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The Prevalence of *Helicobacter pylori babA2, iceA1* and *iceA2* Genes and Their Association with Clinical Outcomes in Patients with Chronic Gastritis, Ulcerative Diseases and Non-Ulcer Dyspepsia in South East of Iran

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A B S T R A C T

Background: *Helicobacter pylori* virulence factors are important in development of the clinical outcomes. The initial stage of colonization is binding of *H. pylori* to gastric epithelial cells through the *babA* protein. Heterogeneity among *H. pylori* strains in presence and expressing the babA gene may be a factor in the variation of clinical outcomes. Likewise, another recently *H. pylori* described virulence factor; *iceA* has been shown to be a marker for ulcerative diseases.
Objectives: We investigated the presence of *babA2, iceA1* and *iceA2 H. pylori* virulence factors in patients with clinical outcomes in the southeast of Iran.
Patients and Methods: In this study, 63 positive culture samples out of 191 biopsies examined to determine of *babA2, iceA1* and *iceA2* genes by PCR. DNA extracted from 63 *Helicobacter* positive specimens including 46 chronic active gastritis, 6 ulcerative diseases and non-ulcer dyspepsia 11.
Results: The frequency of the *babA2, iceA1* and *iceA2* genes in the total isolates were 34 (54%), 14 (22.2%) and 34 (54%), respectively. The association of these virulence factors based on sex and age groups were not statistically significant ($P > 0.05$). There was a borderline significant association between *iceA1* and the clinical outcomes ($P = 0.094$).
Conclusions: Our study showed that the prevalence of *babA2, iceA1* and *iceA2* virulence factors were lower than the other studies that highlighted the role of the geographic factor.

Keywords: Helicobacter pylori; Genes; Iran

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Implication for health policy/practice/research/medical education:
The results of this study can assist the medical microbiologists to know the prevalence of these three genes and their association with the clinical outcomes in our local isolates and measure with the rest of the world.

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1. Background

Helicobacter pylori is a Gram-negative spiral-shaped, microaerophilic bacterium colonizing the human stomach, and infects more than a half of the world's population (1). The outcomes of this infection are; gastric inflammation, peptic ulcer, gastric ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (2). According to the serological evidences the prevalence of this infection is in the range of 60-80% in Iran (3). The International Agency for Research on Cancer, now classifying *H. pylori* as a class I carcinogenic agents (4). The development of the infection depends on bacterial virulence factors, host susceptibility features and environmental factors such as smoking and diet (5).

The most defined genes associated with virulence in *H. pylori* are the vacA and cagA genes. VacA is a cytotoxin that could be found in 50-65% of the isolates. This cytotoxin induces vacuolation in HeLa or primary gastric epithelial cells *in vitro*. The *cagA* gene is present in about 60 to 70% of *H. pylori* strains and encodes a high-molecular-weight protein. It's found that cagA gene is more associated with PUD than in any other clinical outcomes (6).

The adherence of *H. pylori* to the gastric mucosa is considered to play a vital role in the initial colonization and long-term persistence in the human gastric mucosa (7). The *BabA* adhesin mediates binding of *H. pylori* to the fucosylated Lewis b histo-blood group antigen present on the surface of gastric epithelial cells. In animal models, attachment of *H. pylori* to gastric epithelial cells by *babA* protein is associated with increased severity of inflammation, development of parietal cell autoantibodies, and parietal cell loss (8). Interactions between *BabA* and Lewis b related antigens are the best characterized adhesin-receptor interactions in *H pylori* (9).

Alternatively a recently described *H.pylori* putative virulence factor is *iceA* (induced by contact with the epithelium), which exists in two allelic forms, *iceA1* and *iceA2*. *iceA1* is up regulated by contact of *H. pylori* with the gastric epithelium and has been suggested as a marker for peptic ulcer disease (10). *iceA1*is replaced by *iceA2* at the same locus in many strains. *iceA2* is unrelated to *iceA1* or other known proteins (11).

The role of the *iceA*gene product during human infection remains unrecognized and the translational start site of *iceA1* has not yet been confirmed, but interestingly, carriage of *iceA1* was shown to be weakly but significantly associated with pepticulcer in studies of *H. pylori* strains in the western countries (12).

2. Objectives

The aim of this present study was to determine the frequency of *babA2*, *iceA1* & *iceA2* virulence factors among our local *H. pylori* isolates and their association with the gastric outcomes, including: chronic gastritis, ulcerative disease such as peptic ulcer disease, duodenal ulcer disease and non- ulcer dyspepsia (NUD).

3. Patients and Methods

Sixty-three *H. pylori* isolates were obtained from 191 patients' biopsy samples referred to the endoscopy unit of Afzalipour hospital in Kerman, (southeast of Iran) during 2009. The biopsy samples were cultivated in Brucella agar medium (Merck, Germany), supplemented with 10% defibrinated sheep blood and three antibiotics (vancomycin 10mg/L, amphotericin B 10mg/L and trimetoprim 5mg/L). The inoculated plates were incubated at 37°C under microaerophilic atmosphere provided by anerocult C (Merck, Germany) for 3-5 days. The isolates were recognized as *H. pylori* by urease, catalase, oxidase positive and Gram- negative staining tests (13).

3.1. DNA Extraction and Amplification

DNA was extracted from all 63 *H. pylori* isolates by genomics kit (Bioneer, South Korea) according to the manufacturer's instruction. PCR conditions were as follows; Reactions were carried out in MWG thermo cycler in 25μl mixtures containing 12.5μl PCR master mix (Cinna gen, Iran), 9.5μl sterile deionized water, 1μl template DNA and 1μl of each oligonucleotide primers (*Table 1*).

Table 1. Primers Used to Amplification

Primer Name	5'-3' Sequence	Expected Fragment	Reference
BabA	Forward:AATCCAAAAAGGAGAAAAAGTATGAAA	833	18
	Reverse:TGTTAGTGATTTCGGTGTAGGACA		
iceA1	Forward: GTTGGGTAAGCGTTACAGAATTT	567	23
	Reverse: CATTGTATATCCTATCATTAC		
iceA2	Forward: GTTGGGTATATCACAATTTAT	229 or 334	23
	Reverse: TTR [a]CCCTATTTTCTAGTAGGT		

[a] R= A or G

The cycle temperatures were as followed: Initial denaturation at 95°C for 5mins followed by 30 cycles of denaturation at 95°C for 1min, extension at 72°C for 1min. The final extension step was extended to 5min at 72°C. For *babA2* annealing for 1min at 57°C: For *iceA1* annealing for 1min at 41°C for *iceA2* annealing for 1min at 43°C.

3.2. Electrophoresis

The PCR products were separated on 1 % agarose gels (CinnaGen, Iran) in TBE 1X (Tris/borate/EDTA) buffer. Bands were visualized under UV gel documentation and photographed. Ethidium bromide (Merck, Germany) as a stain has been added to the agarose gel during preparation to give a concentration of 0.2μl/mL.

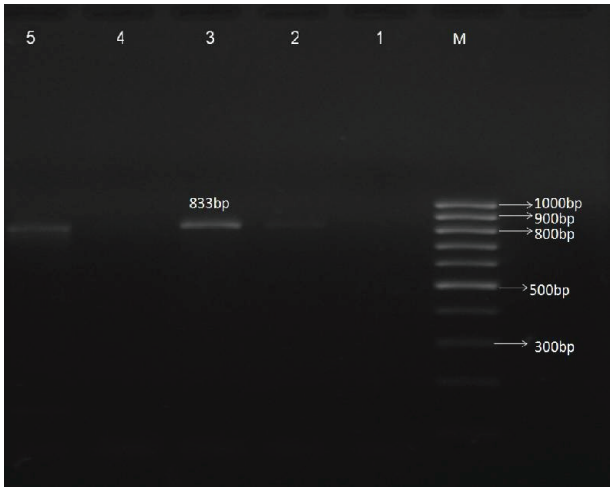
3.3. Statistical Analysis

We used absolute and relative frequency to present descriptive statistics. Chi square test as well as the Fisher exact test (when necessary) used to explain analytical statistics. Data were analyzed by SPSS version 16.0.

4. Results

DNA extracted from 63 (33%) *H. pylori* positive culture specimens biopsies obtained from 25 males and 38 females of whom; 46 (73%) had chronic active gastritis, 6 (9.5%) ulcerative disease includes PU & DU and 11 (17.5%) non-ulcer dyspepsia (NUD). The prevalence of the *babA2* was 34 (54%) (*Figure 1*) and the prevalence of *iceA1* and *iceA2* genes were 14 (22.2%) (*Figure 2*) and 34 (54%) (*Figure 3*), respectively. In this study, the *iceA2* amplicons yielded both the 229bp and 334bp fragments (*Figure 3*).

Figure 1. PCR Products Gel Electrophoresis for *babA2* Gene



M:1000bp DNA Marker (Fermentas, lituania), line1: negative control, lines 2,3,5: *babA2* gene positive samples, line 4: *babA2* gene negative sample

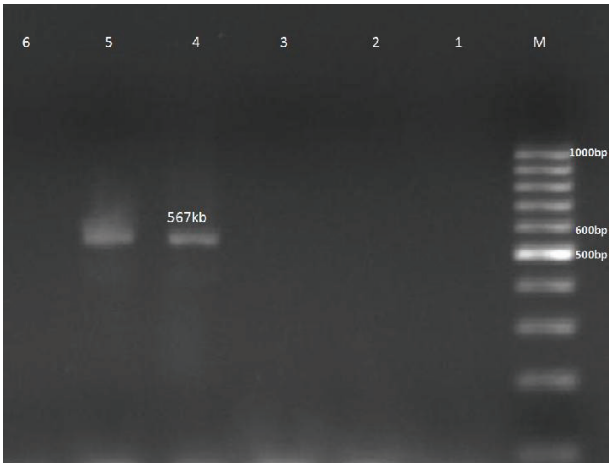
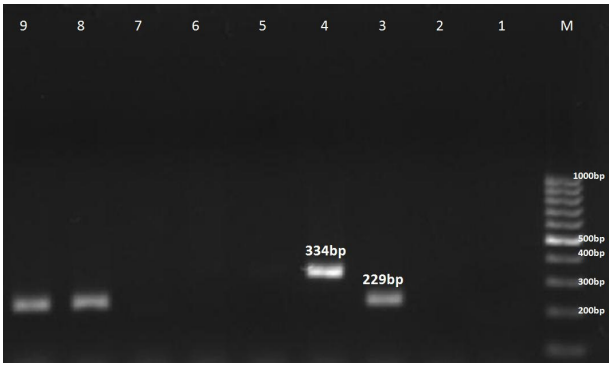


Figure 2. PCRGel Electerophoresis for *iceA1* Gene

Figure 3. PCR Products Gel Electrophoresis for *iceA2* Gene.



Line 1: negative control. Lines 2, 5, 6 and 7: *babA2* negative samples. Lines 3, 8 and 9: *babA2* positive samples (229bp fragment). Line 4: *babA2* positive sample (334bp fragment)

5. Discussion

This study showed that there was no statistically significant association between *babA2* and *iceA2* genes and the gastric outcomes, but there was a borderline significant association between *iceA1* gene and the gastric outcomes (P value = 0.094). *iceA1* prevalence in patients with chronic gastritis and ulcerative diseases was 26.1% and 33.3% respectively while there was no presence of *iceA1* gene in isolates from patients with NUD. In addition, *babA2* was more common in patients with chronic gastritis and ulcerative diseases; however, it was not statistically significant, but these results could be confirmed as an important role in the beginning of the gastric outcomes (*Table 2*). There was no association between the age and the sex of the patients and the prevalence of the *H.pylori* virulence factors (*Table 3*)

The exact role of the *iceA* gene is not yet fully understood, nevertheless we detected that *iceA2* positive *H. pylori* isolates and they are more common in patients with NUD (72.7%) however, this finding perhaps due to small

sample size was not statistically significant. On the other hand, there was no presence of *iceA1* in patients with NUD and only 45.5% of the NUD patients had the *babA2* gene.

Table 2. Association of Virulence Factors With Clinical Outcomes

	babA2, No. (%)		*iceA1*, No. (%)		*iceA2*, No. (%)	
	Positive	Negative	Positive	Negative	Positive	Negative
Gastritis (n=46)	24(52.2)	22(47.8)	12(26.1)	34(73.9)	24(52.2)	22(47.8)
Ulcerative disease (n=6)	5 (83.3)	1 (16.7)	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)
Non-Ulcer dyspepsia (n=11)	5 (45.5)	6 (54.5)	0 (0)	11 (100)	8 (72.7)	3 (27.3)
P value	0.31	0.31	0.094	0.094	0.28	0.28

Table 3. Prevalence of Virulence Factors Based on Age and Sex Groups

	Age, No. (%)		P value	Sex, No. (%)		P value
	≤40 y (n=34)	>40 y (n=29)		Male (n=25)	Female (n=38)	
babA2	17 (50)[a]	17 (58)	0.49	14 (56)	20 (52.6)	0.79
iceA1	10 (29.4)	4 (13.8)	0.13	6 (24)	8 (21.1)	0.78
iceA2	18 (52.9)	16 (55.2)	0.85	11 (44)	23 (60.5)	0.19

In the present study, the *iceA2* amplification yielded both the 229bp and 334bp fragments, This difference in the fragment size is due to the presence of a 105bp in-frame amplicons present in the 334bp fragment that is absent in the 229bp fragment (14). Ghasemian *et al.* announced 71.6% for *babA2* gene while we detected this gene in 54% of our local isolates that is controversial but identically as in our study they could not find any significant association between this gene and the clinical outcomes (15).

Talebi Bezmin Abadi *et al.* detected IceA1 genotype in 64% of their cases and *babA2* genotypes in 21.3% while in our study *babA2* was more frequent than *iceA1* (54% versus 22.2%). As same as our study they did not find a significant association between these two genotypes and the clinical outcomes (16). Probably due to the small sample size in our study, we could not show a statistically significant association between *babA* gene and the clinical outcomes in spite of its apparent high frequency in certain clinical outcomes but many studies confirm its close relation with the gastric outcomes.

Grehard *et al.* (17) indicated that the presence of *babA* was significantly associated with duodenal ulcer (P = 0.0002) and adenocarcinoma (P = 0.033) and would be a useful marker to identify patients who are at higher risk for specific *H. pylori*-related diseases. In another study in Brazil, Oleviera *et al.* showed that the *babA2* and *cagA* positive *H.pylori* strains are associated with duodenal ulcers and gastric adenocarcinoma (18). Likewise, our study, Yamaoka *et al.* did not observe a significant association between the *babA2* gene and duodenal ulcer (19).

Mizushima *et al.* announced that, *babA* prevalence in Japan is higher than the western countries. They detect the *babA* gene in approximately 85% of their local isolates while the rate is about 66% to 72% for the western countries. No significant correlation was found between the *babA2* genotype and the clinical outcomes (20). In our study, babA gene was detected in 54% of isolates, which is somewhat lower than the above-mentioned geographical locations. Whether such a difference is meaningful or is due to errors, remains to be investigated further.

For the first time Peek *et al.* introduced the *iceA* gene (induced by contact with epithelium) and showed that *iceA1* strains were significantly associated with peptic ulceration and increased mucosal concentrations of IL-8 (21). Likewise Van doorn *et al.* announced that isolates with *iceA1* are more leading to ulcerative diseases (14). In contrast with Smith *et al.* study that detected the *iceA* gene in all of the *H.pylori* isolates from Nigerian patients; we detected the *iceA1* gene in only 22.2% and the *iceA2* gene in 54% of our cases, but in accordance to our study they detect no association between the *iceA1* allele and peptic ulcer disease (22).

Ito *et al.* showed that there was no significant difference in the proportions of strains with the *iceA1* genotype between peptic ulcer and chronic ulcer groups. They also showed that the *iceA1* genotype did not appear to be a reliable marker of peptic ulcer disease among Japanese subjects (23).

Our study showed that the prevalence of *babA2*, *iceA1* and *iceA2* virulence factors were lower than the other studies that highlighted the role of the geographic factors. Likewise the lack of a significantly association between *babA2*, *iceA1* and *iceA2* virulence factors and the clinical outcomes is maybe due to this fact that the disease development is a multi-factorial process.

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Author's Contribution

None declared.

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None declared.

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