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Research article

Determination of *CagA* EPIYA motif in *Helicobacter pylori* strains isolated from patients with digestive disorder

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ABSTRACT

This study was conducted to identify patterns of *cagA* EPIYA motifs in *H. pylori* strains isolated from patients with gastrointestinal diseases in Hospitals of Shahrekord, and investigate the association between these biomarkers and clinical outcomes of gastrointestinal diseases due to *H. pylori*. In this study, 253 patients with gastrointestinal diseases were studied within 1395–1396. Histopathological investigations and urease test showed that 207 isolates were *H. pylori*-positive. Then, screening using a molecular technique, PCR, confirmed that 159 isolates had cagA. Finally, the pattern and prevalence of the motifs were determined by PCR and identified a number of motifs were sequenced.

Results of this study showed that the pattern of motifs was as follows: ABC (140 isolates) (93/7%), ABCC (6 isolates) (3/77%), ABCCC (4 isolates) (2/5%), AB (7 isolates) (4/4%), AC (1 isolate) (0/6%), and BC (1 isolate) (0/6%). Sequencing results showed the presence of changed EPIYA motif in some isolates. CM motif sequence was also seen in all isolates. In this study, no significant association was seen between the prevalence rate of different patterns and clinical symptoms ($p = 0.71$). There is a slight association between the presence of ABC motifs and the type of digestive disorder ($p = 0.056$). Results indicated that ABC was the most frequently seen pattern however, in such that positive cases of ABC motifs were more common in gastritis. All isolates had kinase phosphorylation region, and the observed pattern in this region was a generally western type (ABC).

1. Introduction

Helicobacter pylori is a gram-negative and microaerophilic bacillus that invades human gastric mucosa and is the cause of chronic gastritis and an effective risk factor for certain diseases such as gastric and duodenal ulcers and gastric adenocarcinoma [1]. Many bacteria, including Enterobacteriaceae (*Shigella*, *E. coli*, *Salmonella*, etc.) are involved in causing chronic gastritis, although *H. pylori* is known as the most common cause of chronic gastritis [1, 2, 3]. Only a few number of the people with *H. pylori* infection develop peptic, duodenal and gastric ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma [4]. Over half of the world's population are infected with *H. pylori*. The prevalence of *H. pylori* infection is comparatively higher in developing countries such that over 80% of the people in these countries are infected with *H. pylori* infection [5]. Environmental factors, host genetic characteristics, and bacterial pathogenic factors contribute to the final outcome of bacterial pathogenesis [6].

Although various characteristics of *H. pylori* including urease, flagellum, adhesins, vaccine cytotoxin and cag pathogenicity island can be involved in pathogenicity, the most important factor involved in pathogenicity is likely the genes belonging to the cag pathogenicity island [7]. The strains type I whose genomes have cag pathogenicity island, have been found to be more likely to cause peptic ulcers and cancer [8]. Pathogenic islands (cag PAI) approximately 40kb in size encode type IV secretion system in *H. pylori* through which the *cagA* gene is transported into the host cell, and then the cell progresses toward anarchy through the phosphorylation of the end of the *cagA* gene (the EPIYA sequence) on the cag PAI [9].

This protein is also able to exert various effects on host cells in two phosphorylation-dependent and -independent ways, ultimately leading

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the infected cell to malignancy [10]. *CagA* is one of the genes of cag PAI that is not observed in all *H. pylori* strains, and thus it is used as a marker of cag PAI presence [11]. The carboxyl ends of the cagA protein, containing repeat phosphorylation components [Glu-Prp-Ile-Tyr-Ala (EPIYA) motifs], are widely varied in different *H. pylori* strains. The numbers and combinations of different motifs vary depending on geographical regions and determine the clinical outcome of the disease [5]. The *cagA* gene contains EPIYA motifs (including four classes A, B, C, and D) that have been detected in different strains of *H. pylori* based on the presence of repeat regions. EPIYA fragments can occur as the combinations ABC, ABCC, ABCCC and ABBDC [1]. EPIYA motifs are indeed phosphorylated motifs whose sequence is Glu-Prp-Ile-Tyr-Ala and that are classified as EPIYA-A, -B, -C, and -D according to the amino acids of their structures [12]. The main phosphorylation site of EPIYA motif is the Tyr of the EPIYA-C or -D motif. The phosphorylation of these motifs is necessary for binding to Src homology region 2 domain-containing phosphatase (SHP2) and exerting pathogenicity [13].

To date, two types of combinations of these motifs, i.e. western *cagA* and eastern *cagA*, have been detected. The EPIYA-A and -B motifs are preserved in both western and eastern types, while EPIYA-C is exclusive to the western combination and EPIYA-D to the eastern combination [13]. Our aim was to identify the patterns of the EPIYA motifs of the *cagA* gene in the *H. pylori* strains isolated from the patients with gastrointestinal diseases in Kashani and Hajar Hospitals, Shahrekord, southwest Iran as well as to investigate the association between these biomarkers and the clinical outcomes of the gastrointestinal diseases due to *H. pylori*.

2. Experimental section

2.1. Clinical sampling

A total of 253 patients with gastrointestinal diseases referring to the studied hospitals were enrolled. For all patients, a questionnaire including items on age, gender and taking antacids and antibiotics was completed. The patients with the history of gastric surgery and *H. pylori* elimination treatment and those receiving H2 receptor blocker, nonsteroidal anti-inflammatory drugs and pomp protein blockers and proton-pump inhibitors were excluded [13]. The study was approved by the human research ethics committee at Shahrekord University of Medical Sciences (Ethical code: IR.SKUMS.1395.76) and informed consent was obtained from each volunteer before participation.

2.2. Collection of tissue samples

Three tissue samples were taken from each patient after endoscopy and confirmation of gastrointestinal disease diagnosis. The first sample was used for rapid urease test. The second sample was immediately 10% formalin-fixed for histological analysis, and the third sample was placed in a buffer solution [Tris (10mM, pH = 8), EDTA (2mM, pH = 8) and 5.5% SDS] and stored at -20 °C till molecular test [14].

2.3. Histopathological investigations

According to the Sydney System guidelines, a tissue section of the biopsy was taken and then the grades of acute inflammation (polymorphonuclear infiltration) and chronic inflammation (mononuclear penetration), granulocarcinoma atrophy, intestinal metaplasia and *H. pylori* colonization (0–3) were determined by hematoxylin and eosin staining and Giemsa Stain Procedure. In addition, the infiltration grade and the number of neutrophils and mononuclear cells in lamina propria at ×200 magnification were counted in five fields. Finally, the number of cells was graded on a four-point (0–3) basis [15].

2.4. DNA extraction

According to Diatom Kit protocol (Bio Flux, Japan), genomic DNA from biopsy samples was extracted and assessed in terms of quantity using Nanodrop 2000 (Thermo Fisher Scientific Inc., USA).

2.5. Detecting H. pylori

The initial diagnosis of *H. pylori* was made by rapid urease test, but the definitive diagnosis based on the molecular detection of the glmM gene using a forward-sequence primer with 5-AAGCTTTTAGGGGTGT-TAGGTTT-3 sequence and a reverse primer with 50-AAGCTTACTTTC-TAACACTAACGC-30 sequence [16].

2.6. CagA gene amplification

H. pylori 16S rRNA gene-positive samples were selected for PCR to identify the *cagA* gene using the primers which previously described by Figura et al. [17]. To amplify a 550- to 850-bp region within the 3′ variable region of the *cagA* gene, the primers cag2 and cag4 were using (Table 1) [18,19]. The reaction mix consisted of 1.5 mM MgCl2, 2.5 mM buffer (KBC), 0.2 mM dNTPs (KBC), 0.5pmol of each primer, 1 U of Taq DNA polymerase (KBC), and 50 ng of total DNA in a total volume of 25 μl. The amplification conditions were used: 1 cycle at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 40 s; and a final extension cycle at 72 °C for 5 min. The PCR products were subjected to electrophoresis on polyacrylamide gel (PAG), followed by staining with silver nitrat. At least 1 of the 2 bands was observed in the samples were considered *CagA*-positive.

2.7. Amplification of the cagA gene 3′ variable region and EPIYA motif prediction

To identify the EPIYA motifs, each cagA-positive sample was subjected to 4 PCR reactions. The Forward primer cag28F was used in all 4 reactions, while the Reverse primers *cagA* P1C, cagAP2TA [18] *CagA* West, and *CagA* East [19] were used in distinct reactions to amplify the

Table 1. Primers used in this study.

Primers	Sequences	Product size (bp)	EPIYA motif	References
glmM	AAGCTTTTAGGGGTG TTAGGTTT AAGCTTACTTTCTAACACTAACGC	294		[14]
cagAF D008 cagAR R008	ACAATGCTAAATTAGACAACTTGAGCGA TTAGAATAATCAACAAACATCACGCCAT	298		[15]
cag2F	GGAACCCTAGTCGGTAATG	550 to 850		[16, 34]
cag4	ATCTTTGAGCTTGTCTATCG	550 to 850		[12, 34]
cagA28F	TTCTCAAAGGAGCAATTGGC		Forward for all EPIYA motifs	[16]
cagA-P1C	GTCCTGCTTTCTTTTTATTAACTTKAGC	264	EPIYA-A	[16, 17]
cagA-P2TA	TTTAGCAACTTGAGTATAAATGGG	306	EPIYA-B	[16, 17]
cagAWest	TTTCAAAGGGAAAGGTCCGCC	501	EPIYA-C	[17]
cagAEast	AGAGGGAAGCCTGCTTGATT	495	EPIYA-D	[17]

EPIYA-A (~264 bp), B (~306 bp), C (~501 bp), and D (495 bp) motifs, respectively, Table 1.

All PCR samples were performed in a final reaction volume of 25 μl, including 0.5mM dNTPs (KBC), 1.5 mM MgCl2, 2.5 mM Buffer (KBC), 0.5 pmol of each primer, 1 U of Taq DNA Polymerase (KBC), and 50 ng of total gastric biopsy DNA. The amplification conditions were used: 1 cycle at 94 °C for 5 min; 35 cycles at 94 °C for 1 min, 59 °C for 30 s, and 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. The PCR products were separated by electrophoresis on polyacrylamide gel, followed by staining with silver nitrate.

2.8. Sequencing and bioinformatics analysis of the cagA gene 3′ variable region

A subset of 9 samples was randomly selected for sequencing to confirm the PCR results. Cag28F and cag4 primers were used to amplify the variable region and generate ~650 to ~850-bp amplicons. The PCR reaction was conducted in a 50-μl volume with 15 pmol of each primer, 0.3 mM dNTPs, 2 mM MgCl2, and 1 U of Platinum® Taq DNA Polymerase (Invitrogen Carlsbad, CA, USA) per reaction. The amplification conditions were as follows: 1 cycle at 94 °C for 5 min; 30 cycles at 94 °C for 40 s, 55.5 °C for 30 s, and 72 °C for 50 s; and a final extension cycle at 72 °C for 7 min. The amplified products were sequenced using a BigDye Terminator Cycle Sequencing kit in an ABI Prism 310 automatic sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed with a Chromas 2.6.4 software (Applied Biosystems). The nucleotide sequences were turned into amino acid sequences using MEGA v5 software. Multiple sequence alignment program of Clustal Omega was used to generate alignments between three or more protein sequences. The partial CagA protein sequence from the *H. pylori* strain 43526 (GenBank: AF001357.1) was used as a reference.

2.9. Statistical analysis

Pearson Chi-square ($\chi 2$), and Fisher's exact test analyses were used to determine significant differences. The Pearson Chi-square ($\chi 2$) and Fisher's exact tests were used to analyze the frequencies of motif types and their associations with the types of gastroduodenal diseases (Gastritis and Peptic ulcer). The influence of the number of type C phosphoryaltion (EPIYA-C) motifs on the risk of Gastritis or Peptic ulcer was evaluated by Fisher's exact test analyses. The corresponding 95% confidence intervals (CIs) were computed. P-value < 0.05 indicated statistical significance. All analyses were conducted with The SPSS statistical version 21.0 (Armonk, IBM Corporation, USA) was used for data analysis.

3. Results and discussion

This study was to phenotype the EPIYA motifs and to investigate the association between these phenotypes and gastrointestinal diseases in Chaharmahal and Bakhtiari province. A total of 253 patients with gastrointestinal diseases were enrolled. The results of rapid urease test indicated that 207 (81.8%) were *H. pylori*-positive and the test *H. pylori*-negative. The gastrointestinal diseases were divided into two groups, gastritis and peptic ulcer; and 144 people (59 men and 85 women) had gastritis and the test (65 males and 44 women) peptic ulcer. The mean age of the participants was 50.1 ± 16.5 (range: 15–91) years and 124 (49%) men.

3.1. Frequency of the cagA gene in the isolated H. pylori

We observed that out of the 207 *H. pylori*-positive isolates, 158 had the *cagA* gene and the test did not; and out of the 46 *H. pylori*-negative isolates, only one isolate had the *cagA* gene. This indicates that phenotypic tests (rapid urease test) are not sufficiently sensitive. The frequency distribution of the *cagA* gene is shown in Table 2.

3.2. The association between the cagA gene and the types of gastrointestinal disease in H. pylori-infected patients

The frequency of the *cagA gene* was 57.2% (91 isolates) in the people with gastritis and 42.8% (68 isolates) in those with peptic ulcer. By chi-square test, no significant association between the *cagA* gene and the type of disease was observed ($p = 0.895$) Table 3.

3.3. The frequencies of the motifs of cagA in gastrointestinal diseases and their association with the type of gastrointestinal disease

The observed motifs were western and no East Asian motif (D) was observed in this study. The most frequent pattern of motifs in the current study was ABC type (140 isolates) followed by AB (7 isolates), ABCC (6 isolates), ABCCC (4 isolates) and AC and BC (1 isolate). The frequency of the ABC type was higher in gastritis (60%) than peptic ulcer (40%), but the frequencies of the motifs were higher in peptic ulcer than gastritis with increasing the C fragment (ABCC, ABCCC). Fisher's exact test did not yield any significant association between the motif types of the *cagA* gene and the type of gastrointestinal disease ($p = 0.071$) Table 4.

3.4. The frequency of the ABC motif in gastrointestinal diseases and its association with the type of gastrointestinal disease

By chi-square test, a partial association were observed between the presence of the ABC motif and the type of gastrointestinal disease ($p = 0.056$) such that the number of cases positive for the ABC motif were higher in the patients with gastritis Table 5.

3.5. Bioinformatic analysis of the CagA amino acid sequence

The 3′ region of the *cagA* gene was amplified by PCR for sequencing. The *H. pylori* isolates from the chronic gastritis patients were 275, 247, 189, 163, 162 and 147 and those isolated from the peptic ulcer patients 265, 245 and 164. The results on the sequences and nucleotides were then converted to amino acids to identify the sequences of the EPIYA motifs and the EPIYA motif-related sequences in the *cagA* gene repeat region. The motif patterns below were derived: EPIYA-A with the sequence EPIYAKVNKKK (A/T/V/S) GQ, EPIYA-B with the sequence EPIY (A/T) (Q/K) VAKKVNAKI, and EPIYA-C with the sequence EPIYATIDDLGGP. But there was no East Asian pattern (motif D) with EPIYATIDFDEANQAG sequence. The results of sequencing confirmed motifs classification based on the PCR test. In the current study, one out of nine samples had a mutated EPIYA-B motif that was observed as EPIYT and EPIYA. Two CRIPA motifs were observed in all sequences: The first one with the sequence FPLK (R/K) H (D/G/S) KVD (D/N) LSKVG at the N-terminal of the EPIYA-C and the second one with the sequence FPLKRHDKVDDLSKVG at the C-terminal of the EPIYA-C (Figure 1).

Table 2. The frequency distribution of the cagA gene in isolated *Helicobacter pylori*.

Total (%)N	Negative (%)N	Positive (%)N	cagA *Helicobacter pylori*
(100)207	49(23/7)	158(76/3)	Positive
(100)46	45(97/8)	1(2/2)	Negative
(100)253	94(37/2)	159(62/8)	Total

Table 3. The frequency distribution of the cagA gene in gastrointestinal diseases.

Total (%)N	Peptic ulcer (%)N	Gastritis (%)N	Gastrointestinal diseases cagA
(100)159	68(42/8)	91(57/2)	Positive
(100)94	41(43/6)	53(56/4)	Negative
(100)253	109(43/1)	144(56/9)	Total

Table 4. The frequency distribution of motifs in gastrointestinal diseases.

Total (%)N	Peptic ulcer (%)N	Gastritis (%)N	Gastrointestinal diseases CagA motif
140 (100)	56 (40)	84 (60)	ABC
(100)6	4(66/7)	2(33/3)	ABCC
(100)4	2(50)	2(50)	ABCCC
(100)7	5(71/4)	2(28/6)	AB
(100)1	0(100)	1(100)	BC
(100)1	1(0)	0(100)	AC
(100)159	68(42/8)	91(57/2)	Total

3.6. The association between EPIYA motifs and Oipa

Oipa was most frequent in the ABC motif [82.3% (n: 65)] and least frequent in the BC (1 isolate) and AC (1 isolate) motifs. Out of the six isolates containing the ABCCC motif, only two isolates had the Oipa gene and out of the four isolates containing the ABCCC motif, three had the Oipa gene, but all seven isolates containing the AB motif had the Oipa gene. According to chi-square test, there was a significant association between the EPIYA motifs and the Oipa gene ($p = 0.003$) Table 6.

3.7. The association between the number of EPIYA motifs and babA2

BabA2 was most frequent in the ABC motif [83.9% (n: 78)] and least frequent in the BC (1 isolate) and AC (1 isolate) motifs. Out of the six isolates containing the ABCC motif, only three isolates had the babA2 gene and out of the four isolates containing the ABCCC motif, three had the babA2 gene, but all seven isolates containing the AB motif had the babA2 gene. According to chi-square test, there was a significant association between the EPIYA motifs and the babA2 gene ($p = 0.013$) Table 7.

3.8. The association between the number of EPIYA motifs and iceA1

IceA1 was most frequent in the ABC motif [80.6% (n: 50)] with 4.8%, 6.5%, and 8.1% frequency rates in the AB, ABCCC, and ABCC motifs, respectively. The iceA1 gene was not seen in the AC and motifs. According to chi-square test, there was not any significant association between the EPIYA motifs and the iceA1 gene ($p = 0.095$).

3.9. The association between the number of the EPIYA motifs and the iceA2 gene

According to chi-square test, there was not any significant association between the EPIYA motifs and the iceA1 gene ($p = 0.803$).

The *cagA* oncoprotein is one of the most important factors for *H. pylori* virulence that play an important part in gastritis due to *H. pylori* [4]. This protein, with a molecular weight of 120–140 kDa, has a high immunogenicity [20, 21]. The size of the cagA protein widely varies due to the difference in the repeat sequences of the EPIYA motif at the C-terminal [20]. The bioactivity of the *cagA* gene is determined by the phosphorylation of motif tyrosine at the 3′ region of this gene [22]. According to the EPIYA pattern, this protein is divided into two types, western (EPIYA-A-B-C) and eastern (EPIYA-A-B-D) [23, 24]. Evidence indicates that the Eastern Asian type is comparatively more associated with cancer development than the western type [25]. This is due to the increased tendency of the EPIYA-D motif to the SHP2 and pathogenicity compared to the eastern type [22]. Because binding to the SHP2 (the cause of cytoskeletal changes and induction of interleukin-8 level changes) leads to biological changes in the infected cells and cell atrophy [26, 27, 28].

The pathogenicity mechanism of the EPIYA is as follows: This pattern affects the signaling pathway of the cell through phosphorylation of its tyrosine via Src and Abi (the kinase family) following the introduction of intragastric *cagA* into the duodenal epithelial cells [22]. The number and variations in the EPIYA patterns depend on the phosphorylation rate in the levels of epithelial cells that occurs via *H. pylori* [25]. The current study was first to investigate the prevalence of the EPIYA motifs and its association with clinical symptoms in central and southwestern Iran. The prevalence rates of the *cagA* gene vary in different regions (89.3% in

Table 5. The frequency distribution of the ABC motif in gastrointestinal diseases.

Total	*gastrointestinal diseases*		ABC Motif
	Peptic ulcer	Gastritis	
140	56	84	Positive
19	12	7	Negative
159	68	91	Total

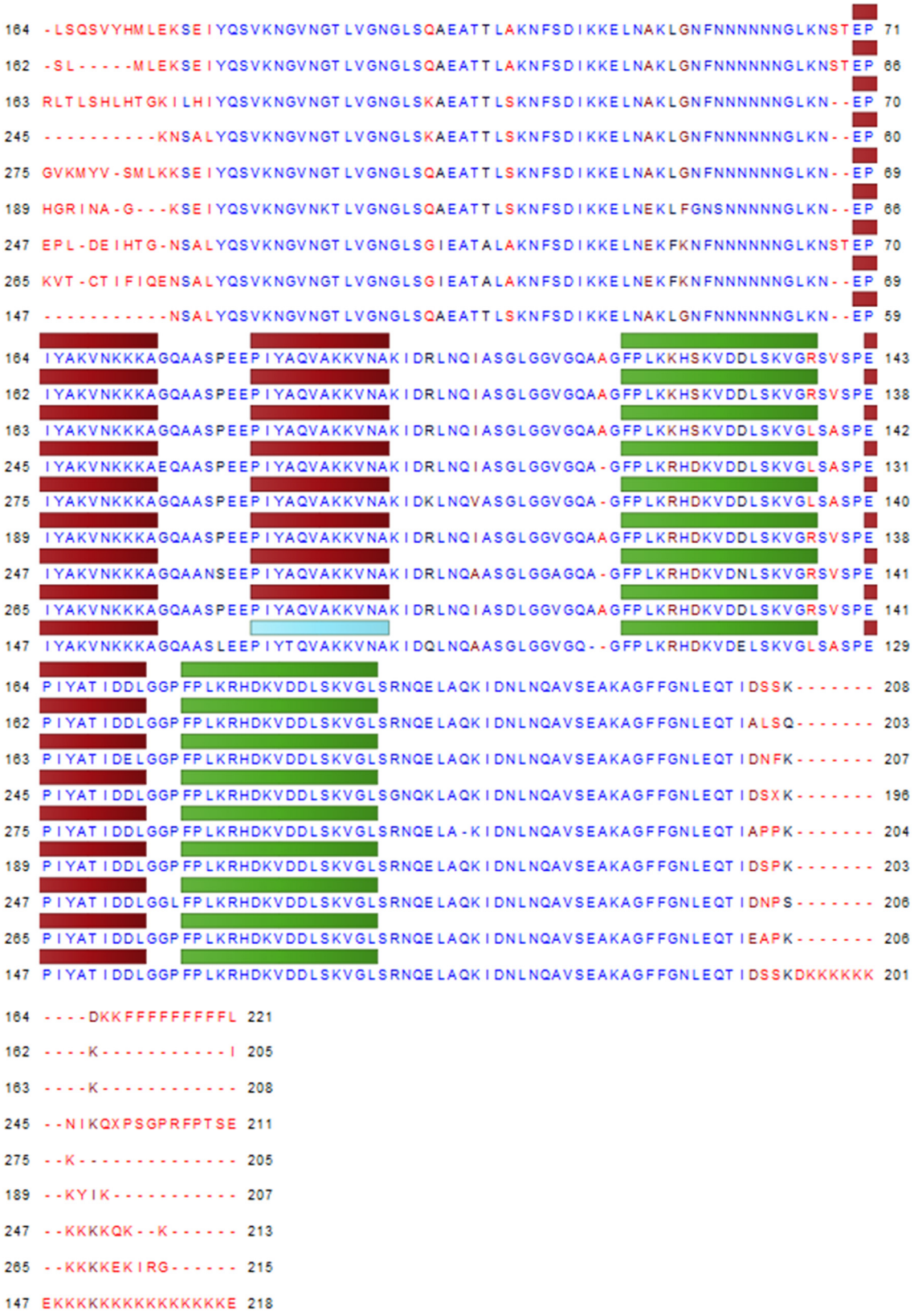

Figure 1. The sequences of EPIYA motifs. The cagA repeat region was also analyzed. Six isolates from gastritis samples (275, 247, 189, 163, 162 and 147) and three isolates from peptic ulcer samples (265, 245 and 164) from the sequence *H. pylori* strain 43526 (GenBank: AF001357.1) were used as the reference sequence. red, green, and blue colors represent the EPIYA, CRIPA, and the mutated EPIYT motifs, respectively.

Table 6. Distribution of the CagA EPIYA-C genotypes according to the *Helicobacter pylori Oipa* gene pool.

cagA motif	*OipA* gene		Total
	Positive	Negative	
ABC	65	74	139
	82.3%	93.7%	88.0%
ABCC	2	4	6
	2.5%	5.1%	3.8%
ABCCC	3	1	4
	3.8%	1.3%	2.5%
AB	7	0	7
	8.9%	.0%	4.4%
BC	1	0	1
	1.3%	0%	0.6%
AC	1	0	1
	1.3%	0%	0.6%
Total	79	79	158
	100.0%	100.0%	100.0%

China, East Asia, 50%, 71%, and 76% in, respectively, Kuwait, Iraq, and Iran, Middle East, and 71.6% and 37% in, respectively, Spain and Turkey, Europe [28, 29, 30, 31]. In our study, the prevalence rate was derived approximately 70% which is consistent with available reports on the prevalence of this gene in the other regions of Iran.

The motif patterns in our study were as follows: BC (140 isolates), ABCC (6 isolates), ABCCC (4 isolates), AB (7 isolates), AC (1 isolate), and BC (1 isolate); the most frequent pattern was ABC, all strains were western, and no D motif was observed, which is consistent with the studies in other regions of Iran [4, 14, 32], as well as those in other countries particularly Europe and Americas (e.g. Colombia, Turkey, USA, Sweden, and Brazil) [33, 34, 35, 36, 37]. However, the observed patterns in the present study are inconsistent with those in South Korea and Viet Nam, two East Asian countries that are mainly of the type D [38, 39]. In India and Philippines, both eastern and western patterns have been observed [40, 41]. In the current study, various patterns of the EPIYA motifs were observed including AB, BC, and AC, consistent with other studies [6, 31, 33].

In addition, the most frequently observed motif pattern, consistent with studies in different countries [1, 12, 14, 35, 42] was ABC. We did not observe any clinical association between the motifs and gastritis and peptic ulcer which is consistent with some studies [26, 32] conducted in Iran but in disagreement with a study in Iran [1]and two studies in Mexico [14]and Brazil [36]. Our study showed that increased C fragments cannot increase pathogenicity; however, the number of motifs with two and more C fragments was very low and the prevalence rates and distributions of gastritis and peptic ulcer very high in our study, which is consistent with Ogordnik and Hossein & and Shekarzadeh [35] studies, but Vianna [36], Salih [34], Gonzalo [43], Basso [42] and Kalaf [44] have reported that increased C fragment was associated with clinical symptoms.

Taken together, geographical and ethnic distribution contributes greatly to the prevalence and variations in motifs due to the influences of environmental conditions and physiological characteristics of populations in different regions. The current study indicated that a highly potent preserved sequence called CRIPA was observed before and after the C motif, which is consistent with Sicinschi [33], Ogorednik [35], Monstein [37], and Beltrán-Anaya [14]studies. CRIPA plays a role in the viability, half-life, and protection of the cagA oncoprotein in epithelial cells [33]. In addition, the changed motif EPIYT, due to the replacement of the threonine amino acid with alanine amino acid [44], leads to EPIYA conversion to EPIYT.

In 2020, Gomez et al. examined the prevalence of *H. pylori* isolates containing the cagA gene with the EPIYA motif and the association of this

Table 7. Distribution of the CagA EPIYA-C genotypes according to the *Helicobacter pylori babA2* gene.

cagA motif	*babA2* gene		Total
	Positive	Negative	
ABC	78	61	139
	83.9%	93.8%	88.0%
ABCC	3	3	6
	3.2%	4.6%	3.8%
ABCCC	3	1	4
	3.2%	1.5%	2.5%
AB	7	0	7
	7.5%	0%	4.4%
BC	1	0	1
	1.1%	0%	0.6%
AC	1	0	1
	1.1%	0%	0.6%
Total	93	65	158
	100.0%	100.0%	100.0%

motif with age-related changes. The results of their study showed that all the isolates identified were of the western type (motif C) and the highest frequency of the EPIYA motif was related to the ABC motif. The results of our study were similar to the findings of the Gomez study. Another example of the similarity of our results with Gomez study is that its prevalence decreases with increasing C components. However in this study a significant correlation is seen between the number of EPIYA-C replicates and the age of the patients. The similarity of the prevalence and distribution of this motif in Iran and Colombia shows the same geographical distribution of this bacterium in the two countries and it can be concluded that the distribution in our study is mainly similar to the distribution in South America [45].

4. Conclusion

Our results showed that the most frequently observed motif pattern was ABC, and the prevalence rates of the patterns were not significantly associated with the clinical symptoms; however, a partial association was found between the ABC motif presence and the type of gastrointestinal disease such that gastritis was more frequent in the ABC-positive cases. All isolates had kinase phosphorylation regions and the main pattern of this region was western.

(ABC)

Identification of EPIYA motifs, their role in pathogenesis, as well as identification of their distribution pattern can be used as specific biomarkers in the diagnosis of cancer and *H. pylori*-related diseases. Therefore, due to the high prevalence of *H. pylori* and the high costs for individuals and the health system, further studies are needed to identify the mechanism of bacterial pathogenesis, screen patients in the early stages of the disease, find appropriate treatment strategies and identification pathogenic markers. As a result, the information obtained from this study and other similar studies can be used to achieve the mentioned goals.

Declarations

Author contribution statement

Mansoor Khaledi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nader Bagheri: Performed the experiments; Analyzed and interpreted the data.

Majid Validi, Behnam Zamanzad, Javad Fathi: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hamed Afkhami: Performed the experiments.

Ghorbanali Rahimian: Conceived and designed the experiments; Performed the experiments.

Abolfazl Gholipour: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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