What is the Relationship Between Helicobacter Pylori CagA Genotypes and Gastrointestinal Diseases in the Iranian Population? A Systematic Review and Meta-Analysis

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) is one of the most well-known risk factors for getting the gastric cancer disease. In recent studies, the relationship between its virulence factors, specially CagA (cytotoxin-associated gene A) toxin and development into the gastrointestinal diseases is taken into consideration. According to review of literature, despite the presence of four motifs A, B, C, and D in CagA toxin, two motifs C and D are more associated with gastrointestinal complications in patients who are infected by *H. pylori*.

Methods: In the present study, we researched about these ambiguities using a comprehensive meta-analysis study. In this study, we assessed the information of 1762 Iranian patients for potential relationship between all genotypes of *cagA* gene and gastrointestinal diseases.

Results: According to statistical analysis, the abundance of *cagA* genotypes AB, ABC, ABCC, ABCCC, and ABD in Iranian population is 5.52%, 80.18%, 22.81%, 2.76%, and 0% respectively. In addition, it was determined that there is a significant relationship between *cagA* genotypes ABCC and ABCCC on the one hand and *cagA* genotype ABCCC on the other hand with susceptibility to chronic gastritis and gastric cancer respectively.

Conclusions: Overall, it can be concluded that the higher number of EPIYA-C copy numbers lead to the higher risk of gastric cancer. According to our results, it seems that the presence of EPIYA-ABCCC motif in strains of *H. pylori* should be considered as an appropriate marker in preventing the gastric cancer among the Iranian population.

1. Background

*Helicobacter pylori* (*H. pylori*) is a microaerophilic, spiral, and motile gram negative bacterium which is resident in human gastric submucosa (1). Creating a persistent infection is one of the main properties of this microorganism, so that in previous studies, it has been shown that *H. pylori* can survive for decades in the human stomach (1–2). This bacterium is the etiologic agent for gastrointestinal complications including acute gastritis, gastric ulcer, duodenal ulcer, and also gastric adenocarcinoma tumors and mucosa-associated lymphoid tissue (MALT) lymphoma (3). Based on the available evidence, *H. pylori* triggers a range of changes in stomach mucus from an acute inflammation to other problems such chronic gastritis, atrophy, intestinal metaplasia, dysplasia, and gastric adenocarcinoma (2–4). Regarding potency of *H. pylori* in creating gastric cancer, the International Agency for Research on Cancer (GLOBOCAN) introduced this pathogen as the human class I carcinogenic agent in 1994 (5). Gastric cancer is among the four global prevalent cancers, and also is accounted as the third deadliest cancer in the world; annually, approximately 700,000 patients die from this disease (6). The statistics in the developing countries, especially in Iran is more worrisome; the rate of infection by *H. pylori* in the Iranian population is about 90%, so that 22 deaths occur per day associated with gastric cancer in Iran (7). Furthermore, *H. pylori* and administration of nonsteroidal anti-inflammatory drugs (NSAIDs) are accounted as the two main predisposing risk factors for gastric cancer. In this regard, the studies show that more than 60% of patients who are colonized by *H. pylori* are affected to gastric cancer (8–9). While about the half of world population are infected by *H. pylori*, however the diseases associated by this bacterium happen in 15–20% of population. Due to the problems such as high colonization rate, poor prognosis of gastric cancer, re-infection, as well as difficult eradication of bacteria, unfortunately the patients are identified in the advanced stages of the disease and this phenomenon is along with high mortality (10–11). Based on various studies, bacterial strains have a lot of genetic diversity, and so the occurrence of different forms of infection such as gastritis, peptic ulcer, gastric adenocarcinoma, and MALT depend on different factors, especially virulence factors which express by different strains of bacterium (12–13). The encoding gene of CagA toxin (s120-140 kDa), *cagA* gene, is one of the most important genes in the *H. pylori* whole genome, and locates in cag pathogenicity islands (cag-PAIs). The presence and expression of *cagA* gene is related with gastric complications such gastritis, peptic ulcer, gastric polyps, precancerous status, cell survival and gastric adenocarcinoma (14–15). Studies in China, Japan, and South Korea showed that 90% of isolated strains from patients had *cagA* gene in their genome (16–20). Based on genomix studies, there are various genetic diversity of *cagA* gene among the different strains of *H. pylori*. The most difference in their open reading frame (ORF) is related to locus which encodes 1147–1181 amino acids, which in turn is associated to nucleotide sequences in the 3’ terminal of *cagA* gene (21–22). According to recent studies, upon the colonization of bacterium into the host stomach, CagA secreted inside the gastric cells via type IV secretory system. Then, this protein is phosphorylated by c-Src and c-Abl of host cell tyrosine kinases. Phosphorylated CagA binds to host proteins and blocks signaling pathway (21, 23–26). Based on surrounding sequences of the EPIYA motifs, CagA is subdivided to four classes, A, B, C, and D (27). While the most East Asian dominant strains are ABD, in the Western countries ABC is the dominant genotype (28–32). In addition, it has been suggested that the East Asian *cagA* genotype (ABD) is significantly associated with the
increased risk of gastric cancer (33–34). The main goal of this meta-analysis was evaluation of abundance EPIYA motifs, and potential association of prevalent genotypes with gastritis, peptic ulcer, and gastric cancer in Iranian population.

2. Methods

In the present systematic review and meta-analysis, all stages of research including literature search, selection criteria, quality assessment, data extraction, and statistical analysis was done according preferred items in the systematic review and meta-analysis guidelines (35).

2.1. Search strategy

To receive all reports about Iranian population associated EPIYA motifs, conducted studies till August 2020 were collected from PubMed, Scopus, Google Scholar, Magium, IranMedex, and ISC databases. Studies were research without language limitation and also we used some keywords such as Iran, \textit{cagA} gene, EPIYA, and \textit{Helicobacter pylori}.

2.2. Selection criteria

Identification of eligible articles (titles, abstracts, and full text) was done by two authors, separately. According to standards protocols, the inclusion criteria included Iranian patients, collected clinical specimens, original articles (cross-sectional, case-control, and cohort), EPIYA motifs (AB, ABC, ABCC, ABCCC, and ABD), and reliable material and methods. In addition, excluded studies included the articles with insufficient information, letter to editors, review articles, case reports, congress abstracts, repetitive samples, and duplicate articles.

2.3. Quality assessment and data extraction

The quality of studies was evaluated by Newcastle-Ottawa quality assessment scale criteria, and articles which received score \( \geq 5 \) were included in present meta-analysis (data not shown). Then, the process of data extraction from eligible studies was done by two authors, separately (Table 1). Extracted information included titles such as first author, publication year, city, numbers of patients, distribution of age and gender in each study, frequency of EPIYA motifs in each study, and diagnostic methods.
Table 1  
Characteristics of included studies

| First Author     | Year  | City     | Age         | No. patients | cagA genotypes | Diagnostic method | Ref |
|------------------|-------|----------|-------------|--------------|----------------|-------------------|-----|
| Shokrzadeh et al.| 2010  | Tehran   | 45.4 ± 1    | 190          | 3 86 3 0 0     | PCR + Sequencing   | 37  |
| Saberi et al.    | 2012  | Tehran   | NR          | 76           | 6 31 23 0 0    | PCR + Sequencing   | 38  |
| Ajami et al.     | 2013  | Sari     | 42.2 ± 3    | 250          | 125 39 54 32 0 | PCR + Sequencing   | 39  |
| Vaziri et al.    | 2013  | Tehran   | 66          | 71           | 44 0 30 4 1    | PCR + Sequencing   | 40  |
| Haddadi et al.   | 2014  | Shiraz   | 45          | 280          | 68 0 67 53 0   | PCR + Sequencing   | 41  |
| Honarmand et al. | 2015  | Tehran   | 33          | 168          | 168 0 157 1 9 0| PCR + Sequencing   | 42  |
| Yadegar et al.   | 2015  | Tehran   | 46          | 61           | 52 0 39 7 1    | PCR + Sequencing   | 43  |
| Farzi et al.     | 2018  | Tehran   | 47.9 ± 2    | 68           | 58 0 41 7 1    | PCR + Sequencing   | 44  |
| Sarrami et al.   | 2018  | Ardabil  | NR          | 206          | 22 0 79 7 2    | PCR + Sequencing   | 45  |
| Sheikh et al.    | 2018  | Ahvaz    | 71          | 201          | 134 0 66 2 0   | PCR + Sequencing   | 46  |
| Abdollahi et al. | 2019  | Kerman   | 47          | 191          | 39 0 46 0 3 0  | PCR + Sequencing   | 47  |

2.4. Statistical analysis

Available information was analyzed by comprehensive meta-analysis (CMA) version 2.0 software. First, heterogeneity of data was assessed by use of $I^2$ and Cochrane Q-test indexes, and then as regard to heterogeneity we used of random effects model. As well as, publication bias was evaluated by funnel plot. Finally, considering limitation of studies, potential relationship between each EPIYA motifs by gastritis, peptic ulcer, and gastric cancer was estimated by Odds Ratio (ORs) with 95% Confidence intervals (CIs) (36).

3. Results

In this study, from all 92 articles, 11 articles met the inclusion criteria (Fig. 1).

Among the included articles, ten was in English language, and 1 was Persian. The studies had been conducted from various cities such as Tehran, Ardebil, Sari, Kerman, Shiraz, and Ahvaz. In the present study, we evaluated the comprehensive information of 1762 patients. Approximately 51.2% of patients were men, and 48.8% were women, and also, mean age of them was 49.2 ± 5. Frequency of cagA gene in isolated strains from different areas of Iran was assessed around 71.34%. Based on statistical analysis, it was found that there is a significant relationship between cagA gene and susceptibility to gastrointestinal complications, especially gastric cancer (ORs: 2.77 with 95% CIs) and peptic ulcer (ORs: 1.05 with 95% CIs) diseases (Fig. 2).
In all include studies, EPIYA motifs was identified by PCR and DNA sequencing techniques. Based on studies, so far no reports has been reported of EPIYA motif D and cagA genotype ABD from Iran. However, in present study, the most prevalent EPIYA motifs, A, B, and C, and cagA genotypes, ABC and ABCC, were assessed. Geographical distribution of CagA genotypes in Iran has been noted in Fig. 3.

According to statistical analysis, abundance of cagA genotypes AB, ABC, ABCC, ABCCC, and ABD in Iranian population is 5.52%, 80.18%, 22.81%, 2.76%, and 0% respectively. In addition, it was determined that there is a significant relationship between cagA genotypes ABCC and ABCCC on the one hand and cagA genotype ABCCC on the other hand, with susceptibility to chronic gastritis and gastric cancer respectively (Table 2). Publication bias was not seen in our study (data not shown).

| cagA genotypes | Gastrointestinal diseases | Random effects mode ORs (95% CIs) | p-value | Heterogeneity of \( \chi^2 \) square index | Frequency (%) in cagA+ strains |
|----------------|---------------------------|----------------------------------|---------|---------------------------------------------|-------------------------------|
| AB             | Gastritis                 | 1.69 (0.71–4.1)                   | 0.23    | 74.7%                                       | 5.52%                         |
|                | Peptic ulcer              | 0.69 (0.26–1.83)                  | 0.46    | 53.1%                                       |                               |
|                | Gastric cancer            | 1.33 (0.66–2.65)                  | 0.42    | 68.4%                                       |                               |
| ABC            | Gastritis                 | 0.66 (0.48–0.89)                  | 0.008   | 54.3%                                       | 80.18%                        |
|                | Peptic ulcer              | 0.000                             | 0.39 (0.28–0.55) | 34.2%                       |                               |
|                | Gastric cancer            | 0.000                             | 0.33 (0.21-051) | 28.3%                       |                               |
| ABCC           | Gastritis                 | 0.001                             | 2.23 (1.39–3.55) | 32.5%                       | 22.81%                        |
|                | Peptic ulcer              | 0.46                              | 1.19 (0.73–1.93) | 90.8%                       |                               |
|                | Gastric cancer            | 0.66                              | 1.13 (0.64–1.99) | 67.4%                       |                               |
| ABCCC          | Gastritis                 | 0.012                             | 1.99 (1.25–3.20) | 61.5%                       | 2.76%                         |
|                | Peptic ulcer              | 0.42                              | 1.18 (0.7–1.9)  | 72.6%                       |                               |
|                | Gastric cancer            | 0.02                              | 1.84 (1.1–3.5)  | 54.8%                       |                               |

### 4. Discussion

The cagA gene is one of the most important virulence factors in H. pylori genome, which locates in the end of I region in PAIs. The GC content this gene is 35%, which is less compared to other bacterial genes (40%) of bacterial genome. This gene can be transferred between bacterial strains through horizontal transmission (48–49). The cagA gene encodes a 128–145 kDa protein in different strains, and its frequency has reported between 40–97% (50–51). According to review of literature, H. pylori strains which carry cagA gene are more virulent (more pathogen). Frequency of cagA positive strains in East Asia (regions with high incidence of gastric cancer) is very high (51–52). For example, frequency of this gene in South-East Asian countries such South Korea (97%), Japan (95%), China (90%) is much more compared to Western countries (16, 53). Also, Iran is one of the regions of the world with high prevalence of gastric cancer. Based on GLOBOCAN the incidence of gastric cancer in Iran has been estimated about 62.3 cases per 100000 population. Based on our statistical analysis, frequency of cagA gene in Iranian population was evaluated about 71.34%, which confirmed previous findings. In addition, the presence of cagA has a meaningful relationship with gastrointestinal diseases such as peptic ulcer, gastric atrophy, and gastric cancer (54–55). In our project, also, abundance of cagA positive strains in peptic ulcer and gastric cancer patients was very high, and we also saw a meaningful relationship between the presence of cagA gene and gastric cancer (ORs: 2.277; p-value: 0.00). In recent, molecular studies have shown that upon translocation of CagA toxin into the cytoplasm of gastric epithelial cells, it is phosphorylated by Src kinases family in its Tyrosine residues of EPIYA motifs (56–57). Phosphorylated CagA reacts with about 20
various proteins in host cell, and depending on host epigenetic situations, alternative signaling pathway leads to intracellular events such as to loss polarity and junction, increase of motility due to alternation in cytoskeletal rearrangement, stimulation of cell proliferation, DNA damage and aberrant cell survival, stimulation of pro-inflammatory response via stimulating of NF-kB signaling pathway and induction of hummingbird phenotype, and finally gastric cancer (58–61). Nowadays, based on difference in surrounding amino acids sequences (32–40 residues), the EPIYA motif is subdivided to four classes A, B, C, and D (62). Each of these motifs effect on different signaling pathways. Even, in recent, it has found that nucleotide sequences and their number of repeats are unique in each EPIYA motif, and this situation in turn effects on binding affinity with host proteins, and has determinative role in final outcomes of *H. pylori* infection (63–65). In addition *H. pylori*, in recent researches it has been demonstrated that EPIYA motifs in other human pathogens such as enteropathogenic *Escherichia coli* (T1), *Haemophilus ducreyi* (LspA), and *Anaplasma phagocytophilum* (AnkA) play as PEIYA effectors which can affect signaling pathway in host cell (57, 66). In recent years, evaluation of EPIYA motifs has attracted a lot of attention. For example, despite of high colonization rate with *H. pylori* in Africa, Latin America and some East Asian countries such Thailand and Malaysia, there is a decreased incidence of gastric cancer in those countries, which considering differences in EPIYA motifs, this paradox was justified (34, 67–69). It seems that EPIYA motifs solving the puzzle of being carcinogenic of *cagA* gene, and also, these days the EPIYA motif can be used as a tool for epidemiologic studies and also monitoring of circulating of *H. pylori* strains worldwide (14). Generally, almost all *H. pylori cagA* positive strains in Western countries harboring EPIYA motifs A, B, and one or more repeats of C, while EPIYA motif patterns in East Asian countries are ABD (63, 70). Li et al. (2017) demonstrated that the C and D motifs can induce hummingbird phenotype. In another study, Argent et al. (2004) also showed that these two motifs can phosphorylate SHP-2; hence both are accounted as risk factor for gastric cancer (71–72). In new discoveries, it has demonstrated that EPIYA-D has high affinity to bind to SHP-2 (pY-[V/T]/A[I/S]-X-[L/I/V]-X-[F/W], while EPIYA-C has less affinity for SHP-2 (merely one amino acid in pY + 5th position) compared to EPIYA-D (73–74). However, according to literature, the presence of the higher number of EPI-X repeats is associated with the greater tendency of binding to SHP-2 (75). According to this, in a study on Mexico population, the risk of gastric cancer due to strains containing two and more than two repeats in EPIYA-C was 32 and 51 fold respectively (76). Studies in Brazil and Columbia showed that the possibility of gastric cancer due to strains with 2, 2 or 3, and 3 repeats of EPIYA-C was 5.9, 3.8, and 12 fold respectively (77–78). Also, EPIYA-A and B bind C-terminal Src kinase (Csk) and p85 (subunit of PI3K) complex respectively, but these motifs have been less studied and their effects remain unknown (66). In general, *cagA* genotypes recognized from around the world include AB, ABC, ABCC, ABCCC, and ABD. In studies from Iran, so far there is no any document based on detection of *cagA* genotypes ABCCC and ABD, which is arising from genetic differences between isolated strains of Iranian patients and East Asian's strains (37–46). Nevertheless, according to studies, in Iran, *cagA* genotypes ABCC and ABCCC are more prevalent in peptic ulcer and gastric cancer. However, based on our statistical analysis, only *cagA* genotype ABCCC had a meaningful relationship with gastric cancer. Like our results, studies in South America, North America, and European showed a significant relationship between infection by multiple EPIYA-C strains and gastric cancer in the patients (71, 75). Recently, Gomez et al. (2020) found a meaningful relationship between EPIYA-ABCC and ABCCC with gastric cancer which confirms our study (75). Furthermore, according our results, it seems that infection by strains possessing EPIYA-ABC motif has a preventive role against gastritis, peptic ulcer, and gastric cancer. Regarding less affinity of EPIYA-C motif to binding to SHP-2, hence, it concluded that the presence of one copy of EPIYA-C has less effect in induction of gastric cancer, especially that EPIYA-D is less be able to induce interleukin 8 (IL-8) compared to EPIYA-C (66, 79). Our country, Iran, locates in Middle East, and recent studies have demonstrated that gastric cancer is the most prevalent cancer in Iran. Based on recent studies, the prevalence of gastric cancer in Iran has been estimated about 13.7 per 100000 population. In addition, the recent studies have shown that north provinces, in particular, northeast provinces are dedicated the most gastric cancer patients to themselves, and this is while based on epidemiologic studies these areas have the most abundance of infection by *H. pylori* (80–81, 12). According to this meta-analysis it was demonstrated that infected patients to *H. pylori* are 2.26 fold more exposed to gastric cancer, which has conformity with received results from Iranian population (82). In developing countries such Iran the age of infection with *H. pylori* is low, and based on recent studies, about 80% of children in the first ten years of their lives are affected to this infection. Vic versa, in developed countries infection with *H. pylori* elevate with increasing age. Therefore, the age is accounted as a determinative factor in increasing of the numbers of gastric cancer patients in developing countries, especially in Iran (82–83). Also, various studies have demonstrated that *cagA* positive strains of *H. pylori* are more virulent compared to the *cagA* negative ones, and hence, these strains are directly related to peptic ulcer and gastric cancer (71). In the present study, frequency of *cagA* gene in patient with peptic ulcer and gastric cancer was reported about 71%. Based on our statistical analysis, infection with *H. pylori* has a meaningful relationship with affecting to gastric cancer in Iranian population. According to Ghotaslou et al. (2018) study, the infection with strains possessing *cagA* and *vacA* s1m1 genes causes the increased risk to gastric cancer in Iranian population, which confirms our declarations (84). However, although the rate of gastric cancer in Iran is like East Asian countries such Japan, Korea, and China, but EPIYA motifs pattern in these countries is different of Iran (34, 37, 85). While in East Asian countries, the *cagA*2a (EPIYA-ABD) genotype is common, but so far in Iran
no report has been issued about detection of EPIYA-D, and this is due to geographical differences of circulating strains (34). In addition, in other regions such as Africa, Latin America, or even East Asian countries e.g. Thailand and Malaysia, despite high colonization rate of \textit{H. pylori} but the frequency of gastric cancer is low; one of the most probable reasons for this difference is diversity in \textit{H. pylori} strains. According to estimates, it was determined that circulating \textit{H. pylori} strains in Iran have five different genetic patterns, which three patterns are like to identified patterns from Arabia, Turkey, and Uzbekistan, while two other patterns specifically are belong to Bandar Abbas and Yazd (86). Latifi-Navid et al. (2010), showed that Iranian strains fall in a same clade with European \textit{H. pylori} (hpEurope) strains (Fig. 4).

According to phylogenetic analysis, Iranian patients’ strains fall into a same clade with isolated strains of England, Spain, Finland, Turkey, and Italy (86). The hpEurope strains are formed from combination of both Ancestral Europe1 (AE1) and Ancestral Europe2 (AE2) population. AE1 is more related to Northern Europe, while AE2 is related to isolated strains from Southern Europe regions (87). Available information show that strains of Central Asia are more AE1, whereas North East Africa strains originate from AE2 (12, 86). It seems that circulating strains among Iranian patients originate from hpEurope strains, and have transferred to Iran from some groups such as Arabs in the 7-8th centuries, Uzbeks fight in 16th century, and Ottoman Empire during the 20th century (86). Existence of cagA2a genotype (EPIYA-ABC motifs) in Iran’s studies confirm this hypothesis (37–47). However, why the number of gastric cancer cases in Iran as is high as cases of Japan, South Korea, and China? It seems that the presence of EPIYA-C motif, especially in strains which have more than one repeat of EPIYA-C can be reinforcement of binding to SHP-2 and is accounted a risk factor for gastric cancer (75,88). In our study, the presence of EPIYA-ABCCC was significantly related with gastric cancer in Iranian population. Moreover, life style, ethnicity, and etc. cause to raise the prevalence of gastric cancer in Iran (81). Finally, it should be noted that our study had several limitations such: high degree of heterogeneity in the analyzed studies, limitation in number of studies, Lack of solidarity between cagA genotypes and other virulence factors with gastrointestinal diseases development. In many of studies it has determined that the simultaneous presence of some alleles of cagA and vacA genes cause increase the risk of gastric cancer in different populations of the world (84). Therefore, further studies will be essential to elucidate the dominant role of cagA genotypes for assessment of susceptibility into gastrointestinal diseases.

5. Conclusions

In the present study, we declared the high prevalence of cagA gene in gastrointestinal diseases of Iranian population. We demonstrated that this gene significantly is related to susceptibility of patients to gastric cancer. Notwithstanding EPIYA-ABD motif is related to increase the risk of gastric cancer in East Asian population, so far no evidence has been found based on identification of EPIYA-ABD in Iranian population, and regarding of high prevalence of gastric cancer in Iran, we are faced with a paradox. This question has also been observed in the Thailand and Malaysian population. Sahara et al. (2012) in their study demonstrated that the frequency of EPIYA-D motif is low in these countries, and this reason justifies the decrease of frequency of gastric cancer in Thai and Malaysian population (34). We observed that there was a significant relationship between EPIYA-ABCCC and gastric cancer in Iranian population. Overall, it can be concluded that the higher number of EPIYA-C copy numbers leads to the higher risk of gastric cancer (55, 88). Therefore, according to our results, it seems that the presence of EPIYA-ABCCC strains of \textit{H. pylori} should be considered as an appropriate marker in prevention of gastric cancer in Iranian population.

Abbreviations

\textit{Helicobacter pylori} (\textit{H. pylori})

Mucosa-associated lymphoid tissue (MALT) lymphoma

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Comprehensive meta-analysis (CMA)

Odds Ratio (ORs)

Confidence intervals (CIs)

Ancestral Europe1 (AE1)

Ancestral Europe2 (AE2)
Declarations

Ethics approval and consent to participate
Not applicable (this paper was provided based on researching in global databases)

Consent for publish
Not Applicable

Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files

Competing interests
There is no any conflict of interest among the all authors.

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Authors' Contributions
1. MK1 have contributed to design of the work and analysis of data
2. MK2 have drafted the work and substantively revised it
All authors read and approved the final manuscript

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Figure 1

Schematic illustration of search strategy process in current meta-analysis.
Figure 2

Forrest plots of the H. pylori cagA gene and its association with gastrointestinal diseases. A) Representative role of cagA gene in susceptibility to gastric cancer; B) representative role of cagA gene in susceptibility to peptic ulcer.
Figure 3

Distribution of cagA genotypes in different regions of Iran. The image taken from https://commons.wikimedia.org/wiki/File:Iran_location_map.svg. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 4

Neighbor-joining analysis of 68 H. pylori strains in Iran to other worldwide which is retrieved from Latifi-Navid et al., 2010 study (86).

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