Research article

Frequency of virulence-associated genotypes of *Helicobacter pylori* and their correlation with clinical outcome and histological parameters in infected patients

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A R T I C L E   I N F O

A B S T R A C T

Helicobacter pylori (*H. pylori*) is a gram-negative which can cause several gastroduodenal diseases, including gastritis and peptic ulcer disease (PUD). *H. pylori* specific genotypes have been related to increased occurrence of gastritis and PUD. The aim of this study was to investigate the clinical relevance of the major virulence factors of *H. pylori* with clinical outcomes and histological parameters in Iranian patients. Totally, 200 subjects with PUD and gastritis disease who underwent gastroduodenal endoscopy were enrolled in this study. The presence of the *cagA, vacA, oipA, babA2,* and *iceA* genes in antral gastric biopsy specimens were determined by polymerase chain reaction (PCR) and the results were compared with clinical outcomes and histological parameters. The frequency of $babA2^+$, $oipA^+$, *vacA s1/m2,* and *vacA m2* genes was significantly higher in patients with peptic ulcer disease compared with patients with gastritis. In contrast, the frequency of *vacA s1/m1* gene was significantly higher in gastritis subjects than PUD subjects. The high-density scores of *H. pylori* were strongly associated with *iceA1,* $babA2^+$, and $oipA^+$ genes. Additionally, the high polymorphonuclear cell infiltration and high mononuclear cell infiltration scores were strongly associated with the $cagA^+$, *iceA1,* $oipA^+$ genes and $cagA^+$, $babA2^+$, $oipA^+$ genes, respectively. Our study indicated that the *vacA, babA2,* and *oipA* virulence factors are related to a higher risk of PUD in subjects with *H. pylori*-infection. Infection with these strains was associated with a more severe gastropathy.

1. Introduction

Helicobacter pylori (*H. pylori*) infection is a major bacterial infection worldwide, leading to various gastroduodenal diseases, including chronic gastritis, peptic ulcer disease (PUD), gastric cancer (GC) and MALT (mucosa-associated lymphoid tissue) lymphoma [1, 2, 3, 4]. However, most infected patients with *H. pylori* remain asymptomatic, and increased disease risk is related to the genetic diversity of *H. pylori* strains or inflammatory responses governed by host genetic diversity, or both [5, 6, 7]. In the hostile acidic environment of the stomach, virulence factors play essential roles in the colonization and survival of *H. pylori. H. pylori's*

ability to cause several gastrointestinal diseases has been related to the expression of various virulence factors such as outer inflammatory protein A (OipA), blood adhesion binding protein A2 (BabA2), induced by contact with epithelium protein A (IceA), vacuolating cytotoxin protein A (VacA), and cytotoxin-associated protein A (CagA) [8]. A main *H. pylori* virulence factor is the *cag* pathogenicity island (*cag*PAI), which contains about 30 genes, encoding a type 4 secretion system (T4SS), that transfers CagA toxin and peptidoglycan into gastric epithelial cells, resulting in increased cellular release of various proinflammatory cytokines such as interleukin 8 (IL-8) [9]. The name of Vaca refers to the capability of the toxin to cause a formation of large vacuoles in cultured epithelial cells

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[10]. Although the *vacA* gene is present in all strains of *H. pylori*, its sequence and expression profile is greatly different [11, 12]. The *vacA* gene has variable regions, including *s1*, *s2*, *m1*, and *m2* [13]. The chimeric strains, such as *vacA s1/m1* have greater vacuolation than *s1/m2* strains, while *s2/m2* strains typically have no vacuolating activity [8]. BabA is one of the best-characterized adhesion molecules of the *H. pylori* that mediate the binding of bacterium to Lewis b (Leb b) antigens on gastric epithelial cells [14, 15, 16]. Three *bab* alleles have been recognized: *babA1*, *babA2*, and *babB*. However, only the *babA2* gene product is functional for the Leb b attachment activity [14]. The *iceA* gene exists in two major allelic sequence variants, *iceA1* and *iceA2*. But, only iceA1 is induced after contact with epithelial cells [11]. Moreover, OipA with a molecular weight of 33–35 kDa is another best-characterized adhesion molecule of the *H. pylori* that mediate the attachment of *H. pylori* to gastric epithelial cells and causes gastric inflammation and gastroduodenal diseases via induction of pro-inflammatory cytokine interleukin (IL)-8 [17]. The role of *babA2*, *iceA1*, *iceA2*, and *oipA* in inflammation shows that they may be important not only in colonization by helping *H. pylori* adhere to host cells and delivering *cagA* and *vacA* toxins into host cells, but also in *H. pylori*-associated severe diseases by being involved in immune response induction. This research was done to study the clinical relevance of the major virulence factors of *H. pylori* with clinical outcomes and histological parameters in Iranian patients.

2. Methods

2.1. Specimen collection and processing

Four antral biopsies specimens were collected from 200 subjects who underwent upper gastrointestinal endoscopy at Hajar Hospital, Shahrekord, Iran, the samples assessed by histological analysis, RUT—Rapid Urease Test and PCR—polymerase chain reaction. This study was approved by the ethical board of Shahrekord University of medical sciences with number: IR.SKUMS.REC.1394.280. Subsequently, the subjects were classified as *H. pylori*-positive subjects with gastritis (n = 55: 26 males, 29 females; mean age: 50.18 ± 15.02 years old) and *H. pylori*-positive subjects with PUD (n = 47: 27 males, 20 females; mean age: 50.16 ± 15.3 years old) according to the results of RUT, histological analysis, and PCR test (detection of "housekeeping genes" such as 16srRNA and glmM). The subjects were classified as *H. pylori*-infected cases if RUT, histology, and PCR (16srRNA and glmM) were positive.

The exclusion criteria were as follows: patients received antibiotics and anti-inflammatory treatments, the presence of chronic inflammatory diseases and patients who have less than four positive tests.

2.2. Histological examination

For histology analysis, tissues were fixed in 200 ml of 10% neutral-buffered formalin at room temperature, and the biopsies were dehydrated through an ethanol series, cleared with xylene. The Sydney's system was followed for grading *H. pylori* infection and gastric pathologies [18]. Subsequently, Sections (4μm thick) from paraffin embedded biopsies were cut for staining with Haematoxylin and Eosin (H and E) and modified Giemsa stain for *H. pylori* visualization using a light microscope. Histological features of gastric inflammation scored as normal:0, mild:1, moderate:2 and severe:3. We described a PUD any circumscribed break of ≥5 mm in diameter with apparent depth covered with exudates occurring in the duodenum or stomach.

2.3. DNA extraction

Genomic DNA from all biopsy specimens was extracted using the Biospin Tissue Genomic DNA Extraction Kit (BioFlux, Japan) according to the manufacturer's instructions. DNA was quantified by determining optical density at 260 nm (OD260) and 280 nm (OD280) (NanoDrop; Thermo Scientific, USA).

2.4. Detection of housekeeping genes and virulence factors of H. pylori

Housekeeping genes and virulence factors of *H. pylori* were detected by polymerase chain reaction (PCR) amplification using a method previously described by Mashak *et al.* [19].

2.5. Statistical analysis

The t test was used for comparing the age of the patients between groups. The chi-square (χ2) test or Fisher's exact test were used to compare virulence factors of *H. pylori* and clinical outcomes. The data were statistically analyzed using SPSS 16. The statistically significant result was *P*-values ≤ 0.05.

3. Results

3.1. Demographic characteristics

The results of 200 subjects with PUD (63 infected patients and 20 uninfected subjects) and gastritis (89 infected patients and 28 uninfected subjects) were reported in this study. 152 (76%) subjects were positive for *H. pylori*, including 89 (76%) of the 117 subjects with gastritis and 63 (75.9%) of the 83 subjects with PUD. The study population in *H. pylori*-infected subjects consisted 54 females and 35 males with gastritis and 24 females and 39 males with 63 PUD. The mean age of subjects with gastritis and PUD was 50.17 ± 16.2 and 50.28 ± 16.3 year, respectively. Table 1 Indicates demographic characteristics of the subjects participated in this study.

3.2. Relation between patient sex and different gastric diseases

Results from chi-square test showed a statistically significant difference between patient sex and different gastric diseases (*P* = 0.006) (Table 2). The frequency of gastritis disease was more in female as compared with male; however, the frequency of PUD in male was more than that of female. Moreover, the frequency of *cagA*-positive in *H. pylori*-infected subjects was more in female as compared with male (*P* < 0.05), but there was no significant relationship between the *vacA*, *oipA*, and *iceA* genes with sex (Table 3).

3.3. Genotyping

The *cagA* gene was detected in 106 (69.7%) isolates of *H. pylori*. In the *vacA m-region*, 11 subjects (7.2%) were *m1+* and *m2+*. In the subjects carrying one single *vacA m* allele, 50 (32.9%) and 62 (40.8%) subjects were *m1+* and *m2+,* respectively. The frequency of *vacA m1* was more prevalent in gastritis compared to PUB. In the *s-region*, 26 subjects (17.1%) were *m1+* and *m2+*. In the subjects carrying one single *vacA s* allele, the *s1* allele was found in 88 subjects (57.9%) and *s2* in 22 subjects (14.5%). The *vacA* genotype *s1/m1*, *s1/m2*, and *s2/m2* were detected in 42 (27.6%), 32 (21.1%), and 18 (11.8%) subjects, respectively. 77 (50.7%) and 93 (61.2%) subjects were positive for *oipA* and *babA2* genes,

Table 1. Demographic characteristics of PUD and gastritis subjects.

Groups [n (%)]	Infection		Age (years)
	Positive [n (%)]	Negative [n (%)]	
G[a] 117 (58.5)	89 (58.6)	28 (58.3)	50.17 ± 16.2
PUD[b] 83 (41.5)	63 (41.4)	20 (41.7)	50.28 ± 16.3
P-value	0.979[c]		0.993[d]

[a] G: gastritis.
[b] PUD: peptic ulcer diseases.
[c] chi squared (χ2) test.
[d] Student t test.

Table 2. Relation between sex and the disease type of in *H. pylori*-infected subjects.

Groups [n (%)]	Sex		P-value
	Male [n (%)]	Female [n (%)]	
G[a] 89 (58.6)	35 (39.3)	54 (60.7)	0.006
PUD[b] 63 (41.4)	39 (61.9)	24 (38.1)	
Total 152 (100)	74 (48.7)	78 (51.3)	

[a] G: gastritis.
[b] PUD: peptic ulcer diseases.

Table 3. Relation between sex and virulence factors in *H. pylori*-infected subjects.

Genotypes	Male [n (%)]	Female [n (%)]	P-value[a]
cagA⁺	45 (60.8)	61 (78.2)	0.02
cagA	29 (39.2)	17 (21.8)	
oipA⁺	38 (49.4)	39 (50.0)	0.868
oipA	36 (48.6)	39 (50.0)	
babA2⁺	46 (62.2)	47 (60.3)	0.81
babA2	28 (37.8)	31 (39.7)	
iceA1	19 (52.8)	17 (47.2)	0.663
iceA2	27 (42.9)	36 (57.1)	
iceA1/iceA2⁺	11 (50.0)	11 (50.0)	
iceA1/iceA2	17 (54.8)	11 (45.2)	
vacA			
s1	41 (46.6)	47 (53.4)	
s2	8 (36.4)	14 (63.3)	0.479
s1s2	14 (53.8)	12 (46.2)	
m1	25 (50.0)	25 (50.0)	
m2	29 (46.8)	33 (53.2)	0.586
m1m2	7 (63.3)	4 (36.4)	
s1m1	19 (45.2)	23 (54.8)	
s1m2	17 (53.1)	15 (46.9)	0.797
s2m1	0 (00.0)	0 (00.0)	
s2m2	8 (38.9)	11 (61.1)	

[a] Chi-square tests.

Table 4. The prevalence of *cagA*, *vacA*, *iceA*, and *oipA* genotypes of *H. pylori* among the groups.

Groups Genotypes	G[a] [n (%)]	PUD[b] [n (%)]	P-value[c]
vacA			
s1	50 (64.1)	38 (65.5)	
s2	14 (17.9)	8 (13.8)	0.78
s1s2	14 (17.9)	12 (20.7)	
m1	36 (54.5)	14 (24.6)	
m2	24 (36.4)	38 (66.7)	0.002
m1m2	6 (9.1)	5 (8.8)	
s1m1	30 (60.0)	12 (28.6)	
s1m2	9 (18.0)	23 (54.8)	
s2m1	0 (00.0)	0 (00.0)	0.001
s2m2	11 (22.0)	7 (16.7)	
cagA⁺	59 (66.3)	47 (74.6)	0.227
cagA	30 (33.7)	16 (25.4)	
iceA1	17 (27.0)	19 (32.8)	
iceA2	34 (54.3)	29 (50.0)	0.785
iceA1/iceA2	12 (19.0)	10 (17.2)	
babA2⁺	47 (52.8)	46 (73.0)	0.012
babA2⁻	42 (47.2)	17 (27.0)	
oipA⁺	39 (43.8)	38 (60.3)	
oipA⁻	50 (56.2)	25 (39.7)	0.045

[a] G: gastritis.
[b] PUD: peptic ulcer diseases.
[c] P-value was calculated by Chi-square test.

respectively. Overall, *iceA1* was detected in 36 subjects (23.1%) of the total 152 subjects and *iceA2* was found in 63 subjects (41.4%), 22 subjects (14.5%) were *iceA1+* and *iceA2+*.

3.4. Relationship between virulence factors and different gastroduodenal diseases

The frequency of *oipA*, *babA2* and *vacA m2* virulence factors was significantly more in *H. pylori*-positive patients with peptic ulcer disease as compared with *H. pylori*-positive subjects with gastritis disease. Also, the frequency of *vacA s1/m1* allele was significantly more in *H. pylori*-positive subjects with gastritis compared with *H. pylori*-positive subjects with PUD ($P < 0.05$), but the relationship between the prevalence of other virulence factors with diseases was not significant ($P > 0.05$) (Table 4).

3.5. Association between vacA genotype and other genotypes

Among 106 infected patients with *H. pylori* who were positive for the *cagA* genotype, a significant relationship was found only between *m1*, *m2*, and *m1/m2* strains of *vacA* genotype with the *cagA* genotype ($P < 0.021$). Among 93 infected patients with *H. pylori* who were positive for the *babA2* genotype, a significant relationship was detected only between *s1*, *s2*, and *s1/s2* strains of *the vacA* genotype with the *babA2* genotype ($P = 0.002$). Among 58 patients with *H. pylori* infection who were positive for the *babA2* genotype, a significant relationship was only found

between *s1/m1*, *s1/m2*, and *s2/m2* strains of the *vacA* genotype with *babA2* genotype ($P = 0.003$). Furthermore, no relationship was found between vacA genotypes in patients with *H. pylori*-infection and *iceA2* and *oipA* (Table 5).

3.6. Relationship between histological parameters and the grade of H. pylori density

Higher density scores of *H. pylori* were significantly associated with *iceA1⁻*, *babA2*⁺ and *oipA*⁺ virulence factors ($P < 0.05$). Other virulence factors exhibited no significant correlation with the grade of *H. pylori* density (Table 6).

3.7. Relationship between histological parameters and the grade of neutrophils activity and mononuclear cell infiltration

The higher polymorphonuclear cell infiltration was strongly associated with the presence of *cagA*-positive, *iceA*-negative and *oipA*-positive virulence factors in the gastric biopsy specimens ($P < 0.05$). Other virulence factors showed no significant relationship with polymorphonuclear cell infiltration (Table 6). The higher mononuclear cell infiltration was strongly associated with the presence of *cagA*-positive, *babA2*-positive and *oipA*-positive virulence factors in the gastric biopsy specimens ($P < 0.05$). Other virulence factors had no significant relationship with mononuclear cell infiltration (Table 6).

4. Discussion

Four important findings were obtained in the present study. First, among patients with peptic ulcer disease the colonization by *m2* and *s1m2* alleles, *babA2*, and *oipA* was significantly more than patients with gastritis, while the frequency of *vacA s1m1* allele was significantly higher in *H. pylori*-positive subjects with gastritis compared with *H. pylori*-positive subjects with PUD. Second, the colonization with *iceA1*, *babA2*⁺, and *oipA*⁺ virulence factors were strongly correlated with high-density

Table 5. Correlation of *vacA* alleles with *cagA*, *babA2*, *oipA* and *iceA* genotypes of the samples studied.

vacA genotype	cagA		babA2		iceA1		iceA2		oipA	
	Positive/Negative		Positive/Negative		Positive/Negative		Positive/Negative		Positive/Negative	
s1m1	32	10	30	12	16	26	30	12	27	15
s1m2	26	6	23	9	22	10	15	17	19	13
s2m2	11	7	17	1	4	14	11	7	11	7
P-value[a]	0.279		0.126		0.003		0.100		0.908	
s1	67	21	59	29	43	45	52	36	51	38
s2	15	7	19	3	7	15	12	10	14	7
s1/s2	15	11	10	16	7	19	16	10	9	17
P-value[a]	0.177		0.002		0.079		0.883		0.07	
m1	39	11	34	16	20	30	33	17	31	19
m2	45	17	45	17	30	32	34	28	36	26
m1/m2	4	7	7	4	4	7	7	4	5	6
P-value[a]	0.021		0.778		0.586		0.472		0.598	

[a] Chi-square test.

scores of *H. pylori* in infected subjects. Third, the colonization with *cagA*[+], *iceA1* and *oipA*[+] virulence factors were significantly correlated with high polymorphonuclear cell infiltration in infected subjects. Four, the colonization with *cagA*[+], *babA2*[+], and *oipA*[+] were strongly related to higher mononuclear cell infiltration scores in infected subjects.

H. pylori is a major human pathogen which produces inflammation of the stomach and is etiologically related to chronic gastritis and PUD, but not all *H. pylori*-positive subjects develop such diseases [20, 21, 22]. Approximately 50 percent of the human population worldwide is chronically infected with *H. pylori* without any clinical symptoms and there are significant variations in its prevalence between different countries [23].

A study by Garcia *et al.* showed that the severity of gastritis is related with the coexistence of the *iceA2* gene with *cagA*, *vacA s1/m1* and *babA2* [24]. Several studies conducted on patients in Iran demonstrated that vacA is not

Table 6. Relationship between histological parameters and virulence factors of *H. pylori*.

Histological parameters Genotypes	polymorphonuclear cell infiltration[a] [N (%)]				mononuclear cell infiltration [N (%)]			H. pylori density[b] [N (%)]		
	None	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
cagA (+)	17 (58.6)	57 (66.3)	20 (80)	12 (100)	20 (57.1)	50 (66.7)	36 (85.7)	50 (61.7)	27 (75)	29 (82.9)
cagA (-)	12 (41.4)	29 (33.7)	5 (20)	0 (0.0)	15 (42.9)	25 (33.3)	6 (14.3)	31 (38.3)	9 (25)	6 (17.1)
P-value[c]	**0.034**				**0.018**			0.055		
vacA m1	7 (33.3)	29 (42.6)	9 (40.9)	5 (41.7)	6 (26.1)	28 (45.2)	16 (42.1)	26 (48.1)	14 (41.2)	10 (28.6)
vacA m2	12 (57.1)	31 (45.6)	12 (54.5)	7 (58.3)	13 (56.5)	29 (46.8)	20 (52.6)	21 (38.9)	17 (50)	24 (68.6)
vacA m1m2	2 (9.5)	8 (11.8)	1 (4.5)	0 (0)	4 (17.4)	5 (8.1)	2 (5.3)	7 (13)	3 (8.8)	1 (2.9)
P-value	0.384				0.359			0.497		
vacA s1	6 (42.9)	24 (47.1)	8 (44.4)	4 (44.4)	15 (53.6)	46 (68.7)	27 (65.9)	47 (68.1)	20 (62.5)	21 (60)
vacA s2	3 (21.4)	16 (31.4)	8 (44.4)	5 (55.6)	6 (21.4)	9 (13.4)	7 (17.1)	6 (8.7)	7 (21.9)	9 (25.7)
vacA s1s2	5 (35.7)	11 (21.6)	2 (11.1)	0 (0)	7 (25)	12 (17.9)	7 (17.1)	16 (23.2)	5 (15.6)	5 (14.3)
P-value	0.311				0.706			0.165		
vacA s1m1	12 (54.5)	12 (54.5)	12 (54.5)	12 (54.5)	4 (26.7)	24 (51.1)	14 (46.7)	23 (59)	11 (45.8)	8 (27.6)
vacA s1m2	5 (22.7)	5 (22.7)	5 (22.7)	5 (22.7)	6 (40)	16 (34)	10 (33.3)	12 (30.8)	7 (29.2)	13 (44.8)
vacA s2m2	5 (22.7)	5 (22.7)	5 (22.7)	5 (22.7)	5 (33.3)	7 (14.9)	6 (20)	4 (10.3)	6 (25)	8 (27.6)
P-value	0.999				0.460			0.096		
iceA1 (+)	8 (27.6)	29 (33.7)	15 (60)	6 (50)	8 (22.9)	30 (40)	20 (47.6)	24 (29.6)	14 (38.9)	20 (57.1)
iceA1 (-)	21 (72.4)	57 (66.3)	10 (40)	6 (50)	27 (77.1)	45 (60)	22 (52.4)	57 (70.4)	22 (61.1)	15 (42.9)
P-value	**0.049**				0.075			**0.02**		
iceA2 (+)	17 (58.6)	48 (55.8)	11 (44)	9 (75)	25 (71.4)	36 (48)	24 (57.1)	47 (58)	19 (52.8)	19 (54.3)
iceA2 (-)	12 (41.4)	38 (44.2)	14 (56)	3 (25)	10 (28.6)	39 (52)	18 (42.9)	34 (42)	17 (47.2)	16 (45.7)
P-value	0.348				0.069			0.849		
babA2 (+)	14 (48.3)	54 (62.8)	16 (64)	9 (75)	15 (42.9)	48 (64)	30 (71.4)	38 (46.9)	25 (69.4)	30 (85.7)
babA2 (-)	15 (51.7)	32 (37.2)	9 (36)	3 (25)	20 (57.1)	27 (36)	12 (28.6)	43 (53.1)	11 (30.6)	5 (14.3)
P-value	0.365				**0.029**			**0.0001**		
oipA (+)	8 (27.6)	41 (47.7)	19 (76)	9 (75)	8 (22.9)	38 (50.7)	31 (73.8)	27 (33.3)	21 (58.3)	29 (82.9)
oipA (-)	21 (72.4)	45 (52.3)	9 (24)	3 (25)	27 (77.1)	37 (49.3)	11 (26.2)	54 (66.7)	15 (41.7)	6 (17.1)
P-value	**0.001**				**0.0001**			**0.0001**		

Statistically significant values were shown in bold.
[a] The histopathological parameters were scored as: 0, none; 1, mild; 2, moderate; 3, severe.
[b] The histopathological parameters were scored as: 1, mild; 2, moderate; 3, severe.
[c] Chi-square test.

associated with clinical outcomes such as PUD, gastritis, and non-ulcer dyspepsia [25, 26, 27]. Our results are in agreement with previous reports in Iranian patients with *H. pylori* that show vacA s1m2 genotype was found to be significantly associated with PUD [28]. Evaluation of clinical relevance of cagA and vacA gene polymorphisms of *H. pylori* in Italy indicated higher levels of epithelial damage, gastric atrophy, lymphocytic and neutrophilic infiltrates, and intestinal metaplasia [29]. In addition, some other studies showed that cagA + *H. pylori* isolates will develop a more severe form of gastritis [30, 31]. Moreover, cagA-positive *H. pylori* strains were related with a higher gastric mucosal infiltration of neutrophils [32]. The cagA status of *H. pylori* could also influence the cytokine patterns of T-helper cells [33]. A study by Jafarzadeh *et al.* showed that the serum levels of IL-17 in duodenal ulcer patients with anti-cagA antibody was significantly higher than that observed in duodenal ulcer patients with negative for anti-cagA antibody [34]. Our previous study demonstrated that the number of suppressor T cells or Foxp3$^+$ T cells and TGF-β1 mRNA levels in *H. pylori*-positive subjects with *vacA s1m1*-positive was significantly higher than those observed in *H. pylori*-positive subjects with *vacA s1m2*-positive and *vacA s2m2*-positive [35].

Several studies have demonstrated that the oipA and cagA produced by *H. pylori* can stimulate the gastric epithelial cell to secrets pro-inflammatory cytokines that can induce local inflammatory reaction by migration and infiltration of high levels of a neutrophilic and mononuclear cell into the site of infection [17, 36, 37]. Our recent study demonstrated that *H. pylori*-positive subjects with *oipA*-positive had a significantly higher number of inflammatory Th17 cells and the expression level of IL-17 and IL-8 compared with the *H. pylori*-positive subjects with *oipA*-negative. Also, the number of inflammatory Th17 cells and the expression level of IL-17 and IL-8 were significantly higher in patients with peptic ulcer disease in compere to patients with gastritis disease [38]. Previous studies have also reported strong associations between the high prevalence of in-frame oipA gene strains (81%), associated significantly with PUD as well as with *cagA*-positive, *vacA s1*, *m1*, *m2*, and, importantly, *i1* genotypes [39].

In the present study, we have shown that the presence of *iceA1* or *iceA2* is not associated with non-ulcer disease and PUD. The results from other Asian countries are in accordance with our study [40]. However, the presence of the *iceA1* allele is associated with PUD in patients with *H. pylori* from Western countries [11]. A meta-analysis study evaluating clinical outcomes associated with *iceA* confirmed that the presence of *iceA2* is inversely associated with PUD (OR 0.76, 95% CI 0.65–0.89) [41]. Furthermore, in accordance with our study, the presence of *babA2* and *oipA* was associated with high density of *H. pylori* and more polymorphonuclear cell infiltration with an increased risk of PUD [17, 42].

In conclusion, determining the prevalence of *H. pylori* genotypes in patients from different countries leads to better understanding of *H. pylori* pathogenesis and the severity of related diseases. Therefore, we evaluated the relationship between different genotypes of *H. pylori* and increased risks of PUD in adult patients from Iran based on type diseases and histopathological findings. Overall, results from this study indicated that the presence of virulence factors *vacA*, *babA2*, and *oipA* is associated with increased risk of PUD in patients with *H. pylori* infection. These results can help physicians in early prognosis of patients with increased risk of peptic ulcer, prevention and management of PUD, and using best treatments for gastric disorders.

Declarations

Author contribution statement

Milad Shahini Shams Abadi: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Korosh Ashrafi-Dehkordi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Reza Ahmadi: Conceived and designed the experiments.

Ghorbanali Rahimian, Yousef Mirzaei: Analyzed and interpreted the data.

Rana Fereidani, Mojtaba Shohan: Contributed reagents, materials, analysis tools or data.

Fatemeh Azadegan-Dehkordi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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