Functional Cytotoxin Associated Gene A in *Helicobacter pylori* Strains and Its Association with Integrity of *Cag*-pathogenicity Island and Histopathological Changes of Gastric Tissue

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Abstract

Objectives: Existence of cytotoxin associated gene A (*cagA*) is considered as a marker for detection of an oncogenic *Helicobacter pylori* strain. Expression of *cagA* in the strains with incomplete delivery system (partial *Cag*PAI) could convert them to less virulent strains. This study was aimed to analyze expression of *cagA* in *H. pylori* strains presenting diverse *cag*PAI and its effect on histopathological changes of the gastric tissue.

Methods: Clinical strains of *H. pylori* and related histopathological data were obtained to examine the presence of 12 *cag*PAI segments by PCR. Expression of *cagA* was analyzed by RT-PCR as well as Immunoblotting on RNA and protein extracts of the *cag*PAI-positive strains, respectively. *In situ* expression of *Cag*A-positive strains was determined in a gastric epithelial cell line.

Results: Intact *cag*PAI was detected among 33% (4/12) of *H. pylori* strains. Out of 7 diverse *cag*PAI genotypes in these strains, expression of *cag*A was confirmed in 2 strains with complete *cag*PAI at both RNA and protein levels. Occurrence of intestinal metaplasia and severe active gastritis were mainly detected in the strains with complete *cag*PAI genotype. No association was detected between EPIYA types of the strains and expression of *cag*A.

Conclusions: These results collectively showed high diversity of *cag*A and *cag*PAI among the *H. pylori* strains. These diversities may provide some reasons to explain distinct disease severity caused by different *H. pylori* strains in the gastric tissue.

Keywords: Histopathological Changes, *Helicobacter pylori*, *cag*PAI

1. Background

Helicobacter pylori (*H. pylori*), a Gram-negative bacterial pathogen, is recognized as an important risk factor for chronic active gastritis, peptic ulcer, gastric cancer, and gastric mucosa associated lymphoid tissue lymphoma (1-4). Approximately half of the world's population is infected with *H. pylori*; however, most of the infected people have no symptoms (3, 5). Although initial colonization of this bacterium occurs in early years of life, occurrence of disease depends on its genetic entity and virulence property in association with host and environmental risk factors (6). Diversity of *H. pylori* strains was demonstrated in several studies and in different populations (7-10). Association of this diversity with the severity of gastric disorders was shown for some structural and functional proteins of *H. pylori* (5, 8, 11, 12).

Cag-pathogenicity Island (*Cag*-PAI) is one of the complex genetic structures in bacteria that encodes approximately 27 to 31 genes (13-15). While involvement of this structure in inflammation of gastric tissue and its role for delivery of *Cag*A oncoprotein into gastric epithelial cells were demonstrated, there is very few data regarding its association with the clinical outcome of the infection (5, 6). At least 17 out of 27 *cag*PAI genes encode main structural proteins that are important for providing a putative type

IV secretion system (T4SS) to represent a needle-like pilus device including CagL, CagY, CagE, CagW, and Cagα. Some of these proteins, especially exterior proteins, including CagT, CagY, and CagX interact with host cell receptors and their presence are essential for delivery of effector protein *CagA* and peptidoglycan into the cells (16-22).

Diversity of *cag*-PAIs among different *H. pylori* strains, encouraged researchers to use *cag*-PAI typing as a molecular typing method to study its association with their pathogenic capacity in infected patients (23-25). Diversity of this PAI could be screened for the presence or absence of noted genes or defined deletions and insertions. Since there are increasing reports regarding sequence diversity of dedicated genes of *cag*-PAIs, data regarding the integrity of this structure using *cag*-PAI typing could not signify its proper function. *cagA* is considered as a more variable gene of *cag*-PAI and contains a variable number of repeat sequences located in the 3' of this gene (known as EPIYA motif) that may affect its biological activity. Recombination of these repeat units during replication may convert it to a less virulent oncoprotein or a pseudogene (5, 26).

Study of clinical isolates of *H. pylori*, at both molecular and functional levels, could provide more valuable data that somewhat explain the diversity in the clinical outcomes of infected patients. However, such correlation was fairly investigated. This study was aimed to analyze the diversity of *H. pylori* strains in capacity of *CagA* expression and its association with EPIYA types, intactness of *cag*-PAI, and variation in promotion of different gastric disorders in human host tissue.

2. Methods

2.1. Bacterial Strains and Culture Conditions

CagA positive *H. pylori* strains were randomly selected from the microbial collection of research institute for gastroenterology and liver diseases in Tehran, Iran. All the strains were obtained from patients with gastric disorders. The identity of the strains was confirmed by the culture and polymerase chain reaction (PCR) using species specific primers for gene glmM (27). Briefly, the isolates were recovered from the stocks on Brucella agar medium supplemented with fetal calf serum (10%) (v/v), horse blood (7%), selective supplement (vancomycin 2.0 mg, polymyxin B 0.05 mg and trimethoprim 1.0 mg, Merck, Germany), and amphotericin B (3 mg/L). The plates were incubated at 37°C for 3 to 5 days in a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2). The grown colonies were used for biochemical characterization, based on their morphology as well as positive reactions for oxidase, catalase, and urease tests. Genomic DNA of the selected strains was extracted from the harvested colonies, using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. *H. pylori* strain RIGLD-OC661 was used as a reference strain for all the experiments.

2.2. Screening of cagPAI Gene Loci by PCR

To analyze the integrity of *cag*PAI among these strains, the presence of cagA was initially determined using specific primers covering the entire 3' region of *cag*A gene, cag3F: 5'-TCCGTTAAAGATGTGATCATCAATC-3'and *cag*3R: 5'-AGATTTTTGGAAACCACCTTTTG-3' (28). Amplification of *cag*A was performed as follows: an initial denaturation at 94°C for 4 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 90 seconds, with a final elongation step at 72°C for 10 minutes. *CagA* EPIYA types was determined based on the size variation of the PCR products in comparison to reference strains for common EPIYA types (Accession numbers JX428788, JX428766, JX428786, JX428784).

All the *cagA* positive strains were subjected to *cag*PAI typing through 11 sets of primers spanning whole *cag*PAI locus, which were designed by Yadegar et al. (29). PCR reaction mixture of 50 μL was used, containing 1X PCR buffer, 1.5 mM $MgCl_2$, 200 mM of each dNTPs, 1 pmol of each primer, 1 μL of genomic DNA (approximately 150 ng), and 0.05 U/mL of SuperTaq™ DNA polymerase (HT Biotechnology Ltd., Cambridge, UK). *cag*PAI typing was done under the following conditions: initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 1 minutes, annealing at the indicated temperature for each reaction for 45 seconds, extension at 72°C for 1 min/kb, and then the final extension at 72°C for 10 minutes. While lack of $\geq$ 1 *cag*PAI locus defined partial *cag*PAI in the studied strains, the *cag*PAI was defined as intact if all the selected gene loci were present. Complete deletion of *cag*PAI in the *ca*gA negative strains were determined using Luni1 and R5280 primers for *cag* empty site as described before (30).

2.3. RNA Extraction and cDNA Synthesis

Total RNA of the strains were extracted from the freshly grown bacteria in BHI broth medium containing the selective supplement and were incubated at 37°C for 3 days at microaerophilic conditions to obtain an optical density of 0.2 at 600 nm ($\sim 3 \times 10^8$ bacteria/mL). Accordingly, pellets of the cultures were prepared after centrifugation (12000 g, 5 minutes) followed by washing with sterile phosphate buffered saline (PBS, pH 8). Total RNA was extracted using RNeasy mini kit (Qiagen, USA), according to the manufacturer's instructors. The RNA concentration was measured for each strain spectrophotometerically. All the RNA extracts were preserved at -70°C until use for cDNA synthesis.

Reverse transcription was performed with Titan One Tube RT-PCR kit (Sigma-Aldrich) using same *cag*A primer, according to the manufacture's protocol.

2.4. Cell culture and Infection Assays

To analyze expression of *Cag*A in the human gastric cancer cell line, AGS (ATCC CRL-1739TM, IBRC, Tehran, Iran) was incubated in Dulbecco's modified Eagle's medium (DMEM) (Gibco, GrandIsland, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA), 1% non-essential amino acid (Gibco, Grand Island, NY, USA), 100 U mL^{-1} of penicillin, and 100 μg mL^{-1} of streptomycin (Gibco, Grand Island, NY, USA) at 37°C in a humidified incubator (Memmert, Dusseldorf, Germany) containing 5% CO$_2$. *H. pylori* strains cagA$^+$ and cagA$^-$ were re-suspended in DMEM and added to 2 × 10^6 AGS cells at a multiplicity of infection (MOI) of 100. After incubation in a 5% CO$_2$/95% air incubator for the required length of time, AGS cells were washed once with PBS (Sigma-Aldrich) to remove non-adherent bacteria. For imoniassy analyses, AGS cells with attached bacteria were collected by Rippa buffer (Sigma-Aldrich), according to the manufacture's protocol.

2.5. Detection of CagA

Expression of *Cag*A was also characterized at protein level. The freshly prepared bacterial pellets were washed with PBS and centrifuged at 4000 g for 15 minutes. The pellets were re-suspended in lysis buffer (PBS containing lysozyme, 10 mg/mL, EDTA, 2 mM, DNase I, 10 μg/mL, and protease inhibitor cocktail table; Roche, Germany). Sonication of the treated bacteria was done after incubation in 37°C for 30 minutes (Ultrasonic UP 200 H, 100 Watt on ice for 2 minutes). To separate soluble proteins from the bacterial cell lysates, the sonicated solution was centrifuged at 12000 g for 20 minutes at 4°C. Supernatant and pellet of each lysate was collected after addition of urea (9 M) and dithiothreitol (DTT, 70 mM) and stored at -70°C until use for immunoblot analysis. Dot blotting was performed to screen presence of *Cag*A in the protein extracts. Briefly after blocking with bovine serum albumin (5%), polyvinylidene fluoride membrane was inoculated with 15 μL of the crude protein extracts (3 separate 5 μL inoculations) from *cag*A positive and negative *H. pylori* strains. The blots were washed 3 times with TBS-T buffer (0.05%, Tris-buffered saline with Tween 20) and incubated with mouse anti-*Cag*A primary antibody (1:1000 dilutions) for 24 hours at 4°C (Abcam, USA). The HRP-conjugated secondary antibody was added to the blots at 1:5000 dilutions (Razi Biotech, Iran) after 4 washing steps, and incubated for 1 h at same conditions. The presence of *Cag*A was determined after washing of the membrane and addition of TMB solution (3,3',5,5'-tetramethylbenzidine, 10 mg/mL). To confirm our results,

immunoblotting was performed. Accordingly, 50 μL of the protein samples from each strain were subjected to electrophoresis in 12% sodium dodecyl sulfate (SDS)- polyacrylamide gels (30 V, overnight at room temperature). Protein bands were then transferred to a polyvinylidene fluoride membrane in a transfer buffer (25 mMTris, 192 mM glycine, pH 8.3) using Mini-Trans-Blot electrophoretic transfer cell (Roche, Germany). The presence of the specific band for *Cag*A was determined through the same procedure that was described above. *Cag*A expression was also analyzed for the strains encoding *cag*A mRNA in a cell culture model (AGS, ATCC CRL-1739TM, IBRC, Tehran, Iran). Accordingly, during the assay, confluent AGS cell line was incubated in antibiotic free Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% non-essential amino acid (Gibco, Grand Island, NY, USA), at 37°C in a humidified incubator (Memmert, Dusseldorf, Germany) containing 5% CO$_2$. Two freshly grown *cag*A$^+$*H. pylori* strains and 1 *cag*A$^-$ strain were suspended in DMEM and added to AGS cells at a multiplicity of infection (MOI) of 100. After incubation for 6 h at the same conditions, the total protein was extracted from the infected cell lines using the Rippa buffer (Sigma-Aldrich), according to the manufacture's instruction. *Cag*A expression was characterized by immunoblotting using same antibodies. All the assays were performed in 2 replicates.

4. Results

Identity of all the bacterial strains that were used in this study was confirmed by PCR results for glmM gene. PCR results showed the presence of *cag*A in 12 strains. One *cag*A negative strain was included in the study as a negative control for gene expression analysis. As was shown in Figure 1, based on diversity of the strains in *cag*A' variable region, different *Cag*A EPIYA motifs were detected. Verification of the EPIYA types was done through sequence analysis of 1 amplicon that showed similar gel migration with the reference amplicon from *H. pylori* strain RIGLD-HC113 (Accession number KU516680). According to these results, we characterized the EPIYA types as follow: ABC, 7 strains (58.3%); ABCC, 4 strains (33.3%), and ABCCC, 1 strain (8.3%).

PCR analysis was performed on all strains using 11 pairs of spanning primers complementary to different *cag*PAI regions. The PCR amplifications yielded high-quality products with the expected sizes and reproducible results (Figure 2). The data presented in Table 1 show diversity of *cag*PAI genes and specified internal deletions (D1 to D5) among the strains with different EPIYA motifs. In total, 7
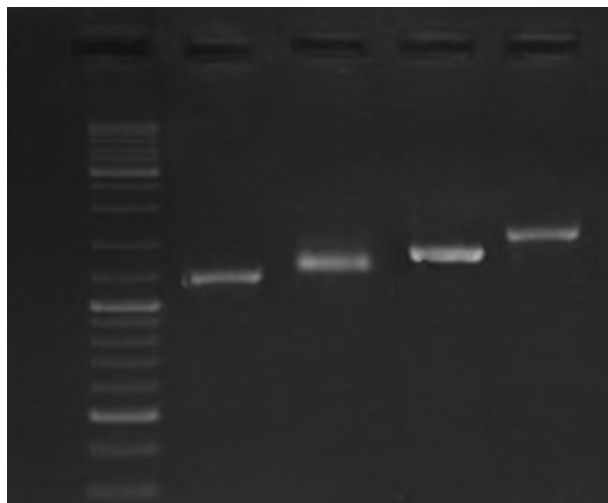
Lane 1, Gene Ruler DNA Ladder Mix (Thermo Scientific); Lane 2, EPIYA type AB; Lane 3, EPIYA type ABC; Lane 4, EPIYA type ABCC; Lane 5, EPIYA type ABCCC.

genotypes of *cag*PAI were determined in these strains. Intact *cag*PAI was detected among 4 strains (33%). Analysis of the results showed deletions D2 (1/12, 8.3%) and D3 (4/12, 33.3%), and occurrence of mutation at loci cagX-cagW (3/12, 25%), cagC-cagA (2/12, 16%), cagL-cagH (3/12, 25%), cagE-cagC (3/12, 25%), cagW-cagT (2/12, 16 %) in these strains.

We further assessed any correlation between EPIYA patterns and intactness of *cag*PAI locus. EPIYA types ABCC (3/12) and ABCCC (1/12) were observed dominantly among strains that showed most conserve *cag*PAI segments, while EPIYA type ABC (7/12) and A-B/C (1/12) was characterized among the strains with mutations at 2 or higher loci (Table 1).

As was shown in Figure 3, *cag*A transcription was confirmed among 2 out of 6 *H. pylori* strains presented the most intact cagPAI genotypes. The 2 strains belonged to those carried intact *cag*PAI (Strain HC113, EPIYA-ABC) and 1 strain with D3 deletion (Strain OC149, EPIYA-ABCCC). Lack of *cag*A expression was detected in 3 strains with intact *cag*PAI, and one strain with mutation at *cag*X-*cag*W and *cag*ζ-*cag*δ (D3) loci (Table 1). *Cag*A expression was confirmed for these 2 strains by both immunoblotting and dot blotting methods and also in cell culture plates (Figure 4).

5. Discussion

H. pylori has several housekeeping and virulence genes that support its persistent colonization and pathogenesis

in the harsh gastric environment for decades. Pathogenesis of *H. pylori* is depended on diversity of these genes and function of their products in the host. *Cag*PAI is one of the main *H. pylori* virulence markers that assumes as a mediator of inflammatory response and carcinogenesis through delivery of *CagA* into host cells (13, 31-33). While diversity of this structural component was described by some recent studies, its correlation with disease outcome is not yet clear. Our results showed different *CagA* EPIYA motifs (ABC, ABCC, ABCCC) in *H. pylori* strains isolated from the patients who suffered from mild gastritis, sever active gastritis, intestinal metaplasia, and gastric cancer. The existence of 7 different genotypes of *cag*PAI among the 12 studied strains confirmed a high rate of diversity in this genetic locus, which may correlate with pathogenicity of the strains. Ahmadzadeh et al. represented 13 different *cag*PAI genotypes among 21 *cag*PAI positive *H. pylori* strains in Iran (34). This diversity is also high in different geographic populations (11, 35-37). Correlation between integrity of *cag*PAI, types of EPIYA motifs, and disease outcome was confirmed by several studies (24, 38-42). However, there are studies that reported contrary results (43-45). Variation in *H. pylori* genome from various areas and presence of polymorphisms in different gene loci of *cag*PAI could be main causes of the differences among the studies. In our study, composition of most important genetic loci of *cag*PAI was determined. The obtained results for genetic structure of *cag*PAI proposed the presence of functional deletions in different segments, including *cag*ζ-δ, *cag*X-W, *cag*L-H, *cag*E-C, *cag*C-A, and *cag*W-T. Most of these segments encode critical structural proteins of *Cag*PAI that are essential for interaction with host cells and delivery of *H. pylori* effector proteins. This finding could somewhat explain the variation in the clinical outcome of infected patients with distinct *H. pylori* strains. While there is some evidence regarding the effects of intact *cag*PAI on the severity of histopathological changes of the gastric tissue, ulceration, and carcinogenesis, establishment of this association needs further studies on different populations (39, 46-48).

Intact *cag*PAI could not solely guarantee correct function of type IV secretion system (T4SS) in *H. pylori* strains, since point mutations in *cag*PAI loci might cause nonsense mutations that disturb its activity. Selbach M. et al. showed that nonsense mutations in cagα (virB11), *cag*Y (virB10), *cag*X (virB9), *cag*W (virB8), *cag*T (virB7), and *cag*E (virB4) could prevent *CagA* translocation, IL-8 induction, and formation of scattering phenotype in gastric epithelial cells (21). Necessity of functional *Cag*Y and *Cag*L for delivery of *CagA* into host cells and secretion of inflammatory cytokine was established by Tegtmeyer N et al. (49). In our study, we found intact cagPAI in the 5 strains, Δ3 was preset in 1 of them. Gene expression analysis of *cag*A in these

Figure 2. Polymerase Chain Reaction Using the Primers Spanning Different *cag*PAI Regions in *H. pylori* Strains
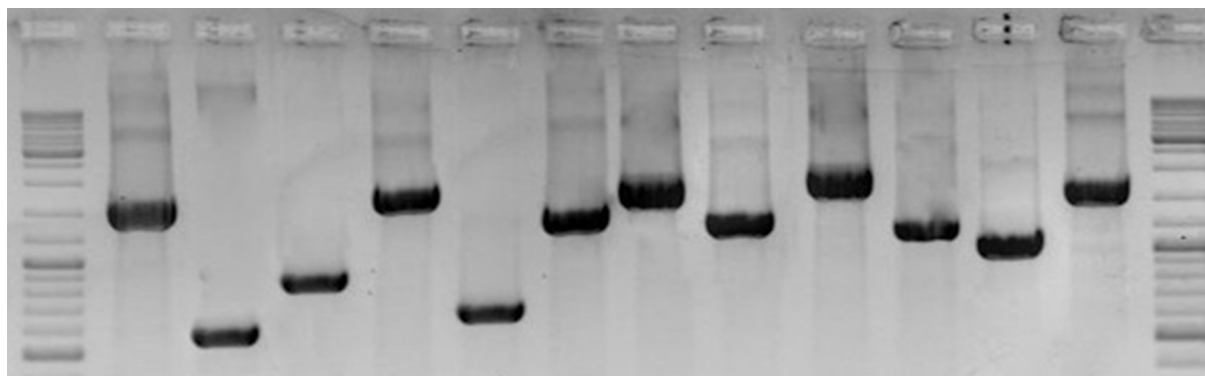
Lane 1 and 14, GeneRuler DNA Ladder Mix (Thermo Scientific); Lane 2, *cagζ-cagδ* (Wild (intact): 1092 bp); Lane 3, D2 (525 bp); Lane 4, D3 (750 bp); Lane 5: *cagQ-cagM* (1721bp); Lane 6, *cagp-cagM* (601 bp); Lane 7, cag*δ*-cag*γ* (1417 bp); Lane 8: *cagα-cagY* (1818 bp); Lane 9, cagX-cagW (1306 bp); Lane 10: *cagW-cagT* (228 bp); Lane 11, cagL-cagH (1329 bp); Lane 12: *cagE-cagC* (1083 bp); Lane 13: cagC-cagA (1917 bp).

Table 1. Diversity of *cag*PAI Genotypes Among *H. pylori* Strains Used in This Study and Their Association with *CagA* Expression

*H. pyloricag*PAI Genotype	ID of Strains (Pathological Findings)	*CagA* Expression	*CagA* EPIYA Type
Intact cagPAI[a]	HC-113 (IM)	Positive	ABC
	HC-168 (SAG)	Negative	ABC
	HC-180 (CAG)	Negative	ABCC
	HC-136 (SAG)	Negative	ABCC
cagζ-cagδ (W)|*cagα-cagY*	cagX-cagW	cagC-cagA⁻	HC-176	Negative	ABC
	HC-174 (Moderate active chronic gastritis)	Negative	ABC
cagζ-cagδ (D2)|cag⁻-cag⁻|cagW-cagT⁻|cagL-cagH⁻|cagE-cagC⁻	OC-180 (Moderate active chronic gastritis)	Negative	ABC
cagζ-cagδ (D3)|*cagX-cagW⁻*	OC-179 (Moderate active chronic gastritis)	Negative	A/B
	HC139	Negative	ABCC
cagζ-cagδ (D3)|*cagQ* (rev. ori.)⁺|*cagQ-cagA⁻*[b]	OC-287	Negative	ABC
cagζ-cagδ (D3)|*cagL-cagH⁻*|*cagE-cagC⁻*	OC-217 (chronic active gastritis)	Negative	ABC
cagζ-cagδ (D3)|*cagQ-cagA*⁺[c]	OC-149 (chronic active gastritis)	Positive	ABCCC

[a]Intact *cag*PAI: *H. pylori* strains with complete *cag*PAIgene segments.
[b]*cag*Q-*cag*A⁻: *H. pylori* strains with mutation at all genetic segments denoted between *cag*Q-*cag*M and *cag*C–*cag*A.
[c]*cag*Q-*cag*A⁺: *H. pylori* strains without mutation at all genetic segments denoted between *cag*Q-cagM and *cag*C-*cag*A. IM, intestinal metaplasia; SAG, severe active gastritis; CAG, chronic active gastritis.

strains proposed a high frequency of nonsense mutation or mutation in promoter of this gene locus. In a study by John T. Loh et al., they revealed that strains expressing high levels of *CagA* were associated with more advanced precancerous lesions compared with those expressing low levels of *CagA* (50). The lower level of *CagA* expression was found to be in relation to the existence of a DNA motif upstream of the translational initiation site of *CagA*. In consistent to these results, high frequency of rearrangement in *cagA* was reported by Kauser F. et al.. They showed diversity in the *cagA* promoter as a main responsible factor affecting its expression in patients with severe pathology (17). The link between existence and higher expression levels of *CagA* and/or T4SS components with progression of gastric pathology and carcinogenesis was similarly depicted by Szkaradkiewicz A et al. (51). While our finding and those presented by other studies strongly indicate that intact *cag*PAI in strains with higher *CagA* expression level are more virulent, the expression of *CagA* and intactness of *cag*PAI may be unrelated, since there are some reports that showed inconsistency between integrity of *cag*PAI and expression of *cagA* (52).

Figure 3. Reverse Transcripase-PCR Analysis of *cagA* Gene Using cag3 Primers in *H. pylori* Strains, Which Had Intactcag-PAI
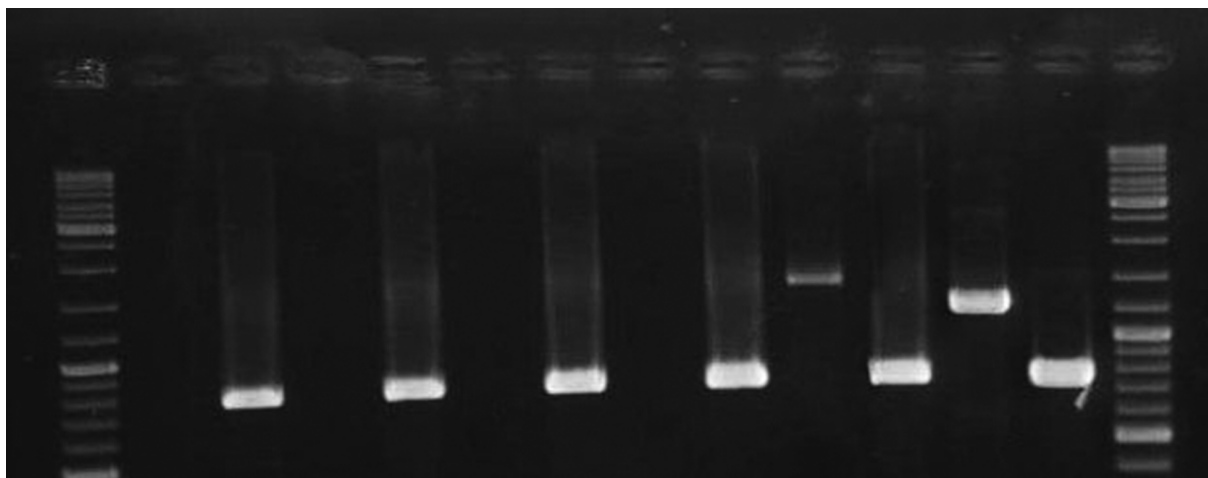
Lane 1 and 14, GeneRuler DNA Ladder Mix (Thermo Scientific, USA); Lanes 2, 4, 6, 8, non-coding *cagA*strains (Strains OC217, HC168, HC136, HC180, respectively); Lanes 10 and 12, *cagA* coding strains (Strains OC149 and HC113, respectively). mRNA expression of *16srRNA* was observed for all the strains; Lanes 3, 5, 7, 9, 11, and 13 represents the RT-PCR product for strains OC217, HC168, HC136, HC180, OC149 and HC113, respectively. Size variation of products for *cagA* is related to the nucleotide sequence diversity in these strains.

Figure 4. Immunoblotting of Protein Extracts from Infected AGS Cell Line with *H. pylori* Strains
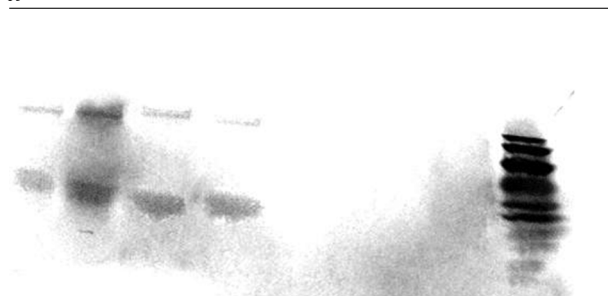
Lanes 1 - 4 represented *CagA* (complete and truncated form) protein in lysates of grown *H. pylori* strains in Brucella broth (Lanes 1 and 3) and total protein extracts of infected cell line (Lanes 2 and 4) after 6 h incubation. MOI of 100 was used in cell culture experiments; Lane 8, PageRuler™ Prestained Protein Ladder (10 - 170 kDa, Thermoscientific, USA); Lane 5, infectedAGS cell with *cagA* negative *H. pylori* strain (Strain OC236); Lane 6, Lysate of grown *H. pylori* strain OC236 in Brucella broth; Lane 7, Protein extracts of non-infected AGS cell line. Size variation for lanes 3- 4 are related to *CagA* EPIYA motif ABCCC.

Increase in EPIYA motifs and existence of some defined EPIYA types are associated with more strengthen intracellular signaling in the infected host cells (13, 38, 53-55). In another study, *H. pylori* strains with higher EPIYA type C-repeats showed higher frequency in the infected patients with gastric cancer (56). According to the previously determined EPIYA types of the studied strains in the previous study, our analysis showed no significant correlation between diversity of *cag*PAI and diversity in *cagA* EPIYA types. However, the presence of 2 or 3 EPIYA type C motifs was mainly observed in the stains with more conserved *cag*PAI

loci, while strains that presented EPIYA type ABC were generally detected among the strains with partial *cag*PAI. This could presume ability of more virulent *H. pylori* strains for preservation of their integrity after the occurrence of the genetic recombination events. Effective interaction of *Cag*PAI with host cell receptors may explain diversity of distinct *H. pylori* pathogenesis in these patients. Our results represented more conserved pathogenicity locus in severe gastritis patients, even among the strains with lower repeats of EPIYA motif. However, involvement of other *H. pylori* virulence factors or host factors also seems probable (25, 57-60).

4.1. Conclusion

In conclusion, our results showed a high frequency of partial *cag*PAI among the *H. pylori* strains in Iran. While all the selected strains were *cagA* positive, the presence of *cag*A didn't predict the integrity of *cag*PAI or its expression. Therefore, assessment of all genetic loci seems to be necessary for detection of intact *cag*PAI in *H. pylori* strains. Lack of *CagA* expression in the *cagA* positive strains proposed high frequency of nonsense mutations in its coding sequence or the promoter region. The pathological findings proposed *H. pylori* strains with intact *cag*PAI and ability of *CagA* expression as more virulent strains than those with disruption at these loci.

Acknowledgments

Footnote

Conflict of Interests: The authors declare that they have no competing interest.

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