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

Milad Shahini Shams Abadi, Korosh Ashrafi-Dehkordi, Reza Ahmadi, Ghorbanali Rahimian, Yousef Mirzaei, Rana Fereidanif, Mojtaba Shohang, Fatemeh Azadegan-Dehkordi

*Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*Department of Molecular Medicine, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*Department of Internal Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*Department of Biogeosciences, Scientific Research Center, Soran University, Soran, Kurdistan Region, Iraq*
*Department of Pathology, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*Department of Immunology, Faculty of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran*

**ABSTRACT**

*Helicobacter 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* 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), vaculating cytotoxin protein A (VacA), and cytotoxin-associated protein A (CagA) [8]. A main *H. pylori* virulence factor is the cag pathogenicity island (cagPAI), 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.
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 vacuolization 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 16s rRNA and glmM). The subjects were classified as *H. pylori*-infected cases if RUT, histology, and PCR (16s rRNA 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 Indicate 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 PUD. 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,

| Groups [n (%)] | Infection | Age (years) |
|---------------|-----------|-------------|
|               | Positive [n (%)] | Negative [n (%)] |
|               | 89 (58.6) | 28 (58.3) | 50.17 ± 16.2 |
| PUD 83 (41.5) | 63 (41.4) | 20 (41.7) | 50.28 ± 16.3 |
| *P*-value     | 0.979     | 0.993d      |
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- | 35 (39.3) | 54 (60.7) | 0.006 |
| PUD | 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 |
|-----------|--------------|----------------|---------|
| cagA + | 45 (60.8) | 61 (78.2) | 0.02 |
| cagA | 29 (39.2) | 17 (21.8) | 0.851 |
| oipA | 38 (49.4) | 39 (50.0) | 0.868 |
| babA2 | 46 (62.2) | 47 (60.3) | 0.81 |
| iceA1 | 27 (37.8) | 31 (39.7) | 0.663 |
| iceA2 | 27 (37.8) | 31 (39.7) | 0.663 |
| oipA | 17 (54.8) | 11 (50.0) | 0.479 |
| oipA | 41 (46.6) | 47 (53.4) | 0.479 |
| s2 | 8 (36.4) | 14 (63.6) | 0.586 |
| s1s2 | 25 (50.0) | 25 (50.0) | 0.586 |
| m2 | 29 (46.8) | 33 (53.2) | 0.797 |
| s1m2 | 7 (36.3) | 4 (36.4) | 0.797 |
| s1m1 | 19 (45.2) | 23 (54.8) | 0.797 |
| s1m2 | 17 (53.1) | 15 (46.9) | 0.797 |
| s2m1 | 0 (00.0) | 0 (00.0) | 0.797 |
| s2m2 | 8 (38.9) | 11 (61.1) | 0.797 |

a: Chi-square tests.

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. Relation 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 (< 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 s1m1 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
scores of \( H. \text{pylori} \) in infected subjects. Third, the colonization with \( cagA^+ \), \( \text{iceA}1^+ \), and \( \text{oipA}^+ \) virulence factors were significantly correlated with high polymorphonuclear cell infiltration in infected subjects. Fourth, the colonization with \( cagA^- \), \( \text{babA}2^- \), and \( \text{oipA}^- \) were strongly related to higher mononuclear cell infiltration scores in infected subjects.

\( H. \text{pylori} \) is a major human pathogen which produces inflammation of the stomach and is etiologically related to chronic gastritis and PUD, but not all \( H. \text{pylori} \)-positive subjects develop such diseases [20, 21, 22]. Approximately 50 percent of the human population worldwide is chronically infected with \( H. \text{pylori} \) 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 \( \text{iceA}2 \) gene with \( cagA \), \( \text{vacA} \text{s1/m1} \) and \( \text{babA}2 \) [24]. Several studies conducted on patients in Iran demonstrated that \( \text{vacA} \) is not

### Table 5. Correlation of \( \text{vacA} \) alleles with \( cagA \), \( \text{babA}2 \), \( \text{oipA} \) and \( \text{iceA} \) genotypes of the samples studied.

| \( \text{vacA} \) genotype | \( cagA \) | \( \text{babA}2 \) | \( \text{iceA1} \) | \( \text{iceA2} \) | \( \text{oipA} \) |
|--------------------------|---------|---------|---------|---------|---------|
|                          | Positive/Negative | Positive/Negative | Positive/Negative | Positive/Negative | Positive/Negative |
| \( \text{s1m1} \)        | 32      | 10      | 30      | 12      | 16      |
| \( \text{s1m2} \)        | 26      | 6       | 23      | 9       | 22      |
| \( \text{s2m2} \)        | 11      | 7       | 17      | 1       | 4       |
| \( \text{P-value}\)      | 0.279   | 0.126   | 0.003   | 0.100   | 0.908   |
| \( \text{s1} \)         | 67      | 21      | 59      | 29      | 43      |
| \( \text{s2} \)         | 15      | 7       | 19      | 3       | 7       |
| \( \text{s1/s2} \)       | 15      | 11      | 10      | 16      | 7       |
| \( \text{P-value}\)      | 0.177   | 0.002   | 0.079   | 0.883   | 0.07    |
| \( \text{m1} \)         | 39      | 11      | 34      | 16      | 20      |
| \( \text{m2} \)         | 45      | 17      | 45      | 17      | 30      |
| \( \text{m1/m2} \)       | 4       | 7       | 7       | 4       | 4       |
| \( \text{P-value}\)      | 0.021   | 0.778   | 0.586   | 0.472   | 0.598   |

* Chi-square test.

### Table 6. Relationship between histological parameters and virulence factors of \( H. \text{pylori} \).

| Histological parameters | polyomorphonuclear cell infiltration\(^a\) | mononuclear cell infiltration\(^b\) | \( H. \text{pylori} \) density\(^c\) |
|-------------------------|--------------------------------|-------------------------------|-----------------|
|                         | [N (%)]                      | [N (%)]                       | [N (%)]         |
|                         | None | Mild | Moderate | Severe | None | Mild | Moderate | Severe | None | Mild | Moderate | Severe |
| \( cagA^+ \)            | 17   | 57   | 66.3     | 20      | 80   | 12   | 100     |
| \( cagA^- \)            | 12   | 29   | 33.7     | 5       | 20   | 0    | 0       |
| \( P-value\)            | 0.034 | 0.018 | 0.055    |          |
| \( \text{vacA} m1 \)    | 7    | 33.3 | 29.2     | 40.9    | 5    | 41.7 | 6      |
| \( \text{vacA} m2 \)    | 12   | 57.1 | 31.4     | 45.6    | 12   | 54.5 | 7      |
| \( \text{vacA} m1/m2 \) | 2    | 9.5  | 11.8     | 4.5     | 0    | 0    | 0      |
| \( P-value\)            | 0.384 | 0.359 | 0.497    |          |
| \( \text{iceA1}^+ \)    | 8    | 27.6 | 29.3     | 33.7    | 15   | 60   | 50     |
| \( \text{iceA1}^- \)    | 21   | 72.4 | 57.6     | 66.3    | 10   | 40   | 65     |
| \( P-value\)            | 0.049 | 0.075 | 0.02     |          |
| \( \text{iceA2}^+ \)    | 17   | 58.6 | 48.5     | 55.8    | 11   | 44   | 9      |
| \( \text{iceA2}^- \)    | 12   | 41.4 | 34.4     | 44.2    | 14   | 56   | 3     |
| \( P-value\)            | 0.348 | 0.069 | 0.849    |          |
| \( \text{babA}2^+ \)    | 14   | 48.3 | 54.2     | 62.8    | 16   | 64   | 9      |
| \( \text{babA}2^- \)    | 15   | 51.7 | 32.3     | 37.2    | 9    | 36   | 3      |
| \( P-value\)            | 0.365 | 0.029 | 0.0001   |          |
| \( \text{oipA}^+ \)     | 8    | 27.6 | 41.7     | 47.7    | 19   | 76   | 9      |
| \( \text{oipA}^- \)     | 21   | 72.4 | 45.2     | 52.3    | 9    | 24   | 3      |
| \( P-value\)            | 0.001 | 0.0001 | 0.0001  |          |

Statistically significant values were shown in bold.

* The histopathological parameters were scored as: 0, none; 1, mild; 2, moderate; 3, severe.

\(^b\) The histopathological parameters were scored as: 0, none; 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 infiltrations, and intestinal metaplasia [29]. In addition, some other studies showed that cagA + H. pylori isolates would 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- [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 mono-nuclear 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 compare 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 Fereidini, 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.

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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.

References

[1] N. Bagheri, et al., Altered Th17 cytokine expression in Helicobacter pylori patients with TLR4 (D299G) polymorphism, Immunol. Invest. (2016) 1–11.
[2] H. Shirzad, et al., New insight into IL-25/IL-17 axis in Iranian infected adult patients with gastritis: effects of genes polymorphisms on expression of cytokines, Acta Gastroenterol. Belg. 78 (2) (2015) 212–218.
[3] M.J. Sanaei, et al., New insights into regulatory B cells biology in viral, bacterial, and parasitic infections, Infect. Genet. Evol. 89 (2021) 104753.
[4] M.N. Menbardi, et al., Evaluation of E-cadherin (CDH1) gene polymorphism related to gastric cancer in Kurdish population, Life Sci. J. 10 (12) (2013).
[5] A. Sanaii, et al., Role of Th22 cells in Helicobacter pylori-related gastritis and peptic ulcer diseases, Mol. Biol. Rep. 46 (6) (2019) 5703–5712.
[6] F. Azadegan-Dehkordi, et al., Increased Indoleamine 2, 3-Dioxygenase expression modulates Th1/Th17/Th22 and Treg pathway in humans with Helicobacter Pylori-Infected gastric mucosa, Hum. immunol. 82 (1) (2021) 46–53.
[7] F. Zandi, et al., Evaluation of IL-17A and IL-17F genes polymorphism in Iranian dyspeptic patients, Life Sci. J. 10 (2013) 544–551 (SPL. IS).
[8] N. Bagheri, et al., Clinical relevance of Helicobacter pylori virulence factors in Iranian patients with gastrointestinal diseases, Microb. Pathog. 100 (2016) 154–162.
[9] N. Tegmeyer, S. Westler, S. Buckert, Role of the cag-pathogenicity island encoded type IV secretion system in Helicobacter pylori pathogenesis, FEMS J. 278 (8) (2011) 1190–1202.
[10] R.D. Leunk, et al., Cytotoxic activity in broth-culture filtrates of Campylobacter pylori, J. Med. Microbiol. 26 (2) (1988) 93–99.
[11] L.J. van Doorn, et al., Clinical relevance of the cagA, vacA, and iecA status of Helicobacter pylori, Gastroenterology 115 (1) (1998) 58–66.
[12] N. Bagheri, et al., Role of regulatory T-cells in different clinical expressions of Helicobacter pylori infection, Arch. Med. Res. 47 (4) (2016) 245–254.
[13] Z. Khodaii, et al., cagA and vacA status and influence of Helicobacter pylori infection on serum oxidative DNA damage in Iranian patients with peptic ulcer disease, Ir. J. Med. Sci. 180 (1) (2011) 155–161.
[14] D. Iyer, et al., Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging, Science 279 (5349) (1998) 373–377.
[15] M.S.S. Abadi, et al., Distribution and diversity of hmvA1 among invasive nontypeable haemophilus influenzae isolates in Iran, Avicenna J. Med. Biotechnol. 8 (2) (2016) 99.
[16] S. Javdan, et al., Agr typing of Staphylococcus aureus species isolated from clinical samples in training hospitals of Isfahan and Shahrekord, BMC Res. Notes 12 (1) (2019) 1–6.
[17] Y. Yamaoka, et al., Importance of Helicobacter pylori oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production, Gastroenterology 123 (6) (2002) 414–424.
[18] M.F. Dixon, et al., Classification and grading of gastritis. The updated Sydney System, Am. J. Surg. Pathol. 20 (10) (1996) 1161–1181.
[19] Z. Mashak, et al., Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat, Infect. Drug Resist. 13 (2020) 257.
[20] N. Bagheri, et al., T-bet(+) cells polarization in patients infected with Helicobacter pylori increase the risk of peptic ulcer development, Arch. Med. Res. 50 (3) (2019) 113–121.
[21] M. Nabili-Samini, et al., Enhanced frequency of CD19(+) B cells in human gastric mucosa infected by Helicobacter pylori, Am. J. Med. Sci. 359 (6) (2020) 347–353.
[22] M.J. Sanaei, et al., Up-regulated CCL18, CCL28 and CXCL13 expression is associated with the risk of gastritis and peptic ulcer disease in Helicobacter pylori infection, Am. J. Med. Sci. 361 (1) (2021) 43–54.

[23] M.A. Mendall, Transmission of Helicobacter pylori, Semin. Gastrointest. Dis. 8 (3) (1997) 113–123.

[24] G.T. Garcia, et al., High prevalence of clarithromycin resistance and cagA, iceA2, and babA2 genotypes of Helicobacter pylori in Brazilian children, J. Clin. Microbiol. 48 (11) (2010) 4266–4268.

[25] Z. Khodaii, et al., cagA and vacA status and influence of Helicobacter pylori infection on serum oxidative DNA damage in Iranian patients with peptic ulcer disease, Ir. J. Med. Sci. 180 (2011) 155–161.

[26] M. Rafeey, et al., Association between Helicobacter pylori, cagA, and vacA status and clinical presentation in Iranian children, Iran J. Pediatr. 23 (5) (2013) 551–556.

[27] M.H. Salari, et al., Frequency of Helicobacter pylori vacA genotypes in Iranian patients with gastric and duodenal ulcer, J. Infect. Publ. Health 2 (4) (2009) 204–208.

[28] N. Farzi, et al., Genetic diversity and functional analysis of oipA gene in association with other virulence factors among Helicobacter pylori isolates from Iranian patients with different gastric diseases, Infect. Genet. Evol. 60 (2018) 26–34.

[29] D. Basso, et al., Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms, Gastroenterology 135 (1) (2008) 91–99.

[30] L.E. Wroblewski, R.M. Peek Jr., K.T. Wilson, Helicobacter pylori and gastric cancer: factors that modulate disease risk, Clin. Microbiol. Rev. 23 (4) (2010) 713–739.

[31] V. ConteDucu, et al., H. pylori infection and gastric cancer: state of the art, Int. J. Oncol. 42 (1) (2013) 5–18 (Review).

[32] T. Ando, et al., Anti-CagA immunoglobulin G responses correlate with interleukin-8 induction in human gastric mucosal biopsy culture, Clin. Diagn. Lab. Immunol. 7 (5) (2000) 803–809.

[33] S.K. Wang, et al., CagA+ H pylori infection is associated with polarization of T helper cell immune responses in gastric carcinogenesis, World J. Gastroenterol. 13 (21) (2007) 2923–2931.

[34] A. Jafarzadeh, et al., Association of the CagA status of Helicobacter pylori and serum levels of interleukin (IL)-17 and IL-23 in duodenal ulcer patients, J. Dig. Dis. 10 (2) (2009) 107–112.

[35] N. Bagheri, et al., Downregulated regulatory T cell function is associated with increased peptic ulcer in Helicobacter pylori infection, Microb. Pathog. 110 (2017) 165–175.

[36] S. Brandt, et al., NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein, Proc. Natl. Acad. Sci. U. S. A. 102 (26) (2005) 9300–9305.

[37] Y. Yamaoka, et al., Induction of various cytokines and development of severe mucosal inflammation by cagA gene positive Helicobacter pylori strains, Gut 41 (4) (1997) 442–451.

[38] N. Bagheri, et al., Up-regulated Th17 cell function is associated with increased peptic ulcer disease in Helicobacter pylori infection, Infect. Genet. Evol. 60 (2018) 117–125.

[39] R. Markovska, et al., Helicobacter pylori oipA genetic diversity and its associations with both disease and cagA, vacA s, m, and i alleles among Bulgarian patients, Diagn. Microbiol. Infect. Dis. 71 (4) (2011) 335–340.

[40] P.Y. Zheng, et al., Association of peptic ulcer with increased expression of Lewis antigens but not cagA, iceA, and vacA in Helicobacter pylori isolates in an Asian population, Gut 47 (1) (2000) 18–22.

[41] S. Shiotani, et al., Helicobacter pylori iceA, clinical outcomes, and correlation with cagA: a meta-analysis, PloS One 7 (1) (2012), e30354.

[42] B. Motzaghi, et al., Helicobacter pylori vacA i region polymorphism but not babA2 status associated to gastric cancer risk in northwestern Iran, Clin. Exp. Med. 16 (1) (2016) 57–63.