ORIGINAL ARTICLE

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

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[9,10]. 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 [11,12]. 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 [13,14]. 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 [15,16]. Studies in China, Japan and South Korea show that 90% of strains isolated from patients have the *cagA* gene in their genome [17–21]. 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 [22,23]. 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 [24–28]. Depending on the sequences around the EPIYA motifs, CagA is divided into four classes: A, B, C and D [29]. Most dominant strains in East Asian contain the EPIYA-ABD allele, but the EPIYA-ABC allele is the predominant genotype in western countries [30–34]. It has also been suggested that the East Asian *cagA* genotype (ABD) is significantly associated with an increased risk of gastric cancer [35,36]. 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 [37].

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 (Table 1). 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 I^2 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 [38]. 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

TABLE 1. Characteristics of included studies

First author	Year	City (province)	Age (years) Female/Male, *n/n*	No. of patients	*cagA*	*cagA* genotypes					Diagnostic method	Ref
						AB	ABC	ABCC	ABCCC	ABD		
Shokrzadeh et al.	2010	Tehran (Tehran)	45.4 ± 1 109/81	190	92	3	86	3	0	0	PCR + Sequencing	[39]
Saberi et al.	2012	Tehran (Tehran)	NR NR	76	60	6	31	23	0	0	PCR + Sequencing	[40]
Ajami et al.	2013	Sari (Mazandaran)	42.2 ± 3 134/116	250	125	39	54	32	0	0	PCR + Sequencing	[41]
Vaziri et al.	2013	Tehran (Tehran)	66 47/24	71	35	0	30	4	1	0	PCR + Sequencing	[42]
Haddadi et al.	2014	Shiraz (Fars)	45 116/164	280	120	0	67	53	0	0	PCR + Sequencing	[43]
Honarmand et al.	2015	Tehran (Tehran)	33 76/92	168	167	0	157	1	9	0	PCR + Sequencing	[44]
Yadegar et al.	2015	Tehran (Tehran)	46 42/19	61	47	0	39	7	1	0	PCR + Sequencing	[45]
Farzi et al.	2018	Tehran (Tehran)	47.9 ± 2 45/23	68	49	0	41	7	1	0	PCR + Sequencing	[46]
Sarrami et al.	2018	Ardabil (Ardabil)	NR 100/106	206	88	0	79	2	7	0	PCR + Sequencing	[47]
Sheikh et al.	2018	Ahvaz (Khuzestan)	71 93/108	201	134	0	66	66	2	0	PCR + Sequencing	[48]
Abdollahi et al.	2019	Kerman (Kerman)	47 98/93	191	49	0	46	0	3	0	PCR + Sequencing	[49]

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

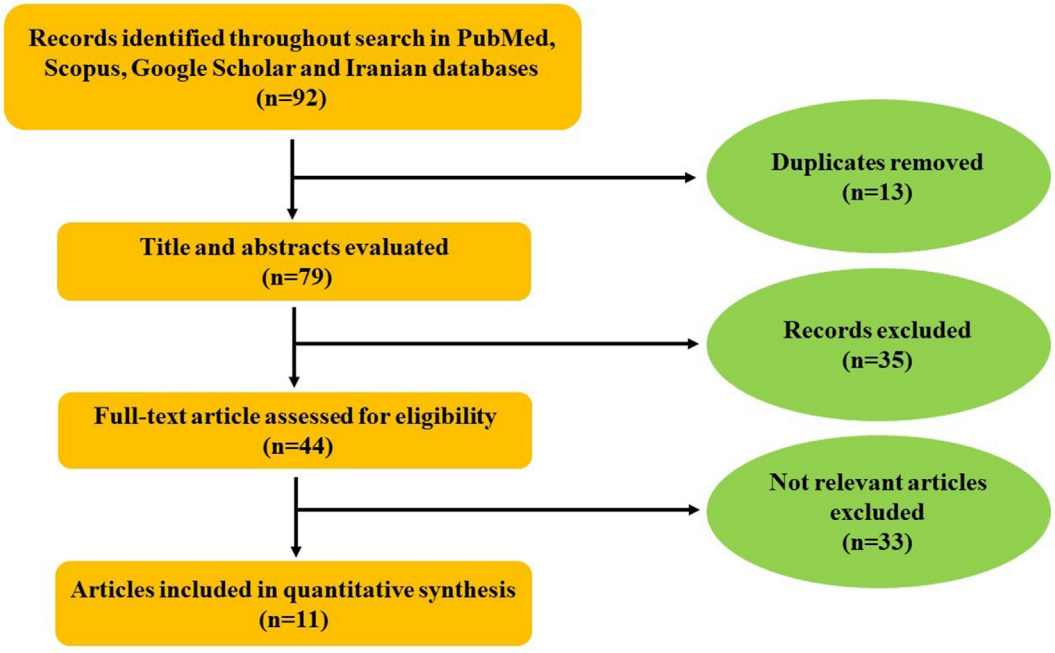

FIG. 1. Schematic illustration of search strategy process in current meta-analysis.

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

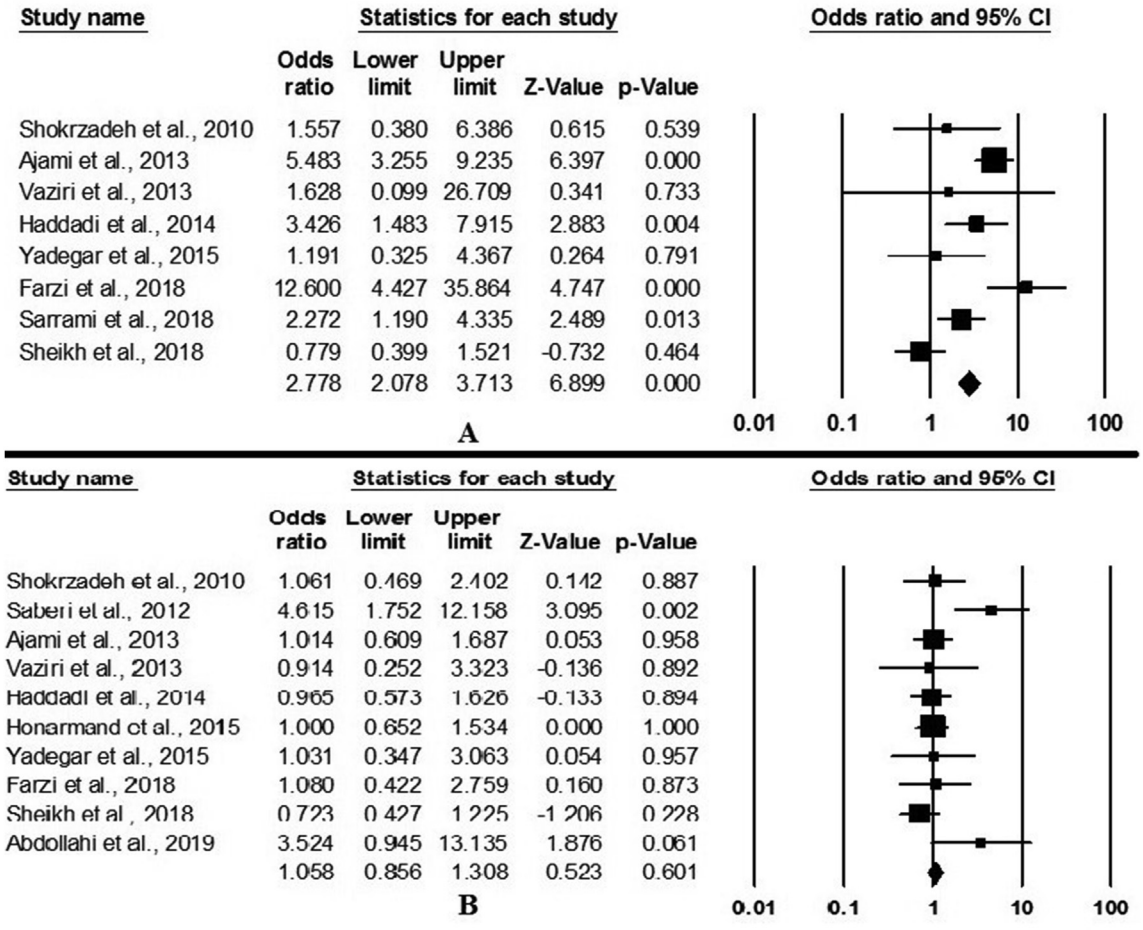

FIG. 2. Forrest plots of meta-analysis on the probable association between infection with *cagA*-positive *Helicobacter pylori* strains and susceptibility to severe clinical outcomes in the Iranian population. (a) Examining the association between infection with *cagA*-positive *H. pylori* strains and 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.

TABLE 2. Summary of ORs with 95% CI for distribution of *cagA* genotypes

cagA genotypes	Random effects mode		Heterogeneity		Publication bias		
Gastrointestinal diseases	ORs (95% CI)	p value	I^2	Q Value	Egger's p value	Begg's p value	Frequency (%) in *cagA* + strains
AB							5.52%
Gastritis	1.69 (0.71–4.1)	0.23	74.7%	79.89	0.50	0.86	
Peptic ulcer	0.69 (0.26–1.83)	0.46	53.1%	54.21	0.10	0.33	
Gastric cancer	1.33 (0.66–2.65)	0.42	68.4%	63.99	0.69	0.74	
ABC							80.18%
Gastritis	0.66 (0.48–0.89)	0.008	54.3%	23.68	0.36	0.76	
Peptic ulcer	0.39 (0.28–0.55)	0.000	34.2%	69.87	0.75	0.46	
Gastric cancer	0.33 (0.21–051)	0.000	28.3%	71.36	0.07	0.13	
ABCC							22.81%
Gastritis	2.23 (1.39–3.55)	0.001	32.5%	25.14	0.37	0.23	
Peptic ulcer	1.19 (0.73–1.93)	0.46	90.8%	69.10	0.51	0.95	
Gastric cancer	1.13 (0.64–1.99)	0.66	67.4%	99.45	0.46	0.53	
ABCCC							2.76%
Gastritis	1.99 (1.25–3.20)	0.012	61.5%	78.66	0.19	0.26	
Peptic ulcer	1.18 (0.7–1.9)	0.42	72.6%	68.12	0.82	0.24	
Gastric cancer	1.84 (1.1–3.5)	0.02	54.8%	99.85	0.05	0.12	

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 [52,58,59]. 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 epigenetic 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 (residuals 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 determinative 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/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 of binding to SHP-2 [78]. Recently, in a study on the Mexican population, it was demonstrated that the risk of gastric cancer due to strains containing two or more repeats of EPIYA-C can be increased in infected patients [79]. Studies in Brazil and Columbia showed that the possibility of gastric cancer due to strains with

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, ABCCC, ABCCCC and ABD. In studies from Iran, so far there is no study based on the detection of *cagA* genotypes ABCCCC and ABD, which is the result of genetic differences between isolated strains of Iranian patients and East Asian strains. Although both ABCC and ABCCC genotypes are more prevalent in Iran in the occurrence of gastric ulcer and gastric cancer, according to our statistical analysis, only the ABCCC 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 *cagA*2a (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 Eroupe2 (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-ABCCC 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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References

[1] Keikha M, Eslami M, Yousefi B, Ghasemian A, Karbalaei M. Potential antigen candidates for subunit vaccine development against *Helicobacter pylori* infection. J Cell Physiol 2019;234:21460–70.

[2] Yousefi B, Mohammadlou M, Abdollahi M, Salek Farrokhi A, Karbalaei M, Keikha M, et al. Epigenetic changes in gastric cancer induction by *Helicobacter pylori*. J Cell Physiol 2019;234:21770–84.

[3] Kusters JG, Van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev 2006;19:449–90.

[4] Helicobacter, Group CC. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 2001;49:347–53.

[5] Youssefi M, Tafaghodi M, Farsiani H, Ghazvini K, Keikha M. *Helicobacter pylori* infection and autoimmune diseases; is there an association with systemic lupus erythematosus, rheumatoid arthritis, autoimmune atrophy gastritis and autoimmune pancreatitis? A systematic review and meta-analysis study. J Microbiol Immunol Infect 2020 [in press].

[6] IARC working group on the evaluation of carcinogenic risks to humans. IARC Monogr Eval Carcinog Risks Hum 1994;61:45–119.

[7] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: A Cancer J Clinicians 2005;55:74–108.

[8] Khademi F, Sahebkar AH, Vaez H, Arzanlou M, Peeridogaheh H. Characterization of clarithromycin-resistant *Helicobacter pylori* strains in Iran: a systematic review and meta-analysis. J Glob Antimicrob Resist 2017;10:171–8.

[9] Mobley HL, Mendz GL, Hazell SL. *Helicobacter pylori*: physiology and genetics. 2001.

[10] Huang J-Q, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet 2002;359(9300):14–22.

[11] Stolte M, Bayerdörffer E, Morgner A, Alpen B, Wündisch T, Thiede C, et al. *Helicobacter* and gastric MALT lymphoma. Gut 2002;50(Suppl. 3): iii19–24.

[12] Argueta EA, Moss SF. Treatment of *Helicobacter pylori*. Curr Opin Gastroenterol 2019;35:544–50.

[13] Bakhti SZ, Latifi-Navid S, Zahri S. *Helicobacter pylori* virulence genes and microevolution in host and the clinical outcome. Tehran Univ Med J 2014;72(9):1–13.

[14] Salama N, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc Natl Acad Sci 2000;97:14668–73.

[15] Yamaoka Y, Kodama T, Kashima K, Graham DY, Sepulveda AR. Variants of the 3′ Region of the cagA Gene inHelicobacter pylori isolates from patients with different *H. pylori*-associated diseases. J Clin Microbiol 1998;36:2258–63.

[16] Sayehmiri F, Kiani F, Sayehmiri K, Soroush S, Asadollahi K, Alikhani MY, et al. Prevalence of cagA and vacA among *Helicobacter pylori*-infected patients in Iran: a systematic review and meta-analysis. J Infect Dev Countries 2015;9:686–96.

[17] Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, et al. Structure of cag pathogenicity island in Japanese *Helicobacter pylori* isolates. Gut 1999;44:336–41.

[18] Miehlke S, Kibler K, Kim JG, Figura N, Small SM, Graham DY, et al. Allelic variation in the cagA gene of *Helicobacter pylori* obtained from Korea compared to the United States. Am J Gastroenterol 1996;91(7): 1322–5.

[19] Pan Z-J, Van der Hulst R, Feller M, Xiao S-D, Tytgat G, Dankert J, et al. Equally high prevalences of infection with cagA-positive *Helicobacter pylori* in Chinese patients with peptic ulcer disease and those with chronic gastritis-associated dyspepsia. J Clin Microbiol 1997;35:1344–7.

[20] Shimoyama T, Fukuda S, Tanaka M, Mikami T, Saito Y, Munakata A. High prevalence of the CagA-positive *Helicobacter pylori* strains in Japanese asymptomatic patients and gastric cancer patients. Scand J Gastroenterol 1997;32:465–8.

[21] Yamaoka Y, Kita M, Kodama T, Sawai N, Kashima K, Imanishi J. Induction of various cytokines and development of severe mucosal inflammation by cagA gene positive *Helicobacter pylori* strains. Gut 1997;41:442–51.

[22] Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci USA 1993;90:5791–5.

[23] Tummuru M, Cover T, Blaser M. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect Immun 1993;61:1799–809.

[24] Murata-Kamiya N, Kikuchi K, Hayashi T, Higashi H, Hatakeyama M. *Helicobacter pylori* exploits host membrane phosphatidylserine for delivery, localization, and pathophysiological action of the CagA oncoprotein. Cell Host Microbe 2010;7:399–411.

[25] Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, et al. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295(5555):683–6.

[26] Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ, Covacci A. c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol 2002;43:971–80.

[27] Tammer I, Brandt S, Hartig R, König W, Backert S. Activation of Abl by *Helicobacter pylori*: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology 2007;132:1309–19.

[28] Karbalaei M, Keikha M. Potential association between the hopQ alleles of *Helicobacter pylori* and gastrointestinal diseases: a systematic review and meta-analysis. Meta Gene 2020:100816.

[29] Panayotopoulou EG, Sgouras DN, Papadakos K, Kalliaropoulos A, Papatheodoridis G, Mentis AF, et al. Strategy to characterize the number and type of repeating EPIYA phosphorylation motifs in the carboxyl terminus of CagA protein in *Helicobacter pylori* clinical isolates. J Clin Microbiol 2007;45:488–95.

[30] Satomi S, Yamakawa A, Matsunaga S, Masaki R, Inagaki T, Okuda T, et al. Relationship between the diversity of the cagA gene of *Helicobacter pylori* and gastric cancer in Okinawa, Japan. J Gastroenterol 2006;41:668–73.

[31] Chomvarin C, Phusri K, Sawadpanich K, Mairiang P, Namwat W, Wongkham C, et al. Prevalence of cagA EPIYA motifs in *Helicobacter pylori* among dyspeptic patients in northeast Thailand. Southeast Asian J Trop Med Public Health 2012;43:105.

[32] Yamazaki S, Yamakawa A, Okuda T, Ohtani M, Suto H, Ito Y, et al. Distinct diversity of vacA, cagA, and cagE genes of *Helicobacter pylori* associated with peptic ulcer in Japan. J Clin Microbiol 2005;43:3906–16.

[33] Naito M, Yamazaki T, Tsutsumi R, Higashi H, Onoe K, Yamazaki S, et al. Influence of EPIYA-repeat polymorphism on the phosphorylation-dependent biological activity of *Helicobacter pylori* CagA. Gastroenterology 2006;130:1181–90.

[34] Nguyen LT, Uchida T, Murakami K, Fujioka T, Moriyama M. *Helicobacter pylori* virulence and the diversity of gastric cancer in Asia. J Med Microbiol 2008;57:1445–53.

[35] Jones KR, Joo YM, Jang S, Yoo Y-J, Lee HS, Chung I-S, et al. Polymorphism in the CagA EPIYA motif impacts development of gastric cancer. J Clin Microbiol 2009;47:959–68.

[36] Sahara S, Sugimoto M, Vilaichone R-K, Mahachai V, Miyajima H, Furuta T, et al. 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;12:1–13.

[37] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009;62:e1–34.

[38] Lee J. Odds ratio or relative risk for cross-sectional data? Int J Epidemiol 1994;23:201–3.

[39] Shokrzadeh L, Baghaei K, Yamaoka Y, Dabiri H, Jafari F, Sahebekhtiari N, et al. Analysis of 3′-end variable region of the cagA gene in *Helicobacter pylori* isolated from Iranian population. J Gastroenterol Hepatol 2010;25:172–7.

[40] Saberi S, Douraghi M, Azadmanesh K, Shokrgozar MA, Zeraati H, Hosseini ME, et al. A potential association between *Helicobacter pylori* CagA EPIYA and multimerization motifs with cytokeratin 18 cleavage rate during early apoptosis. Helicobacter 2012;17:350–7.

[41] Ajami A, Shadman M, Rafiei A, Hosseini V, Talebi BA, Alizadeh A, et al. Prevalence of EPIYA motifs in *Helicobacter pylori* strains isolated from patients with dyspeptic disorders in Northern Iran. Res Mol Med 2013;1(1):30–5.

[42] Vaziri F, Peerayeh SN, Alebouyeh M, Mirzaei T, Yamaoka Y, Molaei M, et al. Diversity of *Helicobacter pylori* genotypes in Iranian patients with different gastroduodenal disorders. World J Gastroenterol 2013;19:5685.

[43] Haddadi MH, Bazargani A, Khashei R, Fattahi MR, Lankarani KB, Moini M, et al. Different distribution of *Helicobacter pylori* EPIYA-cagA motifs and dupA genes in the upper gastrointestinal diseases and correlation with clinical outcomes in Iranian patients. Gastroenterol Hepatol Bed Bench 2015;8(Suppl. 1):S37.

[44] Honarmand-Jahromy S, Siavoshi F, Malekzadeh R, Sattari TN, Latifi-Navid S. Multiple repeats of *Helicobacter pylori* CagA EPIYA-C phosphorylation sites predict risk of gastric ulcer in Iran. Microb Pathog 2015;89:87–92.

[45] Yadegar A, Alebouyeh M, Zali MR. Analysis of the intactness of *Helicobacter pylori* cag pathogenicity island in Iranian strains by a new PCR-based strategy and its relationship with virulence genotypes and EPIYA motifs. Infect Genet Evol 2015;35:19–26.

[46] Farzi N, Yadegar A, Aghdaei HA, Yamaoka Y, Zali MR. Genetic diversity and functional analysis of oipA gene in association with other virulence factors among *Helicobacter pylori* isolates from Iranian patients with different gastric diseases. Infect Genet Evol 2018;60:26–34.

[47] Sarrami S, Latifi-Navid S, Zahri S, Abdi E, Yazdanbod A. Multiple repeats of *Helicobacter pylori* CagA C-terminal motifs predict the risk of gastric cancer in Iran. GOVARESH 2018;23:146–51.

[48] Sheikh AF, Yadyad MJ, Goodarzi H, Hashemi SJ, Aslani S, Assarzadegan M-A, et al. CagA and vacA allelic combination of *Helicobacter pylori* in gastroduodenal disorders. Microb Pathog 2018;122:144–50.

[49] 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 Rep 2019;16:100458.

[50] Akopyants NS, Fradkov A, Diatchenko L, Hill JE, Siebert PD, Lukyanov SA, et al. PCR-based subtractive hybridization and differences in gene content among strains of *Helicobacter pylori*. Proc Natl Acad Sci USA 1998;95:13108–13.

[51] Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci 1996;93:14648–53.

[52] Karbalaei M, Khorshidi M, Sisakht-pour B, Ghazvini K, Farsiani H, Youssefi M, et al. What are the effects of IL-1β (rs1143634), IL-17A promoter (rs2275913) and TLR4 (rs4986790) gene polymorphism on the outcomes of infection with *H. pylori* within an Iranian population; a systematic review and meta-analysis. Gene Rep 2020:100735.

[53] Selbach M, Moese S, Hurwitz R, Hauck CR, Meyer TF, Backert S. The *Helicobacter pylori* CagA protein induces cortactin dephosphorylation and actin rearrangement by c-Src inactivation. EMBO J 2003;22:515–28.

[54] 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;96(17):e6620.

[55] Ferreira RM, Machado JC, Leite M, Carneiro F, Figueiredo C. The number of *Helicobacter pylori* CagA EPIYA C tyrosine phosphorylation motifs influences the pattern of gastritis and the development of gastric carcinoma. Histopathology 2012;60:992–8.

[56] Zhang C, Xu S, Xu D. Risk assessment of gastric cancer caused by *Helicobacter pylori* using CagA sequence markers. PLoS One 2012;7(5):e36844.

[57] Kim SY, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, et al. Genotyping CagA, VacA subtype, IceA1, and BabA of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. J Korean Med Sci 2001;16:579.
[58] Kamogawa-Schifter Y, Yamaoka Y, Uchida T, Beer A, Tribl B, Schöniger-Hekele M, et al. Prevalence of *Helicobacter pylori* and its CagA subtypes in gastric cancer and duodenal ulcer at an Austrian tertiary referral center over 25 years. PloS One 2018;13(5):e0197695.
[59] El Khadir M, Alaoui Boukhris S, Benajah D-A, El Rhazi K, Ibrahimi SA, El Abkari M, et al. VacA and CagA status as biomarker of two opposite end outcomes of *Helicobacter pylori* infection (gastric cancer and duodenal ulcer) in a Moroccan population. PloS One 2017;12(1):e0170616.
[60] Kalaf EA, Al-Khafaji ZM, Yassen NY, Al-Abbudi FA, Sadwen SN. Study of the cytoxin-associated gene a (CagA gene) in *Helicobacter pylori* using gastric biopsies of Iraqi patients. Saudi J Gastroenterol 2013;19(2):69.
[61] Safari F, Murata-Kamiya N, Saito Y, Hatakeyama M. Mammalian Pragmin regulates Src family kinases via the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif that is exploited by bacterial effectors. Proc Natl Acad Sci 2011;108:14938–43.
[62] Bridge DR, Blum FC, Jang S, Kim J, Cha J-H, Merrell DS. Creation and initial characterization of isogenic *Helicobacter pylori* CagA EPIYA variants reveals differential activation of host cell signaling pathways. Sci Rep 2017;7:1–14.
[63] Backert S, Tegtmeyer N, Selbach M. The versatility of *Helicobacter pylori* CagA effector protein functions: the master key hypothesis. Helicobacter 2010;15:163–76.
[64] Segal E, Cha J, Lo J, Falkow S, Tompkins L. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc Natl Acad Sci USA 1999;96:14559–64.
[65] Suzuki N, Murata-Kamiya N, Yanagiya K, Suda W, Hattori M, Kanda H, et al. Mutual reinforcement of inflammation and carcinogenesis by the *Helicobacter pylori* CagA oncoprotein. Sci Rep 2015;5:1–14.
[66] Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, et al. Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 2002;99:14428–33.
[67] Argent RH, Zhang Y, Atherton JC. Simple method for determination of the number of *Helicobacter pylori* CagA variable-region EPIYA tyrosine phosphorylation motifs by PCR. J Clin Microbiol 2005;43:791–5.
[68] Lind J, Backert S, Hoffmann R, Eichler J, Yamaoka Y, Perez-Perez GI, et al. Systematic analysis of phosphotyrosine antibodies recognizing single phosphorylated EPIYA-motifs in CagA of East Asian-type *Helicobacter pylori* strains. BMC Microbiol 2016;16:1–16.
[69] Selbach M, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the *Helicobacter pylori* CagA protein in vitro and in vivo. J Biol Chem 2002;277:6775–8.
[70] Hayashi T, Morohashi H, Hatakeyama M. Bacterial EPIYA effectors – where do they come from? What are they? Where are they going? Cell Microbiol 2013;15:377–85.
[71] Yamaoka Y, Orito E, Mizokami M, Gutierrez O, Saitou N, Kodama T, et al. *Helicobacter pylori* in North and South America before Columbus. FEBS Lett 2002;517:180–4.
[72] van Doorn L-J, Schneeberger P, Nouhan N, Plaisier A, Quint W, De Boer W. Importance of *Helicobacter pylori* cagA and vacA status for the efficacy of antibiotic treatment. Gut 2000;46:321–6.
[73] Sugimoto M, Zali M, Yamaoka Y. The association of vacA genotypes and *Helicobacter pylori*-related gastroduodenal diseases in the Middle East. Eur J Clin Microbiol Infect Dis 2009;28:1227–36.
[74] Pachathundikandi SK, Gutiérrez-Escobar AJ, Tegtmeyer N. Tailor-made detection of individual phosphorylated and non-phosphorylated EPIYA-motifs of *Helicobacter pylori* oncoprotein CagA. Cancers 2019;11:1163.
[75] Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*. Gastroenterology 2004;127:514–23.
[76] Higashi H, Yokoyama K, Fujii Y, Ren S, Yuasa H, Saadat I, et al. EPIYA motif is a membrane-targeting signal of *Helicobacter pylori* virulence factor CagA in mammalian cells. J Biol Chem 2005;280:23130–7.
[77] Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nat Rev Cancer 2004;4:688–94.
[78] Rodríguez Gómez ER, Otero Regino W, Monterrey PA, Trespalacios Rangel AA. cagA gene EPIYA motif genetic characterization from Colombian *Helicobacter pylori* isolates: standardization of a molecular test for rapid clinical laboratory detection. PloS One 2020;15(1):e0227275.
[79] Beltrán-Anaya FO, Poblete TM, Román-Román A, Reyes S, de Sampedro J, Peralta-Zaragoza O, et al. The EPIYA-ABCC motif pattern in CagA of *Helicobacter pylori* is associated with peptic ulcer and gastric cancer in Mexican population. BMC Gastroenterol 2014;14:1–11.
[80] Batista SA, Rocha GA, Rocha AM, Saraiva IE, Cabral MM, Oliveira RC, et al. Higher number of *Helicobacter pylori* CagA EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. BMC Microbiol 2011;11:1–7.
[81] Quiroga AJ, Huertas A, Cómbita AL, Bravo MM. Variation in the number of EPIYA-C repeats in CagA protein from Colombian *Helicobacter pylori* strains and its ability middle to induce hummingbird phenotype in gastric epithelial cells. Biomedica 2010;30:251–8.
[82] Papadakos KS, Sougleri IS, Mentis AF, Hatziloukas E, Sgouras DN. Presence of terminal EPIYA phosphorylation motifs in *Helicobacter pylori* CagA contributes to IL-8 secretion, irrespective of the number of repeats. PloS One 2013;8(2):e56291.
[83] Roshandel G, Ghanbari-Motlagh A, Partovipour E, Salavati F, Hasanpour-Heidari S, Mohammadi G, et al. Cancer incidence in Iran in 2014: results of the Iranian national population-based cancer registry. Cancer Epidemiol 2019;61:50–8.
[84] Malekzadeh R, Sotoudeh M, Derakhshan M, Mikaeli J, Yazdanbod A, Merat S, et al. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the northwest of Iran. J Clin Pathol 2004;57:37–42.
[85] Abdi M, Hakemi-Vala M, Naji T, Nejadeh AH, Tajik A. Seroepidemiology of the *Helicobacter pylori* infection among people of Pishva city of Varamin. Iran J Med Microbiol 2015;8:20–7.
[86] Crew KD, Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol 2006;12(3):354–62.
[87] 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. Microb Pathog 2018;118:214–9.
[88] Latifi-Navid S, Ghorashi SA, Siavoshi F, Linz B, Massarrat S, Khegay T, et al. Ethnic and geographic differentiation of *Helicobacter pylori* within Iran. PloS One 2010;5(3):e9645.
[89] Devi SM, Ahmed I, Francalacci P, Hussain MA, Akhter Y, Alvi A, et al. Ancestral European roots of *Helicobacter pylori* in India. BMC Genom 2007;8:1–11.
[90] Schmidt HMA, Goh KL, Fock KM, Hilmi I, Dhamodaran S, Forman D, et al. Distinct cagA EPIYA motifs are associated with ethnic diversity in Malaysia and Singapore. Helicobacter 2009;14:256–63.
[91] Keikha M, Ali-Hassanzadeh M, Karbalaei M. Association of *Helicobacter pylori* vacA genotypes and peptic ulcer in Iranian population: a systematic review and meta-analysis. BMC Gastroenterol 2020;20:1–11.
[92] Keikha M. Is there a relationship between *Helicobacter pylori* vacA i1 or i2 alleles and development into peptic ulcer and gastric cancer? A meta-analysis study on an Iranian population. New Microb New Infect 2020;36:100726.