that *H.pylori* virulence factors were more relevant to severity of gastritis diseases. Diversity of CagA and babA₂ virulence factors contributes to the clinical outcomes in these patients. CagA is highly frequent in Iranian patients and it can be considered an important virulence factors and high prevalence of babA2 positive was in active phase of gastritis. Helicobacter pylori strains with combination of cagA and babA2 are closely associated with gastritis & ulcer. This article suggests that more studies are necessary to better identify of the bacterial genotype diversity in our region and their relation with clinical outcomes.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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