**EPIYA motifs of *Helicobacter pylori* cagA genotypes and gastrointestinal diseases in the Iranian population: a systematic review and meta-analysis**

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**Abstract**

*Helicobacter pylori* is one of the best risk factors for gastric cancer. Recent studies have examined the relationship between virulence factors, in particular CagA toxin, and the development of gastrointestinal diseases. According to the literature, there is a significant relationship between the polymorphism of cagA-EPIYA motifs and progression to severe clinical outcomes. The main goal of our study was to determine the possible association between cagA genotypes and the risk of severe clinical outcomes in the Iranian population. We investigated these ambiguities using a comprehensive meta-analysis study, in which we evaluated data from 1762 Iranian patients for a potential correlation between all cagA gene genotypes and gastrointestinal diseases. According to statistical analysis, the frequencies of cagA genotypes including ABC, ABCC, AB and ABCCC in the Iranian population were estimated at 80.18%, 22.81%, 5.52% and 2.76%, respectively; the ABD genotype was not detected in these PCR-based studies. There was a significant relationship between cagA genotypes ABCC and ABCCC and severe clinical outcomes of infection such as peptic ulcer and gastric cancer. Overall, it can be concluded that there is a positive correlation with the number of copies of EPIYA-C and the increase of gastric cancer. Therefore, according to our results, it seems that the EPIYA-ABCCC motif has a strong positive relationship with gastric cancer in the Iranian population.

Keywords: cagA genotypes, gastric cancer, *Helicobacter pylori*, Iran, peptic ulcer

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**Background**

*Helicobacter pylori* is a Gram-negative, microaerophilic, helical, motile bacterium that colonizes the submucosal layer in the human stomach [1]. Persistent infection is one of the main characteristics of this microorganism; previous studies have shown that *H. pylori* can survive in the human stomach for decades [2]. This bacterium is the aetiological agent for gastrointestinal complications including acute gastritis, gastric ulcer, duodenal ulcer, gastric adenocarcinoma tumors and mucosa-associated lymphoid tissue lymphoma [3]. Based on the available evidence, *H. pylori* triggers a wide range of changes in the stomach from acute inflammation to other problems such as chronic gastritis, atrophy, intestinal metaplasia, dysplasia and gastric adenocarcinoma, as well as autoimmune diseases [2–5]. Regarding the potency of *H. pylori* in creating gastric cancer, the International Agency for Research on Cancer (GLOBOCAN) introduced this pathogen as a class I human carcinogenic agent in 1994 [6]. Gastric cancer is one of the four most common cancers in the world, and is also the third deadliest cancer in the world; approximately 700,000 individuals die from this disease each year [7]. Statistics are more worrying in developing countries, especially in Iran, where the rate of *H. pylori* infection in the Iranian population is about 90%, and 22 deaths from cancer occur daily [8]. In addition, *H. pylori* and the administration of non-steroidal anti-inflammatory drugs are two major risk factors for gastric cancer. Studies show that more than 60% of patients infected with *H. pylori* are affected by gastric cancer.
Although about half of the world’s population is infected with H. pylori, however, diseases associated with this bacterium occur in 15%–20% of the population. Due to problems such as high colonization rate, poor prognosis of gastric cancer, re-infection and the difficulty in eradication of bacteria, unfortunately patients are identified in the advanced stages of the disease and this phenomenon is associated with high mortality. Based on various studies, bacterial strains have a large genetic diversity, so the occurrence of different forms of infection such as gastritis, peptic ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma depends on various factors, especially the virulence factors expressed by different strains. Cytotoxin-associated gene A (CagA) antigen is encoded by the cagA gene, which in turn is one of the most important genes in the whole genome and locates in cag pathogenicity islands. The presence and expression of the cagA gene is related to gastric complications such as gastritis, peptic ulcer, gastric polyps, precancerous status, cell survival and gastric adenocarcinoma. Studies in China, Japan, and South Korea show that 90% of strains isolated from patients have the cagA gene in their genome. Based on genomic studies, there is genetic diversity of the cagA gene among the different strains of H. pylori. Most differences in their open reading frame are related to a locus that encodes 1147–1181 amino acids, which in turn is associated with nucleotide sequences in the 3’-terminal of the cagA gene. According to recent studies, upon bacterial colonization in the host stomach, CagA is secreted into the gastric cells through a type IV secretion system. This protein is then phosphorylated by c-Src and c-Abl of the host cell tyrosine kinases. Phosphorylated CagA binds to host proteins and blocks the signalling pathway. Depending on the sequences around the EPIYA motifs, CagA is divided into four classes: A, B, C and D. Most dominant strains in East Asian contain the EPIYA-ABD allele, but the EPIYA-ABC allele is the predominant genotype in western countries. It has also been suggested that the East Asian cagA genotype (ABD) is significantly associated with an increased risk of gastric cancer. The main purpose of this meta-analysis was to evaluate the association between cagA genotypes and the risk of susceptibility to severe clinical outcomes, particularly peptic ulcer and gastric cancer, in the Iranian population.

Materials and methods

In the present meta-analysis, all stages of research including literature search, selection criteria, quality assessment, data extraction and statistical analysis were performed according to the guidelines of systematic review and meta-analysis.

Search strategy

To receive all reports of EPIYA motifs related to the population of Iran, studies conducted up to August 2020 were collected from databases such as PubMed, Scopus, Google Scholar, Magiarn, IranMedex and ISC. All studies were researched regardless of language limitations; we used some keywords such as Iran, cagA gene, EPIYA and Helicobacter pylori.

Selection criteria

Eligible original articles (cross-sectional, case-control and cohort) were identified by the two authors separately. According to the standards protocols, the inclusion criteria were: (a) Iranian case-control studies evaluating the association between cagA genotypes (EPIYA motifs) and severe clinical outcomes, (b) studies with data of both case (peptic ulcer and gastric cancer) and control (gastritis) groups, (c) studies on clinical specimens and (d) articles with reliable material and methods. Excluded studies included studies with insufficient information, articles such as letters to editors, review articles, case reports, congress abstracts, studies with repetitive samples, and duplicate articles.

Quality assessment and data extraction

The quality of studies was evaluated using the Newcastle-Ottawa quality assessment scale criteria, and articles that received a score of five or more were included in the present meta-analysis (data not shown). The process of extracting data from qualified studies was performed separately by the two authors. Extracted information included terms such as first author, publication year, city, numbers of patients, age and sex distribution, frequency of EPIYA motifs and diagnostic methods.

Statistical analysis

Available information was analysed by comprehensive meta-analysis (CMA version 2.0) software. First, heterogeneity of data was assessed using I² and Cochrane Q test indexes, and in the high heterogeneity cases we used a random effects model. Publication bias was measured using Egger’s p value test, Begg’s p value test and asymmetry of funnel plot. Finally, we pooled the data to determine the potential association between cagA genotypes (each EPIYA motif) and the risk of developing severe clinical outcomes including peptic ulcer; gastric cancer was estimated by OR with the corresponding 95% CI. In our meta-analysis, patients with gastritis were the control group, and patients with peptic ulcer and gastric cancer were included as the control group.

Results

Initially, 92 potentially relevant studies were retrieved after searching global databases, and 79 articles were evaluated for...
the title and abstract. Review articles and unrelated studies were excluded from the study, and the full texts of 44 documents were evaluated as eligible. Of these, 33 articles were excluded such as non-case–control studies, in vitro studies and studies on children. As a result, in this study, out of the 92 articles, only 11 met the inclusion criteria (Fig. 1) [39–49]. Among the available articles, ten were in English and one was in Persian. The studies had been conducted from different cities such as Tehran, Ardabil, Sari, Kerman, Shiraz and Ahvaz. In the present study, we comprehensively evaluated the information on 1762 patients. All of the eligible studies had evaluated cagA genotypes using PCR as well as sequencing assays. Of the participants, 51.2% were male and 48.8% were female, and their mean age was 49.2 ± 5 years. The frequency of the cagA gene in strains isolated from different regions of Iran was estimated to be 54.82%. Based on statistical analysis, it

### TABLE 1. Characteristics of included studies

| First author      | Year | City (province) | Age (years) | Female/Male, n/n | No. of patients | cagA genotypes | Diagnostic method | Ref |
|-------------------|------|-----------------|-------------|------------------|-----------------|----------------|--------------------|-----|
| Shokrzadeh et al. | 2010 | Tehran (Tehran) | 45.4 ± 1 | 109/81 | 190 | AB 3, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [39] |
| Saberi et al.     | 2012 | Tehran (Tehran) | 42.2 ± 3 | 125/62 | 250 | AB 3, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [40] |
| Ajaei et al.      | 2013 | Sari (Mazandaran) | 42.7 ± 3 | 134/62 | 250 | AB 3, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [41] |
| Vaziri et al.     | 2013 | Tehran (Tehran) | 47/24 | 71 | 35 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [42] |
| Haddadi et al.    | 2014 | Shiraz (Fars) | 45 ± 1 | 120 | 30 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [43] |
| Honarmand et al.  | 2015 | Tehran (Tehran) | 33 ± 1 | 168 | 157 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [44] |
| Yadegar et al.    | 2015 | Tehran (Tehran) | 46 ± 1 | 61 | 47 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [45] |
| Farzi et al.      | 2018 | Tehran (Tehran) | 47/23 | 68 | 49 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [46] |
| Sarrami et al.    | 2018 | Ardabil (Ardabil) | 47/18 | 206 | 107 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [47] |
| Sheikh et al.     | 2018 | Ahvaz (Khuzestan) | 71 | 201 | 134 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [48] |
| Abdollahi et al.  | 2019 | Kerman (Kerman) | 47/93 | 191 | 49 | AB 0, ABC 0, ABCC 0, ABCCC 0, ABD 0 | PCR + Sequencing | [49] |

FIG. 1. Schematic illustration of search strategy process in current meta-analysis.
was found that there is a significant association between infection with *H. pylori* strains expressing CagA and the risk of severe clinical outcomes such as gastric cancer (OR 2.77 with 95% CIs) and peptic ulcer (OR 1.05 with 95% CIs) in the Iranian population (Fig. 2).

In all eligible studies, EPIYA motifs were identified by PCR and DNA-sequencing techniques. According to all the PCR-based studies conducted from Iran, none of studies reported the EPIYA motif D and *cagA* genotype ABD. However, in the present study, the most prevalent EPIYA motifs including A, B and C, and *cagA* genotypes ABC and ABCC were evaluated.

According to statistical analysis, abundances of *cagA* genotypes ABC, ABCC, AB and ABCCC in the Iranian population were at 80.18%, 22.81%, 5.52% and 2.76% respectively. Due to the PCR-based nature of these eligible studies, negative results were obtained from the ABD genotype, although this genotype may occur at a very low frequency. Also, it was found that there was a significant relationship between genotypes ABCC and ABCCC with occurrence of chronic gastritis, whereas the genotype ABCCC was associated with gastric cancer (Table 2).

**Discussion**

The *cagA* gene is one of the most important virulence factors in the *H. pylori* genome, located at the end of region I in the pathogenicity islands. The GC content of this gene is 35%, which is lower than other genes in the bacterial genome, and it is

![FIG. 2. Forrest plots of meta-analysis on the probable association between infection with *cagA*-positive *Helicobacter pylori* strains and susceptibility to severe clinical outcomes in the Iranian population. (a) Examining the association between infection with *cagA*-positive *H. pylori* strains and risk of development of gastric cancer. (b) Examining the association between infection with *H. pylori* *cagA*-positive strains and the risk of development of peptic ulcer. The analysis was conducted using a random-effects model; the black diamond represents the OR for the total population studied. The box sizes are proportional to the precision of the estimates (boxes with larger diameters indicate the higher degree of precision). In addition, p value represents a probability of hypothesis and Z value is an indicator for standard deviations.](image-url)
transferred horizontally between bacterial strains [50–52]. The cagA gene encodes a 128–145-kDa protein in different strains, and its frequency is reported to be between 40% and 97% [53,54]. Based on studies, H. pylori strains that carry the cagA gene are more virulent (more pathogen), and accordingly, the frequency of cagA-positive strains in East Asia (regions with a high incidence of gastric cancer) is very high [55,56]. For example, the frequency of this gene in Southeast Asian countries such as South Korea (97%), Japan (95%) and China (90%) is much higher compared with western countries [17,57]. Iran is also a region with a high prevalence of gastric cancer, so that based on GLOBOCAN, the incidence of gastric cancer in this country is estimated at 62.3 cases per 100,000 people. Based on our statistical analysis, the frequency of the cagA gene in the Iranian population was estimated at 54.82%, which confirmed previous findings: the presence of cagA is significantly associated with gastrointestinal diseases such as gastric ulcer, gastric atrophy and gastric cancer [32,58,59]. In our project, we also saw a meaningful relationship between the presence of cagA gene and gastric cancer (OR 2.77; p 0.00). Recently, molecular studies have shown that by transporting CagA toxin to the cytoplasm of gastric epithelial cells, it is phosphorylated by the Src kinase family in tyrosine residues of EPIYA motifs [60,61]. Phosphorylated CagA reacts with about 20 different proteins in the host cell, and depending on the host epithogenic condition, the alternative signalling pathway leads to intracellular events, such as the loss of polarity and junction, increased motility due to changes in cytoskeletal rearrangement, stimulation of cell proliferation, DNA damage and aberrant cell survival, stimulation of pro-inflammatory response via nuclear factor-κB signalling pathway and induction of hummingbird phenotype, which generally lead to gastric cancer [62–65]. Today, it has been shown that based on differences in the surrounding amino acid sequences (residues 32–40), the EPIYA motif is divided into four classes A, B, C and D [66]. Even more recently, it has been found that amino acid sequences and their number of repeats are unique in each EPIYA motif, which in turn affects the affinity for binding to host proteins and plays a deterministic role in outcomes of H. pylori infection [67–69]. In recent studies it has been demonstrated that, like H. pylori EPIYA motifs, in other human pathogens such as enteropathogenic Escherichia coli (Tir), Haemophilus ducreyi (LspA) and Anaplasma phagocytophilum (AnkA) EPIYA motifs can affect signalling pathways in the host cell [61,70]. Despite the high rate of H. pylori colonization in Africa, Latin America and some East Asian countries such as Thailand and Malaysia, the incidence of gastric cancer in these countries is low; this paradox is related to differences in EPIYA motifs [36,71–73]. It seems that EPIYA motifs solve the puzzle of the carcinogenic effect of the cagA gene, and also, these days the EPIYA motif can be used as a tool for epidemiological studies as well as monitoring the circulating strains [15]. In general, almost all cagA-positive strains in western countries harbour EPIYA motifs A, B and one or more repeats of C, while EPIYA motifs in East Asian countries are mostly ABD [67,74]. Li et al. demonstrated that C and D motifs can induce a hummingbird phenotype, and in another study, Argent et al. also showed that these two motifs can phosphorylate SHP-2; hence, both of these motifs are accounted as a risk factor for gastric cancer [54,75]. It has been demonstrated that EPIYA-D has a high affinity to bind to SHP-2 (pY-(V/I/T/A/I/S)-X-(L/I/V)-X-(F/W) (whereas EPIYA-C has less affinity for SHP-2 (merely one amino acid in pY+5th position) compared with EPIYA-D [76,77]. However, according to literature, the presence of a higher number of EPI-X repeats is associated with the greater tendency for binding to host proteins [78]. In recent studies, it has been demonstrated that, like H. pylori EPIYA motifs, in other human pathogens such as enteropathogenic Escherichia coli (Tir), Haemophilus ducreyi (LspA) and Anaplasma phagocytophilum (AnkA) EPIYA motifs can affect signalling pathways in the host cell [61,70]. 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However, according to literature, the presence of a higher number of EPI-X repeats is associated with the greater tendency for binding to SHP-2 [78].
two, two or three, and three repeats of EPIYA-C was 5.9-, 3.8- and 12-fold respectively [80,81]. Also, EPIYA-A and -B bind to the C-terminal of Src kinase (Csk) and p85 (subunit of PI3K) complex, respectively, but these motifs have been less studied and their effects remain unknown [69]. In general, so far, cagA genotypes recognized from around the world include AB, ABC, ABCC, ABCCCC, ABCCCCC and ABD. In studies from Iran, so far there is no study based on the detection of cagA genotypes ABCCCCC and ABD, which is the result of genetic differences between isolated strains of Iranian patients and East Asian strains. Although both ABCC and ABCCCC genotypes are more prevalent in Iran in the occurrence of gastric ulcer and gastric cancer, according to our statistical analysis, only the ABCCCC genotype was significantly associated with gastric cancer. Similarly, studies in South America, North America and Europe have shown that there is a significant relationship between infection by multiple EPIYA-C strains and gastric cancer in patients [74,78]. Interestingly, according to our results, it seems that infection by strains possessing the EPIYA-ABC motif has a preventive role against gastritis, peptic ulcer and gastric cancer. Regarding the low affinity of the EPIYA-C motif in binding to SHP-2, it is concluded that the presence of one copy of EPIYA-C has less effect in the induction of gastric cancer; also EPIYA-D is less able to induce interleukin-8 than EPIYA-C [69,82]. Iran, is in the 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 at about 13.7 per 100,000 population [83]. Also, recent studies have shown that northern provinces, in particular the northeast provinces, have the most gastric cancer patients, and it is interesting to note that, based on epidemiological studies, these areas have the highest abundance of infection by H. pylori (83,84). According to this meta-analysis, it was demonstrated that patients infected with H. pylori are 2.26-fold more sensitive to gastric cancer, which matches the seroepidemiology results of Abdi et al. for infection with H. pylori in the Iranian population [85]. In developing countries such as Iran, the age of infection with H. pylori is low, and based on recent studies, about 80% of children in the first 10 years of life are affected by this bacterium; in contrast, in developed countries H. pylori infection increases with age. Therefore, age is accounted for as a determining factor in increasing the numbers of gastric cancer patients in developing countries, especially in Iran [85,86]. Also, studies have demonstrated that cagA-positive strains are more virulent than the cagA-negative strains, and hence, these strains are directly associated with peptic ulcer and gastric cancer [74]. In the present study, the frequency of the cagA gene in patients with peptic ulcer and gastric cancer was reported at 71%. According to a study by Ghotaslou et al., infection with strains harbouring both cagA and vacA s1m1 genes increases the risk of gastric cancer in the Iranian population, which in turn confirms our claims [87]. However, although the rate of gastric cancer in Iran is similar to East Asian countries such as Japan, Korea and China, the pattern of EPIYA motifs in these countries is different from that in Iran [31,36,39]. Whereas in East Asian countries, the cagA2a (EPIYA-ABD) genotype is the predominant genotype, there is no report of the detection of EPIYA-D from Iran, and this appears to be due to geographical differences in circulating strains [36]. Besides, in other regions such as Africa, Latin America or even East Asian countries such as Thailand and Malaysia, despite the high colonization rate of H. pylori, the incidence of gastric cancer is low; one of the most likely reasons for this difference is the diversity in H. pylori strains. It was estimated that H. pylori strains circulating in Iran have five different genetic patterns, three of which are similar to those identified in Saudi Arabia, Turkey and Uzbekistan, whereas the other two belong specifically to Bandar Abbas and Yazd; the Iranian strains fall in the same clade with European H. pylori (hpEurope) strains isolated from England, Spain, Finland, Turkey and Italy [88]. The hpEurope strains are derived from a combination of both Ancestral Europe1 (AE1) and Ancestral Europe2 (AE2) populations. AE1 is mostly related to northern Europe, while AE2 is related to strains isolated from southern European regions [89]. Available data indicate that strains of Central Asia originate from AE1, whereas northeast African strains belong to AE2 [13,88]. It seems that the circulating strains among Iranian patients originate from hpEurope strains, and have been transferred to Iran from groups such as Arabs in the seventh and eighth centuries, Uzbeks fighting in the sixteenth century and the Ottoman Empire during the twentieth century [88]. However, why is the number of gastric cancer cases in Iran as high as that in Japan, South Korea and China? It seems that the presence of the EPIYA-C motif, especially in strains with more than one repeat of EPIYA-C, can enhance the binding to SHP-2 and is considered a risk factor for gastric cancer [78,90]. According to our study, the presence of EPIYA-ABCCCC was significantly related to gastric cancer in the Iranian population. In addition, lifestyle and ethnicity can increase the prevalence of gastric cancer in Iran [84]. Finally, it should be noted that our study had several limitations including a high degree of heterogeneity in the analysed studies, limitations in the number of studies, publication bias and lack of correlation between cagA genotypes and other virulence factors with the development of gastrointestinal diseases. Many studies have shown that the coexistence of some cagA and vacA gene alleles increases the risk of gastric cancer in different populations around the world [87,91,92]. Therefore, further studies are needed to elucidate the predominant role of cagA genotypes in assessing the frequency of gastrointestinal diseases.
Conclusions

In the present study, we declared the high prevalence of the cagA gene in gastrointestinal diseases of the Iranian population. We demonstrated that this gene is significantly related to the occurrence of gastric cancer in infected patients. The EPIYA-ABD motif is associated with the increased risk of gastric cancer in the East Asian population, but we observed that there was a significant relationship between EPIYA-ABCCC and gastric cancer in the Iranian population. Therefore, according to our results, it seems that the presence of EPIYA-ABCCC strains of H. pylori should be considered as an appropriate marker for the progression of primary infection to gastric cancer in the Iranian population.

Conflicts of interest

The authors have no conflicts of interest.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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Authors’ contributions

MKe contributed to the design of the work and analysis of data; MKa drafted the work and substantively revised it. Both authors read and approved the final manuscript.

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