Association of Helicobacter pylori vacA genotypes and peptic ulcer in Iranian population: a systematic review and meta-analysis

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Abstract

Background

*Helicobacter pylori* is accounted as the most etiologic agent for digestive disorders, in particular, the most important of them i.e. peptic ulcer and gastric cancer. In the recent years, association of *vacA* genotypes and gastrointestinal disorders has attracted a lot of attention. In present study, we assessed the correlation between *vacA* genotypes (s1, s2, m1, m2, s1m1, s1m2, s2m1 and s2m2) and development to peptic ulcer in Iranian population.

Methods

In our study, first, 23 original articles containing of information of 3328 patients were evaluated. Statistical analysis was done by Comprehensive Meta-Analysis version 2.0 software (Biostat, Englewood, NJ, USA). In this regards, we used from fixed-effects model for analysis of data with low heterogeneity, while for analysis of data with high heterogeneity (*I^2* statistic index >25%, Cochrane Q statistic *p* value < 0.05), random-effects model was used.

Results

Abundance of each of s1, s2, m1, m2, s1m1, s1m2, s2m1, and s2m2 was estimated 36.24%, 28.32%, 42.90% 29.86%, 32.34%, 15.70%, and 25.94%, respectively. According to the results, the m1, s1, and s1m2 genotypes were among the most prevalent genotypes among the Iranian patients, whereas, s2m1 genotype had the lowest frequency.

Conclusions

Finally, we demonstrated that there is a significant relationship between infection of stomach with m1, s1m1, and s2m1 genotypes and development to peptic ulcer.

1. Introduction

In the gastrointestinal tract, peptic ulcer is induced following damage to mucosa and sub-mucosa tissues, which occurs due to the imbalance between invasive factors (secretion of gastric acid, pepsin, bile salts, increase of oxygen free radicals, consumption of non-steroidal anti-inflammatory drugs, and infection with *H. pylori* and host defensive mechanisms (mucus, bicarbonate, prostaglandin, antioxidant, and blood circulation) (1–4). While ulcers occur in gastric epithelium, is called gastric ulcer, and when lesions happen in the first part of duodenum, so called duodenal ulcer (1, 5). The prevalence of peptic ulcer in different areas of world has been estimated 6–15%. Based on reports from The Ministry of Health and Medical Education (MOHME) of Iran, of all eight Iranians, one person has experienced peptic ulcer in
his/her life, however, the frequency of duodenal ulcer is more than gastric ulcer (6–8). According to review of the literature, infection with both \textit{H. pylori} and non-steroidal anti-inflammatory drugs (NSAIDs) are considered as the most important causing agents for peptic ulcers, but the role of \textit{H. pylori} is more prominent, so that this bacterium has isolated from 60–80\% of peptic ulcer cases (9–11). \textit{H. pylori} and NSAIDs by independent mechanisms, but synergistically lead to severe inflammation and consequently peptic ulcer (12–13). \textit{H. pylori} is a microaerophilic, S shaped, gram negative, and motile (by lophotrichous flagella) bacterium which is able to be colonized in human stomach (14). Almost half of world population are infected to \textit{H. pylori}, nevertheless, the rate of colonization in developing countries is more compared to western countries; most of population in developing countries first time infected with this bacterium in childhood ages (14–15). The International Agency for Research on Cancer (IARC) introduced this bacterium as the main causing enemy of gastric cancer (15–16). Also, this bacterium is accounted for some diseases such as primary gastric non-Hodgkin's lymphoma, mucosa-associated lymphoid tissue lymphoma (MALT), gastritis, and peptic ulcer (17). In recent years, virulence factors of \textit{H. pylori}, and above all, cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) have more considered. VacA antigen is one of the well-known virulence factors of this pathogen which its gene, \textit{vacA}, is present in all strains. The mosaic-like structure of \textit{vacA} gene has both conserved and variable allelic sequences. These variable sequences are found in different regions from N-terminal side including signal sequence (s1 and s2) region, intermediate (i1 and i2) region, deletion (d1 and d2) region, and mid (m1 and m2) region, respectively. Whilst the cytotoxicity power of all genotypes differs from each other, in addition, two s1 and m1 regions in turn comprise several subtypes including s1a, s1b, s1c, m1a, m1b and m1c (14, 18–20). This antigen through induction of cytoplasmic vacuolation and apoptosis in infected cells can lead to the death of host gastric epithelial cells (14, 21). In addition, the toxin causes dysregulation of normal signaling pathway via happens such as alternation in the mitogen-activated protein kinases (MAPKs) pathway, polarization, suppression of proliferation and migration, as well as cytoskeletal changes (20–21). Evidence show that there is a significant relationship between the presence of \textit{vacA} gene and progression of disease to peptic ulcer and gastric cancer (22–23). However, some studies have also rejected this correlation (24–25). Despite about 25 years from introduction of VacA antigen by Cover et al., but so far its properties has no recognized correctly. In Iran the abundance of peptic ulcer is about 41\% (95\% confidence interval, or 95\% CI), which is much more compared to the global average (6–15\%) (6–7, 18). Some characteristics such as high colonization by \textit{H. pylori} (about 90\%) and genetic diversity in \textit{H. pylori} strains are influential in this phenomenon (26–27). The main goal of this study was the determination of frequency of \textit{vacA} (s1, s2, m1, m2, s1m1, s1m2, s2m1 and s2m2) alleles and also their relationship with creation of peptic ulcer in Iranian population.

2. Methods

2.1. Search strategy

In the beginning, all studies (English and Persian) were received untill March 2020 from global databases such as Google scholar, Scopus, PubMed, EMBASE, and also Iranian databases of IranMedex, SID, ISC.
We used from keywords based on MeSH including "Helicobacter pylori", “peptic ulcer”, “genotypes”, “Iran”, “VacA protein”, and “gastric ulcer”. In final, based on our inclusion criteria eligibility of articles was evaluated by two authors, separately (Fig. 1). The inclusion criteria were included original articles (cross-sectional, case–control, and cohort studies) associated with vacA genotypes (s1, s2, m1, m2, s1m1, s1m2, s2m1, and s2m2) in Iranian patients with peptic ulcer, and also original articles about the identification of H. pylori and its vacA genotypes. Whilst, other studies such as reviews, letter to editor, congress abstracts, laboratory animals studies, case reports, studies of other countries, ambiguous studies, and non-clinical studies were excluded from our research.

2.2. Quality assessment and data extraction

Quality assessment of eligible studies was done based on the checklist. Afterwards, the most important information such as first author, publication year, city, age and gender distribution, number of H. pylori strains, number of peptic ulcer patients, and frequency of vacA genotypes (s1, s2, m1, m2, s1m1, s1m2, s2m1, and s2m2) was reported for each study (Table 1).
Table 1
Characteristics of included studies

| First Author | Year | City      | Peptic ulcer isolates | H. pylori | Age | caga | VacA genotypes | Ref |
|--------------|------|-----------|-----------------------|-----------|-----|------|----------------|-----|
| Dabiri       | 2017 | Tehran    | 40/16 0               |           | 4.55 ± 1 | 26   | 24/16/12/28/4/4 | 28  |
|              |      |           |                       |           |      | 5/1  | 09/1  | 8/12/12/7/9    |     |
| Salarii      | 2009 | Tehran    | 50/50 0               |           | 4.55 | NA   | 50/0/31/19/19  | 29  |
|              |      |           |                       |           |      | 21/5 | 0/0   | 1/1/2/9        |     |
| Salehii      | 2011 | Tehran    | 54/10 0               |           | 4.55 | NA   | 42/7/34/15/15  | 30  |
|              |      |           |                       |           |      | 53/2  | 6/2   | NA/NA/NA/NA     |     |
| Doosti       | 2009 | Shahrekord | 15/0 17 8             | NA        | 38.3| NA   | 20/3/8/15/7/13 | 31  |
|              |      |           |                       | NA        |      | 3/36 | 36/43 | 6/7/13/4/7/2 |     |
| Nahezi       | 2008 | Tabriz    | 48/15 0               |           | 38.3| NA   | 20/3/8/15/7/13 | 32  |
|              |      |           |                       | NA        |      | 3/36 | 36/43 | 6/7/13/4/7/2 |     |
| Doouraghi    | 2010 | Tehran    | 12/80 0               | NA        | 43.3| NA   | 7/15/9/8/12/0  | 33  |
|              |      |           |                       | NA        |      | 3/36 | 36/43 | 6/7/13/4/7/2 |     |
| Aliakhani    | 2014 | Hamadan   | 27/13 7               |           | 53.3| NA   | 25/16/5/13/6/10| 34  |
|              |      |           |                       | NA        |      | 2/16 | 16/21/4/16/5  |     |

**Notes:**
- s1/s2: Genotypes of VacA
- m1/m2: Genotypes of caga
| First Author       | Year | City     | Peptic ulcer isolates | H. pylori age | cagA Female/Male | VacA genotypes | Ref |
|-------------------|------|----------|-----------------------|---------------|------------------|----------------|-----|
| Navestani         | 2007 | Shiraz   | 33                    | 69            | 47.2             | NA NA NA NA   | 1/65 6/97 0/65 NA | 35  |
|                   |      |          |                       |               |                  |                |     |
|                   |      |          |                       |               | 12/7/13/7        |                |     |
| Salehi            | 2009 | Rasht    | 77                    | 10            | 41               | 39/5 9/22 31/3 | 18/2 9 NA NA NA NA | 36  |
|                   |      |          |                       |               | 44               | 46/3 45/3 29/29| 29/29 NA NA NA NA |     |
|                   |      |          |                       |               |                  |                |     |
|                   |      |          |                       |               | 48/3 98/9 3      |                |     |
| Abdollahi         | 2019 | Kerman   | 6                     | 12            | 38.4             | 6/45 2/29 3/29 | NA NA NA NA NA NA | 37  |
|                   |      |          |                       |               | 22/0 45/5        |                |     |
|                   |      |          |                       |               |                  |                |     |
|                   |      |          |                       |               | 98/9 3           |                |     |
| Havaei            | 2014 | Isfahan  | 40                    | 10            | 43               | 40/1 NA 21/1   | 19/4 21/5 19/4 19/4 | 38  |
|                   |      |          |                       |               |                  | 45/5 50/0      | 9 9 9 9 |     |
|                   |      |          |                       |               |                  |                |     |
|                   |      |          |                       |               | 90/3 45/5        |                |     |
| Ghotaslou         | 2013 | Tabriz   | 62                    | 11            | NA               | 47             | NA     | 47/8 47/7 14/2 14/2 14/2 14/2 14/2 14/2 | 39  |
|                   |      |          |                       |               |                  | 48/2 47/9      | 4 4 4 4 4 4 4 4 |     |
|                   |      |          |                       |               |                  |                |     |
|                   |      |          |                       |               | 79/3 79/3        |                |     |
| Khodaei           | 2010 | Tehran   | 73                    | 14            | 41.4 ± 6.99/5.8  | 56             | 57/9 16/4 32/4 41/9 16/2 37/6 6/14 14/3 | 40  |
|                   |      |          |                       |               |                  | 57/9 16/4      | 3 7 3 9 3 4 4 3 |     |
|                   |      |          |                       |               |                  |                |     |
|                   |      |          |                       |               |                   |                |     |
|                   |      |          |                       |               |                   |                |     |
|                   |      |          |                       |               |                   |                |     |
|                   |      |          |                       |               |                   |                |     |
| Dabiri            | 2009 | Tehran   | 13                    | 12            | 44.6             | 6/8 5/24 6/22 7/1 2/15 6/35 4/7 1/17 | 41  |
|                   |      |          |                       |               |                  | 65/5 48/5      | 9 9 9 9 9 9 9 9 |     |
|                   |      |          |                       |               |                  |                |     |
| First Author | Year | City       | Peptic ulcer isolates | H. pylori Female/Male | Age | cagA | VacA genotypes | Ref |
|--------------|------|------------|-----------------------|-----------------------|-----|-----|----------------|-----|
| Khodaii      | 2013 | Tehran     | 83                    | 15/7                  | 41  | 56  | s1: 57/9/7; s2: 16/4/3; m: 32/4/7; m1: 41/9/3; m2: 16/2/9; s1 m1: 37/6/4; s2 m1: 14/3/3 | 42  |
| Rezaeian     | 2012 | Jahrom     | 38                    | 16/4                  | 47  | 34  | NA/NA/NA/NA/18/6/3; NA/NA/NA/12/7/3; NA/NA/NA/1/6/7/22 | 43  |
| Sedaghvat    | 2014 | Kashan     | 8                     | 37                    | 44  | 4   | s1: 4/20; s2: 3/13; m: 1/9; m1: 6/23; m2: 0/6; s1 m1: 4/15; s2 m1: 1/2; s2 m2: 2/8 | 44  |
| Rafaeey      | 2013 | Tabriz     | 4                     | 33                    | 8.2 | 2   | 8 | NA | NA/NA/NA/NA | 45  |
| Souodd       | 2013 | Jahrom     | 38                    | 20/1                  | 47  | 34  | s1: 15/1/35; s2: 8/29/7; m: 19/6/7; m1: 19/9/7; m2: 23/1/08; s1 m1: 10/7/3; s2 m1: 1/6; s2 m2: 7/22 | 46  |
| Pajavand     | 2015 | Kermanshah | 20                    | 96                    | 46  | NA  | s1: 19/4/7; s2: 1/49/10; m: 17/8/6; m1: NA/3/8; m2: NA/1/47 | 47  |
| Jafari       | 2008 | Tehran     | 19                    | 96                    | 48  | ±1  | 15 | 10/6/8; 4/30/7; 13/5/9; 2/22/7; 2/40/2; 2/8/6 | 48  |
2.3. Data analysis

In the present meta-analysis, we estimated abundance of each vacA genotypes in Iranian patients with peptic ulcer. Possible relationship between each vacA genotypes and development of peptic ulcer was measured by Odds Ratio (OR) with 95% CIs (17). Statistical analysis was done by Comprehensive Meta-Analysis version 2.0 software (Biostat, Englewood, NJ, USA). In this regards, we used from fixed-effects model for analysis of data with low heterogeneity, while for analysis of data with high heterogeneity ($I^2$ statistic index > 25%, Cochrane Q statistic $p$ value < 0.05), random-effects model was used. On the other hand, for estimation of publication bias, the Egger's regression model was employed.

3. Results

Following initial searches, 155 articles was received from various databases. Finally, after study of titles, abstracts, and conformity with eligible criteria, 23 articles met inclusion criteria and were analyzed in present study. Studies were done during 2003–2019, and from Tehran (43.4%), Tabriz (13%), Shiraz and Jahrom (8.6%), and Shahrekord, Kerman, Kermanshah, Rasht, Isfahan, and Hamadan (each, one study) cities (Table 1).
In the present meta-analysis, information of 3328 patients was evaluated which of them, about 55.05% were men, and about 44.95% of them were women; average age of studied population was about 41.1 ± 2. Among all cultured samples, *H. pylori* was isolated from 3004 (90.26%) cultivated biopsies, and also 1120 (33.65%) cases had peptic ulcer. The result of cultured samples of other patients (324 cases) with peptic ulcer was negative. Peptic ulcer in patients with negative culture could be due to administration of nonsteroidal anti-inflammatory drugs (NSAIDs), and or non-growth of this fastidious bacterium on the culture media. In addition, among of patients with peptic ulcers, frequency of duodenal ulcer cases was more than gastric ulcer ones. Abundance of each of s1, s2, m1, m2, s1m1, s1m2, s2m1, and s2m2 was estimated 36.24%, 28.32%, 42.90% 29.86%, 27.88%, 15.70%, and 25.94%, respectively. Regarding this, it was demonstrated that vacA genotypes such as m1, s1, and s1m2 were the most prevalent vacA alleles among the Iranian patients with peptic ulcer. Finally, based on statistical analysis estimations, a significant relationship was observed between infections by m1, s1m1, and s2m1 alleles and development to peptic ulcer (OR 1.36, 1.24 and 4.82 respectively) in Iranian patients (Figs. 2, 3, and 4).

Full details of statistical analysis for relationship between each of vacA genotypes and peptic ulcer in Iranian population is listed in Table 2.

| vacA genotypes | Odds Ratio | p value | Heterogeneity | Egger’s regression |
|----------------|------------|---------|---------------|---------------------|
|                | 95% CIs    |         | Q-value       | I²-squared          |
| s1             | 0.35; 0.28–0.44 | 0.00   | 69.13         | 75.40               | 0.12 |
| s2             | 1.21; 0.86–1.62 | 0.20   | 30.42         | 53.98               | 0.23 |
| m1             | 1.36; 1.03–1.80 | 0.026  | 62.68         | 72.88               | 0.02 |
| m2             | 0.42; 0.34–0.53 | 0.00   | 97.55         | 81.54               | 0.05 |
| s1m1           | 1.33; 1.00–1.76 | 0.046  | 52.54         | 65.74               | 0.46 |
| s1m2           | 0.73; 0.60–0.90 | 0.003  | 58.24         | 69.09               | 0.02 |
| s2m1           | 4.81; 2.82–8.20 | 0.00   | 8.48          | 0.00                | 0.53 |
| s2m2           | 1.28; 0.94–1.72 | 0.10   | 25.40         | 37.02               | 0.03 |

Furthermore, frequency of coexistence of vacA and cagA genotypes in patients with peptic ulcer was evaluated about 33.35%. We found a meaningful relationship between infection with vacA and cagA
positive strains of *H. pylori* and development to peptic ulcer (OR: 1.63, 1.39–1.91; *Q*-value: 12.15; $I^2$: 0.00; *p* value: 0.00 and Egger’s regression: 0.53) (Fig. 5). In addition, frequency of *cagA* gene in s1, s2, m1, m2, s1m1, s1m2, s2m1 and s2m2 genotypes was estimated 46.08%, 11.14%, 12.34%, 35.24%, 20.18%, 50%, 6.62%, and 15.96%, respectively. Thus, s1m2, s1, and m2 genotypes were the most prevalent genotypes which harboring *cagA* gene, respectively. However, due to limited information, we could not evaluate the frequency of *cagA* gene in each of *vacA* genotypes isolated from Iranian patients with peptic ulcer.

### 4. Discussion

In the present study, we estimated the frequency of peptic ulcer about 33% in Iranian patients infected with *H. pylori*, which despite of higher prevalence than global average, but confirms previous studies from Iran. Perhaps this phenomenon is to be due to some factors such as genetic properties of Iranian population, life style, and characteristics of circulating strains in Iran (6–7). Among the patient possessed peptic ulcer, frequency of duodenal ulcer was more than gastric ulcer, and also, the majority of patients were male. Further, like previous studies, age average of studied cases was measured about 41 years old (12, 52). Regarding the present results, *vacA* genotypes m1, s1, and s1m2 were the three most prevalent isolated genotypes from Iranian patients involved with peptic ulcer. As well as, we demonstrated that there is a significant relationship between infection by strains containing m1, s1m1, and s2m1 genotypes and progression to peptic ulcer. Besides, in this meta-analysis, frequency of strains containing coexistence of *vacA* and *cagA* genes in peptic ulcer patients was assessed about 33.35%. We showed that there is a meaningful relationship between infections by *cagA/vacA* positive strains and development to peptic ulcer. *H. pylori* possesses some unique characteristics which cause to persist of bacterial infection in acidic condition of stomach and also evading from immune system (53). The colonization by this bacterium is different in various regions worldwide; for example in Iran, 90% of population are infected with *H. pylori* (54). Nevertheless, most of infected people remain as an asymptomatic carriers throughout the life of themselves; peptic ulcer and gastric cancer happen in 10–15% and 2% of infected cases, respectively (54–55). Therefore, it seems that host genetic properties and pathogenicity power of *H. pylori* strains are as two determining factors in the onset of disease and final outcomes (55–56). According to review of the literature, global prevalence of peptic ulcer has been estimated about 10%, and this bacterium isolated from 90–100% and 60–90% cases of duodenal ulcer and gastric ulcer, respectively (55). Nonetheless, frequency of peptic ulcer in Iran is much more than world average, which is related to host genetic characteristics and virulence factors of bacterium (6, 55). Both surface antigens and cytotoxic enzymes such as VacA and CagA are accounted as the two main virulence factors of *H. pylori* (14, 57). Based on previous meta-analysis, some virulence factors of bacterium e.g. OipA, BabA, DupA, IceA, CagA, and VacA are related to progress to peptic ulcer disease (53, 57–64). Also, it seems that type of colonization can be effective in formation of peptic ulcer, in general, duodenal ulcer is create following antral colonization, but gastric ulcer is the result of corporal and pan-gastritis (55, 56). Although *vacA* gene is present in all *H. pylori* strains, but its functional protein, VacA toxin, expressed in only 50% of those. The VacA protein forms a channel in membrane of bacterium, which be able to uptake of different ions and metabolites to the inside the cytoplasm, and causes to
survival of bacterium in stomach mucosal layer. Endocytosis of VacA into the host cell leads to some events such as vacuoles formation, releasing cytochrome c from mitochondria, and apoptosis. In addition, VacA toxin by impressing on different receptors leads to alteration in signaling pathways of MAPK/p38 and extracellular signal-regulated kinases 1 and 2 (ERK1/2) (18, 66–68). Functional weight of VacA toxin is about 88 kDa, and forms two subunits p33 and p55. The p33 domain which contains residues 1–33 in N-terminal region (as signal sequence) of VacA toxin, and creates vacuole in host cell (66, 69). On the other, p55 domain acts as binding domain of toxin to the cell surface (66). The length of \( \text{vacA} \) gene is 3860–3940 bp, and contains both conserved and variable regions. Nowadays, it has been cleared that the variable regions can be effective in variations of \( \text{vacA} \) gene expression, and directly are related to clinical outcomes of infection by \( h. \ pylori \) (66, 69). For example, McClain et al. in 2001 showed that the hydrophobic amino acids near the cleavage site of \( s2 \), could integrated the VacA toxin with host cell membrane (70). According to literature, \( \text{vacA} \) gene possesses variable sequences in \( s \) (\( s1 \) and \( s2 \)) and \( m \) (\( m1 \) and \( m2 \)) regions. It is notable that \( \text{vacA} \) \( s1m1 \) has the most expression rate, and therefore high vacuolating, but \( \text{vacA} \) \( s1m2 \) is a moderate vacuolating genotype, as well as \( \text{vacA} \) \( s2m2 \) is not toxic, and finally, \( \text{vacA} \) \( s2m1 \) genotype is rare and non-toxic (71–74). Recently, two additional variable regions, \( i1/i2 \) and \( d1/d2 \), have recognized in \( m \) region, and also, each of \( s1 \) and \( m1 \) regions subdivided to different types such \( s1a, s1b, s1c, m1a, m1b \) and \( m1c \) (75–76). In the recent present, we showed that there is a significant relationship between infection by \( cagA \) positive \( H. \ pylori \) strains and peptic ulcer disease. Given that studies in this field, expression of \( cagA \) gene leads to increase of pathogenicity, and directly related to severity of diseases of bacterium (55). Our study confirmed previous studies (77–78). Moreover, we demonstrated that \( m1 \), \( s1m1 \), and \( s2m1 \) genotypes have direct correlation with peptic ulcer in Iranian population. But, due to limit information about the both \( d \) and \( i \) genotypes, we could not assessed the effect of these genotypes on development of infection to peptic ulcer. In 2014, Basiri et al. showed that the infection by \( d1 \) genotype of \( \text{vacA} \) gene raises the risk of primary infection towards gastric adenocarcinoma and peptic ulcer in Northwestern of Iran (79). In another study in 2014, Mottaghi et al. studied on correlation between infection by \( i1 \) allele and development of infection into the gastric cancer and peptic ulcer in Azerbaijan, Iran; they found that \( \text{vacA} \) \( i1 \) genotype is significantly related to gastric cancer, however in their study, they did not find a meaningful relationship between infection by \( \text{vacA} \) \( i1/2 \) alleles and peptic ulcer gastric cancer diseases (80). According to various European studies, it has been demonstrated that there is a significant correlation between \( \text{vacA} \) genotypes of \( s1 \) and \( m1 \) with \( H. \ pylori \)-related gastrointestinal diseases (71, 76, 81–84). It is notable that due to decrease or absence of vacuolating activity, \( s2 \) and \( m2 \) genotypes rarely are related to peptic ulcer (76). In our analysis, we observed a similar correlation about frequency of \( s1 \) and \( m1 \) alleles in patients involved by peptic ulcer with other studies, which is due to some properties of these bacterial strains such as increased binding capacity, vacuolating activity, and alternation in normal signaling pathway (71). In addition, it is known that the origin of Iranian circulating strains is like to Western countries, in that, in 2010 Latifi-Navid et al. proved that the origin of Iranian strains is belonging to European \( H. \ pylori \) (hpEurope) strains. It seems that following migration of European to Iran, the hpEurope strains have been transferred to Iran, and this phenomenon can be effective in similarity of results of both our studies and Western countries (85). Overall, most recent studies have confirmed an intimate relationship between infection by \( s1m1 \) strains...
and progression to gastrointestinal diseases (76, 86–88). In 2005, Martins et al. represented that a significant relevance between colonization by vacA genotype s1m1 and peptic ulcer in Brazilian population (89). Likewise, several separate studies have confirmed relationship between H. pylori vacA s1m1 infection and peptic ulcer (87–90). Based on our results, s1, m1, and s1m2 were proposed as the most prevalent genotypes in peptic ulcer disease. In a study that was done by Sugimoto et al., in 2009, they demonstrated that the frequency of s1 and m1 genotypes in Middle East patients is more than 50%; they found that s1m1 and s2m1 were the most and lowest common genotypes in Middle East regions, respectively, which in turn their results were according to our results (91). While, based on Sugimoto et al. study, s1, m1, and s1m1 genotypes were related to peptic ulcer, but in our findings, m1, s1m1, and s2m1 were accounted as risk factor for peptic ulcer. It may be due to difference in distribution of patients; we only studies on Iranian patients’ samples (91). We declared that there is a direct association between vacA s2m1 with peptic ulcer in Iranian patients; this is while, it seems that the strains which harboring s2m1 are non-toxic, or low capacity of vacuolation, and this finding was challenging (92). Although due to limit information, we could not evaluate the presence of other virulence factors in s2m1 strains, it seems that these strains possess cagA gene or other required virulence factors for development to peptic ulcer. However, isolated strains from patients involved to peptic ulcer and gastric cancer in some regions such as Mexico, Latin America, Africa, and Western countries were contain vacA s2m1 genotype (83–84, 93). In the same year, Sugimoto et al. demonstrated that abundance of vacA s2m1 in Mexican population is about 12.2% (94). Furthermore, Zhang et al. displayed that infection by vacA s2m1 genotype and duodenal ulcer are significantly related with each other (OR: 2.30; 95% CIs: 1.17–4.50) (87). Yet, it needed to more study about the effect of vacA s2m1 strains on creation of peptic ulcer. The limitations of our study were including: 1) limited information of patients; 2) spatial constraints, so that most of the studies were conducted in Tehran; 3) limited information of vacA d and i alleles; 4) limited information of cagA and other bacterial virulence factors; 5) publication bias in some studies. Anyway, we showed that there is a significant relationship between vacA genotypes m1, s1m1, and s2m1, and development of infection to peptic ulcer disease in Iranian population.

Abbreviations

*Helicobacter pylori (H. pylori)*

Ministry of Health and Medical Education (MOHME)

Non-steroidal anti-inflammatory drugs (NSAIDs)

International Agency for Research on Cancer (IARC)

Mucosa-associated lymphoid tissue lymphoma (MALT)

Cytotoxin-associated gene A (CagA)

Vacuolating cytotoxin A (VacA)
Mitogen-activated protein kinases (MAPKs)

Declarations

Compliance with Ethical Standards

• Finding:
We have not received any funding for this research.

• Conflict of Interest:
There is no any conflict of interest among the all authors.

• Ethical approval:
Because this paper is provided based on research in global databases such as Scopus, PubMed, and Web of Science, so it was not necessary for receiving of ethical approval.

• Informed Consent:
All authors have informed consent about the content of this paper.

• Availability of data and material
All data will be available for anyone who requests those

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References

1. Chan FK, Leung WK. Peptic-ulcer disease. The Lancet. 2002 Sep 21;360(9337):933 – 41.
2. Keikha M, Eslami M, Yousefi B, Ghasemian A, Karbalaei M. Potential antigen candidates for subunit vaccine development against Helicobacter pylori infection. Journal of cellular physiology. 2019 Dec;234(12):21460 – 70.3. Yuan Y, Padol IT, Hunt RH. Peptic ulcer disease today. Nature Clinical Practice Gastroenterology & Hepatology. 2006 Feb;3(2):80 – 9.
3. Charpignon C, Lesgourgues B, Pariente A, Nahon S, Pelaquier A, Gatineau-Sailliant G, Roucayrol AM, Courillon-Mallet A, Group de l’Observatoire National des Ulcères de l'Association Nationale des HépatoGastroentérologues des Hôpitaux Généraux (ANGH). Peptic ulcer disease: one in five is
related to neither Helicobacter pylori nor aspirin/NSAID intake. Alimentary pharmacology & therapeutics. 2013 Oct;38(8):946 – 54.

4. Molloy RM, Sonnenberg A. Relation between gastric cancer and previous peptic ulcer disease. Gut. 1997 Feb 1;40(2):247 – 52.

5. Sayehmiri K, Tavan H. Systematic review and meta-analysis methods prevalence of peptic ulcer in IRAN. Journal of Govaresh. 2015 Nov;20(4):250–8.

6. Sayehmiri K, Abangah G, Kalvandi G, Tavan H, Aazami S. Prevalence of peptic ulcer in Iran: Systematic review and meta-analysis methods. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2018;23.

7. Sung JJ, Kuipers EJ, El-Serag HB. Systematic review: the global incidence and prevalence of peptic ulcer disease. Aliment Pharmacol Ther. 2009 May;29(9):938–46.

8. Cullen DJ, Hawkey GM, Greenwood DC, Humphreys H, Shepherd V, Logan RF, Hawkey CJ. Peptic ulcer bleeding in the elderly: relative roles of Helicobacter pylori and non-steroidal anti-inflammatory drugs. Gut. 1997 Oct 1;41(4):459 – 62.

9. Ford AC, Marwaha A, Sood R, Moayyedi P. Global prevalence of, and risk factors for, uninvestigated dyspepsia: a meta-analysis. Gut. 2015 Jul 1;64(7):1049-57.

10. Tanih NF, Okeleye BI, Ndip IM, Clarke AM, Naidoo N, Mkwetshana N, Green E, Ndip RN. Helicobacter pylori prevalence in dyspeptic patients in the Eastern Cape Province–race and disease status. South African Medical Journal. 2010;100(11):734–7.

11. Huang JQ, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. The Lancet. 2002 Jan;5(9300):14–22. 359(.

12. Papatheodoridis GV, Sougioultzis S, Archimandritis AJ. Effects of Helicobacter pylori and nonsteroidal anti-inflammatory drugs on peptic ulcer disease: a systematic review. Clinical Gastroenterology and Hepatology. 2006 Feb 1;4(2):130 – 42.

13. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clinical microbiology reviews. 2006 Jul 1;19(3):449 – 90.

14. Yousefi B, Mohammadalou M, Abdollahi M, Salek Farrokhi A, Karbalaei M, Keikha M, Kokhaei P, Valizadeh S, Rezaianesh A, Arabkari V, Eslami M. Epigenetic changes in gastric cancer induction by Helicobacter pylori. Journal of cellular physiology. 2019 Dec;234(12):21770–84.

15. Song H, Michel A, Nyrén O, Ekström AM, Pawlita M, Ye W. A CagA-independent cluster of antigens related to the risk of noncardia gastric cancer: associations between Helicobacter pylori antibodies and gastric adenocarcinoma explored by multiplex serology. International journal of cancer. 2014 Jun 15;134(12):2942–50.

16. Youssefi M, Ghazvini K, Farsiani H, Tafaghoudi M, Keikha M. A systematic review and meta-analysis of outcomes of infection with Helicobacter pylori dupA + strains in Iranian patients. Gene Reports. 2020 Mar;14:100650.
17. Cover TL. The vacuolating cytotoxin of Helicobacter pylori. Molecular microbiology. 1996 Apr;20(2):241–6.

18. Safaralizadeh R, Dastmalchi N, Hosseinpourfeizi M, Latifi-Navid S. Helicobacter pylori virulence factors in relation to gastrointestinal diseases in Iran. Microb Pathog. 2017 Apr;1:105:211–7.

19. Hosseini E, Poursina F, Van de Wiele T, Safaei HG, Adibi P. Helicobacter pylori in Iran: A systematic review on the association of genotypes and gastroduodenal diseases. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2012 Mar;17(3):280.

20. Cover TL, Blanke SR. Helicobacter pylori VacA, a paradigm for toxin multifunctionality. Nat Rev Microbiol. 2005 Apr;3(4):320–32.

21. Matos JI, de Sousa HA, Marcos-Pinto R, Dinis-Ribeiro M Helicobacter pylori CagA and VacA genotypes and gastric phenotype: a meta-analysis. European journal of gastroenterology & hepatology. 2013 Dec 1;25(12):1431-41.

22. Ghotaslou R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A. Risk of gastric cancer in association with Helicobacter pylori different virulence factors: A systematic review and meta-analysis. Microbial pathogenesis. 2018 May 1;118:214-9.

23. PERNG CL, LIN HJ, SUN FACG. IC, TSENG GY. Helicobacter pylori cagA, iceA and vacA status in Taiwanese patients with peptic ulcer and gastritis. Journal of gastroenterology hepatology. 2003 Nov;18(11):1244–9.

24. Karlsson A, Ryberg A, Dehnoei MN, Borch K, Monstein HJ. Association between cagA and vacA genotypes and pathogenesis in a Helicobacter pylori infected population from South-eastern Sweden. BMC microbiology. 2012 Dec;12(1):129.

25. Jafari F, Shokrzadeh L, Dabiri H, Baghaei K, Yamaoka Y, Zojaji H, Haghazali M, Molaei M, Zali MR. vacA genotypes of Helicobacter pylori in relation to cagA status and clinical outcomes in Iranian populations. Japanese journal of infectious diseases. 2008 Jul;61(4):290.

26. Vaziri F, Peerayeh SN, Alebouyeh M, Mirzaei T, Yamaoka Y, Molaei M, Maghsoudi N, Zali MR. Diversity of Helicobacter pylori genotypes in Iranian patients with different gastroduodenal disorders. World Journal of Gastroenterology: WJG. 2013 Sep;14(34):5685. 19.

27. Dabiri H, Jafari F, Baghaei K, Shokrzadeh L, Abdi S, Pourhoseingholi MA, Mohammadzadeh A. Prevalence of Helicobacter pylori vacA, cagA, cagE, oipA, iceA, babA2 and babB genotypes in Iranian dyspeptic patients. Microbial pathogenesis. 2017 Apr 1;105:226 – 30.

28. Salari MH, Shirazi MH, Hadaiti MA, Daryani NA. Frequency of Helicobacter pylori vacA genotypes in Iranian patients with gastric and duodenal ulcer. Journal of infection and public health. 2009 Jan 1;2(4):204-8.

29. Salehi Z, Akhshabi F, Talachian E. Prevalence of vacA and cagA Genotypes of Helicobacter pylori in Iranian Children with Peptic Ulcer Disease. WASJ. 2011;12(6):840–4.

30. Doosti A, Ghasemi-Dehkordi P. Helicobacter pylori vacA genotypes in Shahrekordian (Iran) H. pylori-positive patients. Res j Biol Sci. 2009;4(1):11–5.
31. Nahaei MR, Sharifi Y, Akhi MT, Asgharzadeh M, Nahaei M, Fatahi E. Helicobacter pylori caga and vaca genotypes and their relationships to peptic ulcer disease and non-ulcer dyspepsia. Res J Microbiol. 2008;3(5):386–94.
32. Douraghi M, SABERI KS, Shokrgoazar MA, OGHALAEI A, ESMAEILI M, BABABEYK M, Shirazi MH, Mohagheghi MA, Mohammadi M. Characterization of the vacuolating cytotoxin in Helicobacter pylori strains isolated from Iran.
33. Alikhani MY, Arebestani MR, Khorasani MS, Majlesi A, Jaefari M. Evaluation of Helicobacter pylori vacA and cagA genotypes and correlation with clinical outcome in patients with dyspepsia in hamadan province, Iran. Iranian Red Crescent Medical Journal. 2014 Nov;16(11).
34. KAMALI SE, Farsiani H, Shamoon PM, Bazargani A, BAGHERI LK, Taghani AR. SABERI FM. Association of myeloperoxidase-463 G/A polymorphism with clinical outcome of Helicobacter pylori infection in Iranian patients with gastrointestinal diseases.
35. Salehi Z, Abadi AS, Ismail PB, Kqueen CY, Jelodar MH, Kamalidehghan B. Evaluation of Helicobacter pylori vacA Genotypes in Iranian Patients with Peptic Ulcer Disease. Digestive diseases and sciences. 2009 Nov 1;54(11):2399.
36. Abdollahi H, Hashemzadeh M, Khoshnood S, Savari M. Characterization of Helicobacter pylori genotypes from Iranian patients with gastric clinical diseases: Predominance of vacA s1a and cagA EPIYA-ABC genotypes. Gene Reports. 2019 Sep;1:16:100458.
37. Havaei SA, Mohajeri P, Khashei R, Salehi R, Tavakoli H. Prevalence of Helicobacter pylori vacA different genotypes in Isfahan, Iran. Advanced biomedical research. 2014;3.
38. Ghotaslou R, Milani M, Akhi MT, Nahaei MR, Hasani A, Hejazi MS, Meshkini M. Diversity of Helicobacter pylori cagA and vacA genes and its relationship with clinical outcomes in Azerbaijan, Iran. Advanced pharmaceutical bulletin. 2013;3(1):57.
39. Khodaii Z, Ghaderian SM, Najar RA, Nejati H, Panah AT. cagA and vacA status and influence of Helicobacter pylori infection on serum oxidative DNA damage in Iranian patients with peptic ulcer disease. Irish journal of medical science. 2011 Mar 1;180(1):155 – 61.
40. Dabiri H, Maleknejad P, Yamaoka Y, Feizabadi MM, Jafari F, Rezadehbashi M, Nakhjavani FA, Mirsalehian A, Zali MR. Distribution of Helicobacter pylori cagA, cagE, oipA and vacA in different major ethnic groups in Tehran, Iran. Journal of gastroenterology hepatology. 2009 Aug;24(8):1380–6.
41. Evaluation of the prevalence of VacA and CagA in patients with peptic ulcer. Scientific-Research. Journal of Shahed University Twenteeth Year, No.104 April-May,2013.
42. Rezaeian AA, Kargar M, Souod N, Ghorbani Dalini S. Genetic Polymorphisms of CagA and VacA Genes in Helicobacter Pylori Isolates from Chaharmahal and Bakhtiari Province, Iran. Journal of Isfahan Medical School. 2012 Sep 17;30(197).
44. Sedaghat H, Moniri R, Jamali R, Arj A, Zadeh MR, Moosavi SG, Rezaei M. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA, babA2, and oipA genotypes in patients with upper gastrointestinal diseases. Iranian journal of microbiology. 2014 Feb;6(1):14.

45. Rafeey M, Ghotaslou R, Milani M, Farokhi N, Ghojazadeh M. Association between Helicobacter pylori, cagA, and vacA status and clinical presentation in Iranian children. Iranian journal of pediatrics. 2013 Oct;23(5):551.

46. Souod N, Kargar M, Doosti A, Ranjbar R, Sarshar M. Genetic analysis of cagA and vacA genes in Helicobacter pylori isolates and their relationship with gastroduodenal diseases in the west of Iran. Iranian Red Crescent Medical Journal. 2013 May;15(5):371.

47. Pajavand H, Alvandi A, Mohajeri P, Bakhtyari S, Bashiri H, Kalali B, Gerhard M, Najafi F, Abiri R. High frequency of vacA s1m2 genotypes among Helicobacter pylori isolates from patients with gastroduodenal disorders in Kermanshah, Iran. Jundishapur journal of microbiology. 2015 Nov;8(11).

48. Jafari F, Shokrzadeh L, Dabiri H, Baghaei K, Yamaoka Y, Zojaji H, Haghazali M, Molaei M, Zali MR. vacA genotypes of Helicobacter pylori in relation to cagA status and clinical outcomes in Iranian populations. Japanese journal of infectious diseases. 2008 Jul;61(4):290.

49. Mohammadi M, Oghalaie A, Mohajerani N, Massarrat S, Nasiri M, Bennedsen M, Colding H, Andersen LP. Prevalence of Helicobacter pylori vacuolating cytotoxin and its allelic mosaicism as a predictive marker for Iranian dyspeptic patients. Bulletin de la Societe de pathologie exotique (1990). 2003 Mar;96(1):3–5.

50. Falsa T, Khani A, Mahjoub F, Asgarani E, Sotoudeh N. Analysis of vacA/cagA genotypes/status in Helicobacter pylori isolates from Iranian children and their association with clinical outcome. Turkish journal of medical sciences. 2015 Jan 27;45(1):170-7.

51. Kamali-Sarvestani E, Bazargani A, Masoudian M, Lankarani K, Taghavi AR, Saberifiroozi M. Association of H pylori cagA and vacA genotypes and IL-8 gene polymorphisms with clinical outcome of infection in Iranian patients with gastrointestinal diseases. World journal of gastroenterology: WJG. 2006 Aug 28;12(32):5205.

52. Zamani M, Ebrahimtabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, Derakhshan MH. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. Alimentary pharmacology & therapeutics. 2018 Apr;47(7):868 – 76.

53. Yamaoka Y, Miftahussurur M. Helicobacter pylori virulence genes and host genetic polymorphisms as risk factors for peptic ulcer disease. Expert Rev Gastroenterol Hepatol. 2015;9(12):1535.

54. Massarrat S, Saberi-Firoozi M, Soleimani A, Himmelmann GW, Hitzges M, Keshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. Eur J Gastroenterol Hepatol. 1995 May;7(5):427–33.

55. Miftahussurur M, Yamaoka Y. Helicobacter pylori virulence genes and host genetic polymorphisms as risk factors for peptic ulcer disease. Expert review of gastroenterology & hepatology. 2015 Dec 2;9(12):1535-47.
56. Lee AD, Fox J, Hazell S. Pathogenicity of Helicobacter pylori: a perspective. Infect Immun. 1993 May;61(5):1601.
57. Ghotaslou R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A. Risk of gastric cancer in association with Helicobacter pylori different virulence factors: A systematic review and meta-analysis. Microbial pathogenesis. 2018 May 1;118:214-9.
58. Liu J, He C, Chen M, Wang Z, Xing C, Yuan Y. Association of presence/absence and on/off patterns of Helicobacter pylori oipA gene with peptic ulcer disease and gastric cancer risks: a meta-analysis. BMC infectious diseases. 2013 Dec 1;13(1):555.
59. Chen MY, He CY, Meng X, Yuan Y. Association of Helicobacter pylori babA2 with peptic ulcer disease and gastric cancer. World journal of gastroenterology: WJG. 2013 Jul;19(26):4242. 19(.
60. Hussein NR. The association of dupA and Helicobacter pylori-related gastroduodenal diseases. European journal of clinical microbiology & infectious diseases. 2010 Jul 1;29(7):817 – 21.
61. Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R, Yamaoka Y. Helicobacter pylori iceA, clinical outcomes, and correlation with cagA: a meta-analysis. PloS one. 2012;7(1).
62. Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H, Furuta T, Yamaoka Y. Role of Helicobacter pylori cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC Infect Dis. 2012 Dec;12(1):223.
63. Matsunari O, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, Fujioka T, Kinjo F, Yamaoka Y. Association between Helicobacter pylori virulence factors and gastroduodenal diseases in Okinawa, Japan. Journal of clinical microbiology. 2012 Mar 1;50(3):876 – 83.
64. Yordanov D, Boyanova L, Markovska R, Gergova G, Mitov I. Significance of Helicobacter pylori vacA intermediate region genotyping—a Bulgarian study. Diagnostic microbiology and infectious disease. 2012 Nov 1;74(3):253-7.
65. Meining A, Kiel G, Stolte M. Changes in Helicobacter pylori-induced gastritis in the antrum and corpus during and after 12 months of treatment with ranitidine and lansoprazole in patients with duodenal ulcer disease. Alimentary Pharmacology and Therapeutics. 1998 Aug 1;12(8):735 – 40.
66. Fahimi F, Tohidkia MR, Fouladi M, Aghabeygi R, Samadi N, Omidi Y. Pleiotropic cytotoxicity of VacA toxin in host cells and its impact on immunotherapy. BioImpacts: BI. 2017;7(1):59.
67. Boquet P, Ricci V, Galmiche A, Gauthier NC. Gastric cell apoptosis and H. pylori: has the main function of VacA finally been identified?. Trends in microbiology. 2003 Sep 1;11(9):410-3.
68. Arents NL, Van Zwet AA, Thijs JC, Kooistra-Smid AM, Van Slochteren KR, Degener JE, Kleibeuker JH, Van Doorn LJ. The importance of vacA, cagA, and iceA genotypes of Helicobacter pylori infection in peptic ulcer disease and gastroesophageal reflux disease. The American journal of gastroenterology. 2001 Sep 1;96(9):2603-8.
69. Talebkhan Y, Mohammadi M. Vacuolating cytotoxin of Helicobacter pylori. Iranian Journal of Biotechnology. 2003 Apr 1;1(2):73–81.
70. McClain MS, Cao P, Iwamoto H, Vinion-Dubiel AD, Szabo G, Shao Z, Cover TL. A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin
activity and alters membrane channel formation. Journal of bacteriology. 2001 Nov 15;183(22):6499–508.

71. Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori association of specific vacA types with cytotoxin production and peptic ulceration. Journal of Biological Chemistry. 1995 Jul 28;270(30):17771–7.

72. Strobel S, Bereswill S, Balig P, Allgaier P, Sonntag HG, Kist M. Identification and Analysis of a New vacA Genotype Variant of Helicobacter pylori in Different Patient Groups in Germany. Journal of clinical microbiology. 1998 May 1;36(5):1285-9.

73. El-Shenawy A, Diab M, Shemis M, El-Ghannam M, Salem D, Abdelnasser M, Shahin M, Abdel-Hady M, El-Sherbini E, Saber M. Detection of Helicobacter pylori vacA, cagA and iceA1 virulence genes associated with gastric diseases in Egyptian patients. Egyptian Journal of Medical Human Genetics. 2017;18(4):365–71.

74. Pinto-Ribeiro I, Ferreira RM, Batalha S, Hlaing T, Wong SI, Carneiro F, Figueiredo C. Helicobacter pylori vacA genotypes in chronic gastritis and gastric carcinoma patients from Macau, China. Toxins. 2016 May;8(5):142.

75. Martins LC, Corvelo TC, Demachki S, Araujo MT, Assumpção MB, Vilar SC, Freitas FB, Barbosa HP, Fecury AA, Amaral RK, Santos SE. Clinical and pathological importance of vacA allele heterogeneity and cagA status in peptic ulcer disease in patients from North Brazil. Memórias do Instituto Oswaldo Cruz. 2005 Dec;100(8):875–81.

76. Sugimoto M, Yamaoka Y. The association of vacA genotype and Helicobacter pylori-related disease in Latin American and African populations. Clinical Microbiology and Infection. 2009 Sep 1;15(9):835 – 42.

77. Li Q, Liu J, Gong Y, Yuan Y. Association of CagA EPIYA-D or EPIYA-C phosphorylation sites with peptic ulcer and gastric cancer risks: a meta-analysis. Medicine. 2017 Apr;96(17).

78. Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R, Yamaoka Y. Helicobacter pylori iceA, clinical outcomes, and correlation with cagA: a meta-analysis. PloS one. 2012;7(1).

79. Basiri Z, Safaralizadeh R, Bonyadi MJ, Somi MH, Mahdavi M, Latifi-Navid S Helicobacter pylori vacA d1 genotype predicts risk of gastric adenocarcinoma and peptic ulcers in northwestern Iran. Asian Pac J Cancer Prev. 2014 Jan 1;15(4):1575-9.

80. Mottaghi B, Safaralizadeh R, Bonyadi M, Latifi-Navid S, Somi MH. Helicobacter pylori vacA i region polymorphism but not babA2 status associated to gastric cancer risk in northwestern Iran. Clinical and experimental medicine. 2016 Feb 1;16(1):57–63.

81. Yamaoka Y, Orito E, Mizokami M, Gutierrez O, Saitou N, Kodama T, Osato MS, Kim JG, Ramirez FC, Mahachai V, Graham DY. Helicobacter pylori in north and south america before columbus. FEBS letters. 2002 Apr 24;517(1–3):180–4.

82. Miehlke S, Kirsch C, Agha-Amiri K, Günther T, Leh N, Malfertheiner P, Stolte M, Ehninger G, Bayerdörffer E. The Helicobacter pylori vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. International journal of cancer. 2000 Aug 1;87(3):322-7.
83. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries. Journal of clinical microbiology. 1999 Jul 1;37(7):2274-9.
84. Van Doorn LJ, Figueiredo C, Méraud F, Pena S, Midolo P, Queiroz DM, Carneiro F, Vanderborght B, Maria Da Glória FP, Sanna R, De Boer W. Geographic distribution of vacA allelic types of Helicobacter pylori. Gastroenterology. 1999 Apr 1;116(4):823 – 30.
85. Latifi-Navid S, Ghorashi SA, Siavoshi F, Linz B, Massarrat S, Khegay T, Salmanian AH, Shayesteh AA, Masoodi M, Ghanadi K, Ganji A. Ethnic and geographic differentiation of Helicobacter pylori within Iran. PloS one. 2010;5(3).
86. Ghotoslou R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A. Risk of gastric cancer in association with Helicobacter pylori different virulence factors: A systematic review and meta-analysis. Microbial pathogenesis. 2018 May 1;118:214-9.
87. Zhang BB, Li Y, Liu XQ, Wang PJ, Yang B, Bian DL. Association between vacA genotypes and the risk of duodenal ulcer: a meta-analysis. Molecular biology reports. 2014 Nov 1;41(11):7241-54.
88. Román-Román A, Martínez-Carrillo DN, Atrisco-Morales J, Azúcar-Heziquio JC, Cuevas-Caballero AS, Castañón-Sánchez CA, Reyes-Ríos R, Betancourt-Linares R, Reyes-Navarrete S, Cruz-del Carmen I, Camorlinga-Ponce M. Helicobacter pylori vacA s1m1 genotype but not cagA or babA2 increase the risk of ulcer and gastric cancer in patients from Southern Mexico. Gut pathogens. 2017 Dec 1;9(1):18.
89. Martins LC, Corvelo TC, Demachki S, Araujo MT, Assumpção MB, Vilar SC, Freitas FB, Barbosa HP, Fecury AA, Amaral RK, Santos SE. Clinical and pathological importance of vacA allele heterogeneity and cagA status in peptic ulcer disease in patients from North Brazil. Memórias do Instituto Oswaldo Cruz. 2005 Dec;100(8):875–81.
90. Karlsson A, Ryberg A, Dehnoei MN, Borch K, Monstein HJ. Association between cagA and vacA genotypes and pathogenesis in a Helicobacter pylori infected population from South-eastern Sweden. BMC microbiology. 2012 Dec;12(1):129.
91. Sugimoto M, Zali MR, Yamaoka Y. The association of vacA genotypes and Helicobacter pylori-related gastroduodenal diseases in the Middle East. European journal of clinical microbiology & infectious diseases. 2009 Oct 1;28(10):1227-36.
92. Chambers MG, Pyburn TM, González-Rivera C, Collier SE, Eli I, Yip CK, Takizawa Y, Lacy DB, Cover TL, Ohi MD. Structural analysis of the oligomeric states of Helicobacter pylori VacA toxin. Journal of molecular biology. 2013 Feb 8;425(3):524 – 35.
93. Letley DP, Lastovic A, Louw JA, Hawkey CJ, Atherton JC. Allelic Diversity of the Helicobacter pylori Vacuolating Cytotoxin Gene in South Africa: Rarity of thevacA s1a Genotype and Natural Occurrence of an s2/m1 Allele. Journal of clinical microbiology. 1999 Apr 1;37(4):1203-5.
94. Sugimoto M, Yamaoka Y. The association of vacA genotype and Helicobacter pylori-related disease in Latin American and African populations. Clinical Microbiology and Infection. 2009 Sep 1;15(9):835 – 42.
Figures

Figure 1

Flowchart of included and excluded articles.
Figure 2

Forrest plot of the vacA genotype m1. The association between vacA genotype m1 and development to peptic ulcer in Iranian populations.
Forrest plot of the vacA genotype s1m1. The association between vacA genotype s1m1 and development to peptic ulcer in Iranian populations.

| Study name  | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|-------------|------------|-------------|-------------|---------|---------|
| Dabiri1     | 1.769      | 0.408       | 7.671       | 0.762   | 0.446   |
| Salari      | 47.000     | 2.734       | 807.942     | 2.653   | 0.008   |
| Doosti      | 1.714      | 0.719       | 4.086       | 1.216   | 0.224   |
| Nahaei      | 0.172      | 0.042       | 0.709       | -2.437  | 0.015   |
| Douraghi    | 1.056      | 0.327       | 3.411       | 0.090   | 0.928   |
| Alikhani    | 2.700      | 0.704       | 10.355      | 1.448   | 0.148   |
| Sarvestani1 | 1.188      | 0.073       | 19.366      | 0.121   | 0.904   |
| Havaei      | 1.367      | 0.635       | 2.942       | 0.799   | 0.424   |
| Ghotaslou   | 10.000     | 3.204       | 31.208      | 3.965   | 0.000   |
| Khodai1     | 0.045      | 0.002       | 0.833       | -2.083  | 0.037   |
| Dabiri2     | 6.231      | 0.806       | 48.186      | 1.753   | 0.080   |
| Khodai2     | 1.692      | 0.638       | 4.486       | 1.058   | 0.290   |
| Rezaeian    | 0.024      | 0.001       | 0.436       | -2.521  | 0.012   |
| Rafeey      | 11.333     | 1.835       | 69.982      | 2.614   | 0.009   |
| Pajavand    | 1.000      | 0.258       | 3.737       | 0.000   | 1.000   |
| Jafari      | 1.350      | 0.175       | 10.419      | 0.288   | 0.773   |
| Mohammadi   | 0.933      | 0.359       | 2.429       | -0.141  | 0.888   |
| Falsafi     | 1.000      | 0.326       | 3.067       | 0.000   | 1.000   |
| Sarvestani2 | 0.667      | 0.323       | 1.377       | -1.096  | 0.273   |
|             | 1.331      | 1.005       | 1.761       | 1.998   | 0.036   |
**Figure 4**

Forrest plot of the vacA genotype s2m1. The association between vacA genotype s2m1 and development to peptic ulcer in Iranian populations.

| Study name | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|------------|------------|-------------|-------------|----------|---------|
| Dabiri1    | 3.200      | 0.954       | 10.733      | 1.884    | 0.060   |
| Nahaei     | 7.833      | 0.431       | 142.239     | 1.392    | 0.164   |
| Alkhani    | 8.333      | 0.838       | 82.858      | 1.809    | 0.070   |
| Ghotaslou  | 61.000     | 2.028       | 1835.172    | 2.367    | 0.018   |
| Khodai1    | 8.375      | 2.175       | 32.252      | 3.089    | 0.002   |
| Dabiri2    | 3.000      | 0.447       | 20.153      | 1.130    | 0.258   |
| Khodai2    | 9.625      | 2.506       | 36.964      | 3.298    | 0.001   |
| Rezaeian   | 7.400      | 0.397       | 137.879     | 1.341    | 0.180   |
| Sedaghhat  | 7.000      | 0.217       | 226.005     | 1.098    | 0.272   |
| Scouod     | 7.400      | 0.397       | 137.879     | 1.341    | 0.180   |
| Jafari     | 2.833      | 0.324       | 24.808      | 0.941    | 0.347   |
| Falsafi    | 4.819      | 2.829       | 8.209       | 5.787    | 0.000   |

0.01 0.1 1 10 100
| Study name        | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
|-------------------|------------|-------------|-------------|---------|---------|
| Dabiri1           | 1.576      | 0.859       | 2.895       | 1.468   | 0.142   |
| Salehi            | 2.294      | 1.132       | 4.650       | 2.304   | 0.021   |
| Nahaei            | 1.266      | 0.718       | 2.231       | 0.816   | 0.415   |
| Alikhani          | 1.208      | 0.616       | 2.370       | 0.551   | 0.582   |
| Sarvestani1       | 1.403      | 0.849       | 2.317       | 1.322   | 0.186   |
| Abdollahi         | 1.788      | 0.419       | 7.621       | 0.785   | 0.432   |
| Ghotaslou         | 2.125      | 1.168       | 3.807       | 2.534   | 0.011   |
| Khodaii1          | 2.000      | 1.185       | 3.377       | 2.594   | 0.009   |
| Dabiri2           | 1.619      | 0.490       | 5.350       | 0.790   | 0.429   |
| Khodaii2          | 2.000      | 1.185       | 3.377       | 2.594   | 0.009   |
| Rezaeian          | 1.111      | 0.649       | 1.901       | 0.385   | 0.701   |
| Sedaghat          | 1.737      | 0.389       | 7.756       | 0.723   | 0.470   |
| Rafeey            | 1.409      | 0.184       | 10.779      | 0.330   | 0.741   |
| Souod             | 1.111      | 0.649       | 1.901       | 0.385   | 0.701   |
| Jafari            | 1.397      | 0.633       | 3.081       | 0.827   | 0.408   |
| Sarvestani2       | 2.468      | 1.579       | 3.859       | 3.962   | 0.000   |
|                   | 1.631      | 1.392       | 1.912       | 6.037   | 0.000   |

**Figure 5**

Forrest plot of the association of coexistence vacA/cagA with development of disease to peptic ulcer in Iranian populations.