**EPIYA Motif Genetic Characterization from *Helicobacter pylori* Isolates in Distinct Geographical Regions of Iran**

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**Abstract**

**Background:** This study aimed to determine the current EPIYA motifs of the cagA gene in *Helicobacter pylori* isolates from patients with gastric disorders, and evaluate the association between these patterns and the clinical outcome of *H. pylori* infection in different geographical regions of Iran.

**Materials and Methods:** We examined 150 patients with gastrointestinal disorders from the central and eastern regions of Iran. The detection of *H. pylori* and screening of cagA was performed by polymerase chain reaction (PCR). The pattern of the motifs was determined by PCR followed by sequencing.

**Results:** The overall prevalence of *H. pylori* was 66.3% in eastern (Mashad) and 50.6% in the central (Isfahan) part of Iran. The frequency of cagA-positive strains in Mashad and Isfahan were 63.4% and 56.7%, respectively. The pattern of EPIYA motif was as follows: 43 (79.6%) ABC, 7 (12.9%) AB, 4 (7.4%) ABCC, and one (1.9%) ABCCC. We also identified a novel EPIYA C sequence motif which showed association with gastric cancer (GC). The relationship between the frequency of specific EPIYA motifs and GC was statistically significant (*P* ≤ 0.05).

**Conclusions:** This is the first report for the determination of the cagA EPIYA motif of *H. pylori* in the Northeast and center of Iran. The prevalence of cagA positive *H. pylori* between the two regions was significant (*P* ≤ 0.05). All isolates of the *H. pylori* cagA were western type (ABC). The increase in the number of EPIYA-C repeats was associated with GC (*P* ≤ 0.01).

**Keywords:** CagA, gastric cancer, gastrointestinal diseases, *Helicobacter pylori*, Iran

**Introduction**

*Helicobacter pylori* is a spiral-shaped, Gram-negative, and microaerophilic bacteria that infect human gastric mucosa and cause gastric disorders, including gastric adenocarcinoma, peptic ulcer, and chronic gastritis.¹ Although nearly half of the global population is infected with *H. pylori*, only a small number of people develop severe diseases. This indicates that the environmental factors, diversity of virulence genes in *H. pylori* isolates, and host immune status may be supportive in the outcome of the disease.² Various *H. pylori* genes have been identified, which are involved in the pathogenicity of this pathogen. A major *H. pylori* virulence determinant is cytotoxin-associated gene A (cagA), which is located within the *cag* Pathogenicity Islands.³ The CagA protein is delivered into the gastric epithelial cells. Upon delivery into the gastric epithelial cells, CagA is phosphorylated by the Src kinases family.⁴ Tyrosine CagA phosphorylation sites are characterized by a unique 5

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amino acid sequence (Glu-Pro-Ile-Tyr-Ala), called the EPIYA motif, which exists in variable numbers in the C-terminal cagA region.[3] Then, CagA interacts with host proteins, which are involved in signaling pathways of destroying cell connections, altering epithelial cell polarization, and inducing the inflammatory and mitogen responses.[6] Based on the epidemiological studies, cagA was found to increase the risk for peptic ulceration and gastric cancer (GC). H. pylori cagA-positive is found in 50%–70% of isolates in Western countries and 80%–100% in Asian communities.[13] Extensive polymorphic status of the 3’ end of cagA gene influences the biological activity and has a subsequent exclusive effect on H. pylori-related disorders.[8] According to the amino-acid sequence surrounding the EPIYA motif, four distinct EPIYA types (A-B-C-D) are defined.[9] EPIYA-A and EPIYA-B fragments are present in almost all cagA isolates. In contrast, the EPIYA-C component is characteristic of H. pylori cagA in Europe, North America, and Australia. Therefore, cagA species containing the EPIYA-C component are shown as “Western cagA.” Similarly, the EPIYA-D fragment is specific for H. pylori cagA, which is native to East Asia. Accordingly, cagA with the EPIYA-D section is called “cagA East Asian.”[13] The EPIYA-D motif is phosphorylated more compared to EPIYA-C. However, the potential of inducing GC in EPIYA-ABCCC is similar to EPIYA-D in H. pylori cagA positive patients.[10] East Asian strains increase the risk of gastric ulcer and cancer, although the number of EPIYA-C motifs within the Western-type cagA has been related to an increased risk of stomach cancer.[11]

Iran is located in the Middle East, and according to previous studies, the prevalence of H. pylori infections is 60%–90%.[12-15] Due to migration from eastern countries, the frequency of H. pylori genotypes in a different region could vary. However, the number of previous studies is too small to explain the correlation between different EPIYA motifs and the outcome of the disease in this region.[16] This study aimed to determine the pattern of EPIYA motif of cagA positive strains, with the polymerase chain reaction (PCR)-based sequencing method in H. pylori-positive clinical isolates, and compare the relationship between EPIYA motif typing pattern and the clinical outcomes in the Central and Northeastern region of Iran.

**Materials and Methods**

**Clinical specimens**

During 2017–2019, a cross-sectional study was performed on patients who were living in two different geographical regions: Mashad (the provincial capital in the northeastern) and Isfahan, which is the center of the province in the central part of Iran. All patients with gastrointestinal symptoms who were subjected to upper endoscopy were enrolled in the study. None of the patients had received non-steroid anti-inflammatory drugs, proton pump inhibitors, or antibiotics a month before endoscopy. Patients with a previous history of cancer, chemotherapy treatment were excluded from the experiment. A questionnaire has been completed from all patients according to demographic information before gastric endoscopy at the referral Al-Zahra Hospital in Isfahan and the referral Pathobiology Centre in Mashad. A total of 150 patients affected by gastritis, intestinal metaplasia, and gastric adenocarcinoma were included in the study. Informed consent was obtained from the subjects according to the issued regulations approved by the Ethics Committee of Isfahan University of Medical Sciences (IR. mui. Rec. 1396.3765).

**Isolation of Helicobacter pylori**

Two biopsy were taken from the antrum and the corpus during the endoscopy procedure. The biopsy samples for bacterial culture were placed in transport media (0.9% normal saline) and immediately transported to the microbiology laboratory. The specimens were homogenized and inoculated directly onto H. pylori selective media (Colombia agar, Gibco, U.S.A) supplemented with (5%) defibrinated sheep blood, fetal calf serum (10%), and campylobacter selective supplement (Merck Co., Germany). The plates were incubated at 37°C for 5–7 days under microaerophilic conditions (Anoxomat; MART Microbiology BV, Drachten, the Netherlands). H. pylori were identified by gram stain (spiral red rod), the morphology of colony (small, translucent colonies), and positive reactions to oxidase, catalase, and urease. For histology, other biopsy samples were fixed in 10% formalin and the thin 4 μm sections were stained with H and E. Histological stages of gastrointestinal disease were assessed by a skilled pathologist and classified by the updated Sydney classification system.[17]

**Detection of Helicobacter pylori by polymerase chain reaction**

Genomic DNA was extracted from colonies using the QIAamp DNA mini kit (QIAGEN, Qiagen, Hilden, Germany) based on the manufacturer’s protocol. PCR was performed to confirm H. pylori using the ureA gene primers. The reaction was done in a final volume of 25 μl including (Taq DNA Polymerase 2x Master Mix RED (Ampliqon)/Denmark), 1.5 mM MgCl₂, 0.5 μM of each primer (Forward primer: 5’-AGTGGGTATTGAAGCGATG-3’ and reverse primer: 5’-TGCTTTTCGTGTCTGCTTT-3’, and 2 ng of template DNA. The reaction conditions included an initial denaturation step 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 59°C for 40 s, 72°C for 30 s, and a final extension at 72°C for 5 min.

The PCR product was purified and sequenced bilaterally to verify H. pylori. For all reactions DNA sample of H. pylori (ATCC 26695) was used as a positive control.

**Screening of cagA and typing of EPIYA motif**

H. pylori-positive samples (histology, culture, and ureA-PCR) were subjected to PCR to detect cagA gene, as described previously.[18] Primers cag2 (5’-GGAAACCTAGTCGGTAATG-3’), as a forward primer, and cag4 (5’-ATCTTTGACCTTGCTCATCG-3’), as
a reverse primer, were used to amplify the whole 3′ variable region of cagA gene. The reaction was done in a final volume of 50 µl including (Taq DNA Polymerase 2x Master Mix RED (Ampliqon/Denmark), 1.5 mM MgCl₂, 0.5 µM of each primer, and 2 ng of template DNA. PCR was performed under the following conditions: Initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, 50°C for 40 s and 72°C for 30 s, final extension at 72°C for 5 min.\textsuperscript{[19]} Agarose gel (1.5%) was used for the electrophoresis of the PCR product. For all reactions, a cagA positive strain (H. pylori ATCC 26695) was used as a positive control.

**DNA sequence analysis**

PCR products were purified using the Silica Bead DNA Gel Extraction kit (Fermentas, USA). Sequencing was done on both strands by Niagene noor Co. (Tehran, Iran). Mega 10 software was used for the alignment of partial CagA peptide sequences, and the analysis of the EPIYA specific amino acid sequences was compared with the total CagA protein sequences available in the GenBank database. The nucleotide sequences of the EPIYA motifs have been deposited in the GenBank database under accession no. OK077137-OK077158.

### Table 1: Sociodemographic, disease, and regions

| Diagnosis     | Gender | Northeast | Center | Total |
|---------------|--------|-----------|--------|-------|
|               | Female, n (%) | Male, n (%) | P     | Female, n (%) | Male, n (%) | P     | Female, n (%) | Male, n (%) | P     |
| Gastritis     | 10 (56) | 15 (44) | >0.05\textsuperscript{1} | 8 (30) | 17 (70) | >0.05\textsuperscript{1} | 19 | 32 | >0.05\textsuperscript{1} |
| Metaplasia    | 11 (5)  | 14 (44) |         | 7 (28) | 18 (72) |         | 18 | 32 |         |
| Carcinoma     | 12 (48) | 13 (52) |         | 7 (28) | 18 (72) |         | 19 | 31 |         |

| Diagnosis     | Age (mean, SD) | P     | Age (mean, SD) | P     | Age (mean, SD) | P     |
|---------------|----------------|-------|----------------|-------|----------------|-------|
| Gastritis     | 46.52 (19.38) | ≤0.01\textsuperscript{2} | 49.2 (19.60) | ≤0.01\textsuperscript{2} | 47.9 (14.5) | ≤0.01\textsuperscript{2} |
| Metaplasia    | 59.5 (14.46)  |       | 65.5 (13.66)  |       | 62.5           |       |
| Carcinoma     | 61.24 (13.73) |       | 61.96 (15.49) |       | 61.6 (16.61)  |       |

| Diagnosis     | Education (median, years) | P     | Education (median, years) | P     | Education (median, years) | P     |
|---------------|---------------------------|-------|---------------------------|-------|---------------------------|-------|
| Gastritis     | 9                         | >0.05\textsuperscript{4} | 12            | >0.05\textsuperscript{4} | 10.5 | ≤0.05\textsuperscript{5}  |
| Metaplasia    | 5                         |       | 9                         |       | 7                         |       |
| Carcinoma     | 5                         |       | 9                         |       | 7                         |       |

| Diagnosis     | BMI (mean, SD) | P     | BMI (mean, SD) | P     | BMI (mean, SD) | P     |
|---------------|----------------|-------|----------------|-------|----------------|-------|
| Gastritis     | 24.16 (3.98)  | >0.05\textsuperscript{5} | 24.4 (4.02) | >0.05\textsuperscript{5} | 24.28 (4.0) | >0.05\textsuperscript{5} |
| Metaplasia    | 24.0 (4.1)    |       | 24.2 (5.1)     |       | 24.1 (4.6)     |       |
| Carcinoma     | 25.67 (5.25)  |       | 25.36 (4.74)   |       | 25.51 (4.9)    |       |

\textsuperscript{1}χ² test, \textsuperscript{2}ANOVA test, \textsuperscript{3}Kruskal-Wallis test. SD: Standard deviation, BMI: Body mass index

### Statistical analysis

SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. ANOVA, Chi-square, and Fisher’s exact probability tests were used to determine the association between cagA types and clinical manifestation. A \( P < 0.05 \) was considered statistically significant (\( P < 0.05 \)).

### Results

#### Demographics, gastrointestinal diseases, and regions

A total of 150 patients with gastrointestinal diseases were enrolled in this study. Based on histopathology method, 46 patients had gastritis, 54 had adenocarcinoma, and 49 had intestinal metaplasia. The population consisted of 75 patients from the Mashad (33 women and 42 men, with the age range of 18–95, and mean age of 58 years) and 75 patients from the Isfahan (22 women and 53 men, with the age range of 8–93, and the mean age of 55 years). Patients with adenocarcinoma were significantly older (\( P \leq 0.001 \)). The statistical relationship between age and type of the disease was similar in both regions. Education levels were significant among the groups (\( P < 0.05 \)) [Table 1].

#### Frequency of cagA in Helicobacter pylori infections

The presence of ureA gene of H. pylori was detected in 89 specimens (59.3%). People who were living in the...
Northeast had a relatively higher \textit{H. pylori} infection rate than those in the center (odds ratio [OR] = 2.26 and 0.79, respectively), [Table 2]. Among 89 \textit{H. pylori}-positive patients, 35 (39.3\%) had gastritis, 26 (29.2\%) had adenocarcinoma, and 28 (32.5\%) had intestinal metaplasia [Figure 1a]. There was no statistically significant relationship between the prevalence of \textit{H. pylori} and the clinical outcome of the disease, gender, age, and other sociodemographic in either region [Tables 2 and 3].

Among the 89 \textit{H. pylori}-positive patients, 54 (60.7\%) were cagA positive. The prevalence of the cagA positive strains in Mashad and Isfahan were 61.1\% (33/54) and 56.7\% (21/37), respectively. The prevalence of cagA positive \textit{H. pylori} between the two regions was significant (P ≤ 0.05), [Figure 1b]. There was a significant difference in the frequencies of the cagA gene among the study groups (P ≤ 0.01).

Sequence analysis of 3´ variable region

The PCR products of cagA positive samples were showed four electrophoretic patterns in accordance with the following EPIYA motifs: AB, ABC, ABCC, and ABCCC, [Figure 2]. The results were confirmed by sequencing the 3´ variable region of the cagA gene in 54 samples. The EPIYA ABC motif was detected in 42 (77.7\%) samples, ABCC in 4 (7.4\%), AB in 7 (12.9\%), and ABCCC were found in one patient with adenocarcinoma, Figure 1c. The ABC motif was most prevalent among the cagA positive patients in Isfahan with gastritis (83\%), intestinal metaplasia (85\%), and gastric adenocarcinoma (62.5\%). The frequency of cagA EPIYA-ABC as the most prevalent motif was 77.7\%, 78.6\%, 80\%, in patients with gastritis, gastric adenocarcinoma, intestinal

| Variable          | \textit{H. pylori} positive/total n (%) | OR   | 95\% CI | P  |
|-------------------|----------------------------------------|------|---------|----|
| Place             |                                        |      |         |    |
| Northeast         | 52/75 (60.3)                           | 2.26 | 1.26-4.06 | ≤0.05 |
| Center            | 38/75 (50.7)                           | 0.79 | 0.45-1.37 |     |
| Gender            |                                        |      |         |    |
| Male              | 51/95 (53.6)                           | 1.27 | 1.11-2.17 | >0.05 |
| Female            | 39/55 (51.4)                           |      |         |    |
| Education         |                                        |      |         |    |
| High education    | 37/66 (56)                             | 1034 | 0.69-2.59 | >0.05 |
| Highschool and lower | 53/84 (63)           |      |         |    |

\(P<0.05\), Chi-square, estimate risk by OR and 95\% CI. OR: Odds ratio, CI: Confidence interval, \textit{H. pylori}: \textit{Helicobacter pylori}

### Table 2: Association of sociodemographic, region with \textit{H. pylori} infection

### Table 3: Association of \textit{H. pylori} infection, cagA and and EPIYA pattern with gastrointestinal disease

#### cagA genotype

| Diagnosis   | Northeast | H. pylori | Center | Total | P  | n | OR   | 95\% CI | P  | n | OR   | 95\% CI | P  | n | OR   | 95\% CI |
|-------------|-----------|-----------|--------|-------|----|----|------|---------|----|----|------|---------|----|----|------|---------|
| Gastritis   | 19        | 1.44      | 0.5-4.3|       |    | 16 | 2.26 | 0.8-6   |    | 35 | 1.9  | 1-3.9   |    |    |      |         |
| Metaplasia  | 17        | 1.04      | 0.4-3  |       |    | 12 | 0.8  | 0.3-2.1 |    | 29 | 1    | 1-4.6   |    |    |      |         |
| Carcinoma   | 16        | 0.6       | 0.2-1.8|       |    | 10 | 0.5  | 0.2-1.4 |    | 26 | 0.6  | 0.2-1.8 |    |    |      |         |

#### EPIYA motif

| Diagnosis   | AB   | OR   | 95\% CI  | ABC  | OR   | 95\% CI  | ABCC | OR   | 95\% CI  | P  | n | OR   | 95\% CI |
|-------------|------|------|----------|------|------|----------|------|------|----------|----|----|------|---------|
| Gastritis   | 3    | 2.2  | 0.4-11.2 | 12   | 1.2  | 0.3-5.2  | -    | -    | -        |    |    |      |         |
| Metaplasia  | 3    | 1.8  | 0.4-8.9  | 14   | 1.5  | 0.4-6.4  | -    | -    | -        |    |    |      |         |
| Carcinoma   | 1    | 0.2  | 0.02-1.9 | 16   | 0.5  | 0.1-2    | 4    | 9.41b| 1-87.2   |    |    |      |         |

#### Number of EPIYA-C

| Diagnosis   | 1C   | ≥2C  | OR   | 95\% CI | P  | n | OR   | 95\% CI |
|-------------|------|------|------|---------|----|----|------|---------|
| Gastritis   | 15   | -    | -    | -       |    |    |      |         |
| Metaplasia  | 14   | -    | -    | -       |    |    |      |         |
| Carcinoma   | 16   | 4    | 9.06 | 1-84.5  | ≤0.01c|

\(P<0.05\), Chi-square test, *P<0.05* Fisher’s exact test, †Fisher’s exact test. OR: Odds ratio, CI: Confidence interval, cagA: Cytotoxin-associated gene A, \textit{H. pylori}: \textit{Helicobacter pylori}
metaplasia, in the Northeast [Table 3 and Figure 1c]. Based on the bioinformatic analysis and peptide sequence alignment of the EPIYA motifs it was observed all sequenced cagA variable regions corresponded to the Western-type, with three EPIYA motifs: EPIYA-A EPIYAKVNKKKAGQ; EPIYA-B EPIYA (A/T) QV AKKVNAKI, and EPIYA-C EPIYA TIDDLGGP. No isolate corresponded to the EPIYA-D or East Asian type: EPIYATIDFDEANQAG was detected in the population studied. Two CRIPA motifs (FPLKRHDKVDDLSKVG) were observed in all sequences which were located before and after each EPIYA-C segment.

**Discussion**

The incidence of GC is closely related to the prevalence of *H. pylori* worldwide.\(^{[21]}\) *H. pylori* seem genetically diverse, and its various strains can be dispersed among different ethnic groups or geographical areas, and this could affect the risk of cancer to be variable in different regions.\(^{[22]}\) In Iran, the ethnic-geographic diversity is so high and almost 69% of Iranian people are infected with *H. pylori*.\(^{[23]}\) Although the migration from highly prevalent neighboring countries (Afghanistan, and Pakistan) to the northeast of Iran is high, few studies are available from this region. We have shown that gastrointestinal disorders are related to age, level of education but not to gender, and obesity. In this study, we found that the *H. pylori* infection rate in the Northeast is slightly higher than those in the central region of Iran (OR = 2.26, 95% CI 1.26–4.06; and 0.79,
95% CI 0.45–1.37, respectively). The relationship between the prevalence of *H. pylori* and the clinical outcome of the disease was not statistically significant (*P* > 0.05). Likewise, in some countries such as India (known as Asian enigma) the high prevalence of *H. pylori* infection is not correlated with high frequencies of GC.[24] It seems that the prevalence of GC depends on the pathogenic factors of the bacterial strains, environmental co-factors, and host susceptibility.[25] Therefore, we further examined the presence of *cagA*, as one of the most important virulence factors of *H. pylori*, in the two regions. Similarly, our *cagA* positivity rate (60.7%) for *H. pylori* isolates was in agreement with the global prevalence of *cagA*‑positive strains in western countries, and other regions of Iran.[26‑28] We addressed that the prevalence of *H. pylori*‑*cagA* positive strains in Mashad is higher than in Isfahan (*P* ≤ 0.05). The presence of *cagA*-positive *H. pylori* is significantly associated with the outcome of gastrointestinal diseases such as peptic ulcers, intestinal metaplasia, and GC.[29] In this study, we detailed a strong association between the *cagA*-positive genotype and the severity of the disease (*P* ≤ 0.01). The ORs were increased with the severity of the disease (OR = 0.6 for gastritis and 2.1 for adenocarcinoma). Also, *H. pylori* *cagA* positive genotype increases the risk of GC (OR = 1.6, 95% CI: 0.8–3.3) relative to the risk of *H. pylori* infection alone (OR = 0.6, 95% CI: 0.2–1.8). Similarly, Doohan et al. indicated that the *H. pylori* IgG seropositive with *CagA* IgG low titer was the strongest risk factor for noncardia GC (relative risk [RR], 3.9; 95% CI: 2.1–7.0; *P* < 0.001), compared with *H. pylori* IgG seropositive with *CagA* IgG negative (RR, 2.2; 95% CI: 1.3–3.9; *P* = 0.0052).[30] In another study related to *cagA*, *cagA*-positive strains were significantly associated with GC and PU.[3] In Western countries, the prevalence of *cagA* positive *H. pylori* genotype is 50%–70%. Given the fact that Iranian strains are in the same group as *hpEurope* population,[23] it is likely that the *cagA* positive genotype may be a useful biomarker for the prediction of clinical outcomes in Iran following western countries.

On the other hand, despite a high prevalence of *cagA* positive *H. pylori* genotype (90%–100%) in East Asian countries, this high prevalence of the *cagA* positive genotype is not related to clinical outcomes.[31] It is reported that types of EPIYA have a major role in the development of gastrointestinal diseases.[32] EPIYA motif is used as a tool for identifying the circulating *H. pylori* as well as epidemiological studies.[33] We further analyzed the sequences surrounding the EPIYA for the first
time in these two regions. We did not find EPIYA-D (East Asian type) in these regions, which is compatible with the findings of previous studies in Iran. In 77.7% of the cases, the cagA gene contained an EPIYA-C motif in the typical ABC sequence, and this was more frequent in patients with adenocarcinoma. In other Iranian studies related to EPIYA-C repeats, Vaziri et al. indicated that the H. pylori-positive patients with gastritis had 68% EPIYA-ABC. There is evidence in Western countries that the number of EPIYA motifs, especially section C, increased the risk of intestinal metaplasia and GC. For instance, the frequency of strain rate exceeding one EPIYA-C (ABCC) replication was 51.1% in Colombia and 33.3% in Italy, which is much higher as compared to the present findings. This would explain those countries as higher GC risk regions than Iran. Although in our study the frequency of the ABC motif in gastric adenocarcinoma and intestinal metaplasia was high, the relationship between the ABC motif and the clinical outcome was not statistically significant. The results of this study show only a relationship between the clinical outcome of the disease and the EPIYA ABCC motif, which is consistent with studies in Iran and other countries. The study conducted by Vaziri et al. showed a significant association between ABC motif and duodenitis. The result of Ajami et al. revealed that CagA-positive H. pylori with ABCC motifs are associated with the risk of GC. In our study, number of EPIYA-C repeats increases 9.01 times the risk of adenocarcinoma (95%CI [0.45–42.8], \( P < 0.01 \)). This result was in agreement with other studies in the EPIYA motif of the western genotypes. It has been suggested that CagA-EPIYA ABC might be associated with GC, while EPIYA-AB might be associated with duodenal ulcers. Polymorphisms in the EPIYA motif pattern vary according to geographical and ethnic distributions due to the influences of environmental conditions and physiological characteristics of populations in different regions. We did not find more than one C repeat in other groups than GC. It might be possible that the number of C repeats in the EPIYA motif could be a good biomarker for detecting or predicting GC. Therefore, further study using a large number of participants will be necessary to investigate this relationship. In some studies, a strong association was found between other pathogenic factors and the incidence of H. pylori-related diseases. In the Northwest of Iran, where the GC risk is high in males, the relationship between the virulence factor, vacA (1-1-d1-genotypes), and the risk of GC have been studied. Xue et al. have also previously shown the possible relationship between H. pylori pathogenic factors such as oipA, babA, icaA, and vacA in China and their clinical outcome. Therefore, examining the other virulence factors in cagA-positive strains might improve the geographic origin diversity and could probably better predict the GC risk in different geographical regions. Furthermore for cancer risk, among Western strains, the most important factor is the number of cagA EPIYA-C segments.

The Agency for Research on Cancer (GLOBOCAN, 2018) estimated that the prevalence of GC in Iran is 25.37 cases per 100,000 people. Since more than 80% of cases of GC have been attributed to H. pylori infection, a program including detecting H. pylori, identifying the important virulence factors would be useful to monitor patients with gastroduodenal disease in hospital laboratories.

**Conclusions**

The findings indicated that the Western type of cagA gene is predominant in two geographically different regions of Iran. This is the first report of prevalence and genotyping of H. pylori in the northeast and center (Isfahan) of Iran. The frequent EPIYA motifs of CagA were ABC. We also identified a novel EPIYA C sequence motif which showed association with GC. Further study with a larger number of isolates is needed to confirm the proposed association of the identified sequence motifs and GC. We found a strong association between the number of C repeats in the EPIYA motif of the H. pylori isolated from GC; thus, it might be used as an important biomarker for predicting the GC risk in Iran.

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**Conflicts of interest**

There are no conflicts of interest.

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