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Prevalence of *cagA* and *babA2* positive *Helicobacter pylori* strains in dyspeptic patients in Iran

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Abstract
Background: *Helicobacter pylori* genome encodes a large number of virulence and adhesion factors that involved in bacterial adhesion to host cells and exerts its virulence effect with CagA secretion. In this study, we aimed to examine the relationships between *babA2* and *cagA* genotype and presence of gastric disorders in patients from Iran.
Material and Methods: The presence of *H. pylori* and selected genes (*cagA* and *babA2*) were detected by PCR method from the genomic DNA of 105 patients who had been diagnosed with gastric disorders like chronic gastritis by endoscopic and histopathologic routes.
Results: Presence of *H. pylori* (*glmM gene*) was detected in 85 out of 105 (80.9%) patients. According to our results *cagA* gene was found in 66 out of 85 (77.6%) patients and *babA2* gene was found in 80 out of 85 (94.1%) patient.
Conclusion: In conclusion, the *cagA* and *babA2* genotypes might be considered as useful biomarkers for non-ulcer disease (NUD) patients and gastric disorders in the geographic region of Iran and the presence of *H. pylori* strains with double-positive status is high clinical relevance to *H. pylori*-associated diseases.

Key words: *Helicobacter pylori*, babA2, cagA, gastric disorders.

Introduction
Less than three decades ago, Robin Warren and Barry Marshall definitively identified *Helicobacter pylori* by culturing an organism from gastric biopsy specimens that had been visualized for almost a century by pathologists (1). *H. pylori* is a gram-negative spiral organism that is capable of colonizing the gastric mucosa and form the main cause of chronic active gastritis (2). Colonization with *H. pylori* is the commonest infection worldwide, affecting at least half the world's population (3). Adherence of *H. pylori* to the gastric epithelium facilitates initial colonization, persistence of infection, and delivery of virulence factors to host epithelial cells.

The cytotoxin-associated gene (*cagA*) was the first gene found to be differentially present in *H. pylori* isolates and is considered a marker for the presence of the cag pathogenicity island *cagPAI*. *H. pylori* strains can be divided into CagA positive or negative strains. CagA was identified as the first protein of the *cagPAI* and appeared to be a major virulence factor (4). CagA is an oncoprotein that thought to be involved in cancer development. The recently described blood group antigen-binding adhesin BabA has been shown to mediate adherence of *H. pylori* to Lewis b receptors on gastric epithelium (4).

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Albeit three bab alleles have been recognized (babA1, babA2, and babB), only the babA2 gene product is intransitive blood-group antigen-binding adhesin that mediates attachment of *H. pylori* to human Lewis-b antigens (5, 6). In this investigation, we focused on *H. pylori* BabA2 adhesion factor and CagA virulence factor in patients suffering from chronic gastritis. We sought to examine the relationships between *babA2* and *cagA* genotypes in patients with gastrointestinal disorders and identify the independent markers of non-ulcer disease (NUD) such as dyspepsia and chronic gastritis in patients from Iran.

Material and methods

Sample collection

In this study, a total of 105 biopsy samples from the stomach antrum and corpus were obtained from patients with gastro duodenal disorders like chronic gastritis, dyspepsia and non-ulcer disease (NUD) that, referred to endoscopy department of Imam Reza hospital, Tabriz, Iran. For study the babA2 and cagA genes, PCR method was used. To PCR analysis DNA was extracted from obtained biopsy samples by performing QIAamp DNA Mini Kit (Qiagen Inc., Germany), according to the manufacturer‘s instructions and prepared DNA were stored at −20 °C for using in future studies.

PCR analysis

The *glmM* gene was primarily amplified for detection of *H. pylori* DNA in collected samples. The forward primer (5′-AAG CTT TTA GGG GTG TTA GGG GTT T-3′) and the reverse primer (5′-AAG CTT ACT TTC TAA CAC TAA CGC-3′) were derived from the published "*H. pylori* " glmM (Urea C) sequence and used to amplify a 294-bp segment of *H. pylori urease C gene* (7). PCR analyses were carried out to determine the presence or absence of *cagA* and *babA2* genes in each *H. pylori* positive sample by using specific primers. The sequences of these primers are described in (Table 1).

The amplification reaction consisted of 1 to 2 µl DNA samples in a final volume of 50 µl containing 1× PCR buffer, 200 µM (each) deoxy nucleoside triphosphate, 100 pmol of primers and 2.5 U of Taq DNA polymerase in Thermo cycler (MJ Mini BIO-RAD).

For analysis of the amplified products of each PCR assay, 6 µl of the amplicons were electrophoresed with a 1X tris-acetate-EDTA buffer on 2% agarose gel stained by ethidium bromide (5 µl/100ml). The amplicons were visualized by UV transillumination, and a 100 base pair ladder was used as standard.

The statistical analysis of data was accomplished by using logistic regression, chi-square test with significance set at a P value of < 0.05.

Results

From all of 105 patients, *H. pylori* (presence of *glmM* gene) were detected in 85 patients (80.9%) including 44 (41.90%) men and 61 (58.09%) women with mean age of 53 years old (±30.5). Age, sex, and PCR findings of patients were tabulated and analyzed by Matlab and SPSS 14 statistical package. In our study *cagA* and *babA2* genes were found in 77.6% and 94.1% of *H. pylori* positive samples respectively. The prevalence of *cagA* and *babA2* harboring strains in Iran is similar. The results of this study suggest that, in Iran like some other countries the presence of *cagA* and *babA2* positive *H. pylori* strains is associated with *H. pylori* related diseases such as dyspepsia, NUD and chronic gastritis (Table2).

Table 1. Description of the pairs of primers used in the amplification of *cagA* and *babA2* genes.

Gene/ Primers	Sequence	Size/ (References)
glmM	**F** 5'-AGCTTTTAGGGGTGT TAGGGGTTT-3' **R** 5'-AAGCTTACTTTCT AACACTAACGC-3'	294 bp (7)
cagA	**F 5'**-GATAACAGGCAA GCTTTTGAGG-3' **R 5'**-CTGCAAAAGATT GTTTGGCAGA-3'	349 bp (7,16)
babA2	**F 5'**-AATCCAAAAAG GAGAAAAAGTATGAAA-3' **R 5'**-TGTTAGTGATTTCG GTGTAGGAC-3'	832 bp (15)

Table 2. The statistical analysis and frequency of *babA2* and *cagA* positive *H. pylori* strains.

Genotype	NUD n (%)	χ^2	*p* value
***babA2* positive**	80/85 (94.11)	9.509	0.0020
***cagA* positive**	66/85 (77.64)		

Discussion

In the study of infectious diseases, researchers for recognize a real pathogen from innocuous organisms, often focus on bacterial virulence factors. Since the discovery of *Helicobacter pylori*, many investigations have focused on explaining the microorganism pathogenicity mechanisms that are associated with disease outcome. In studies conducted on *H. pylori*, several virulence factors have been discussed. But one of the most critical property of *H. pylori*, is its ability to remain for many years within the host tissues without any damage. Therefore, colonization is not equivalent with virulence but rather may refer to the persistence of the microorganism in a specific site in the host. The adherence of *H. pylori* to host cells is a pertinent step in the development of gastro duodenal disorders. In our study, we focused on *H. pylori* BabA2 adhesion factor and CagA virulence factor in patients suffer from gastro duodenal disorders like chronic gastritis and dyspepsia. In our study *cagA* and *babA2* genes were found in 77.6% and 94.1% of *H. pylori* positive samples respectively. The prevalence of *cagA* and *babA2* harboring strains in Iran is similar.

In studies of *H. pylori* colonization and its adhesion to the host cells, the BabA adhesion has been the most studied factor (8, 9). BabA2 attaches *H. pylori* to host epithelial cells, permitting the transfer of CagA toxin to the gastric epithelium and so increasing gastric tissue damage. Some authors suggest that, the presence of *babA2* is associated with some disorders like duodenal ulcer and gastric cancer, when found in conjunction with *vacA s1* alleles and cagA (10). In our study *cagA* gene was found in 77.6% of patients with *H. pylori* and *babA2* genotype was positive in 94.1% of patients, we also showed that, the co-presence of these genes can increase the risk of gastro duodenal disorders. The last analyses of *babA2* gene as a marker for diagnosis *H. pylori* associated disease have produced inconsistent data on the useless of this gene expression in anticipating clinical outcomes, which is most likely related with the geographic scope of the *H. pylori* strains. Another author suggests that In Thai populations, *babA2* is not a biomarker for peptic ulcer disease or chronic gastritis (11). But, for strains isolated from Turkey, northern Portugal or Germany, BabA2 expression is related to the severity of gastric duodenal disorders (12, 13 and 14). *H. pylori* isolates from Cuba displayed a high frequency (82.3%) of the *babA2* allele in Cuban patients (15). Our data suggests that in Iran like some other countries the presence of *cagA* and *babA2* positive *H. pylori* strains is associated with *H. pylori*-related diseases such as chronic gastritis and there is a significant association between increased two genes together.

Conclusion

In conclusion, the *cagA* and *babA2* genotypes might be considered as useful markers for monitoring and prognosis of NUD patients and gastric disorders in the geographic region of Iran and the presence of *H. pylori* strains with double-positive status is of high clinical relevance to *H. pylori* associated diseases.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the author.

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