Analysis of babA, cagE and cagA Genes in Helicobacter pylori from Upper Gastric Patients in the North of Iran

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Abstract: **Objective:** Helicobacter pylori is a Gram-negative bacterium which has a serious effect on up to half of the world’s population and has been related to different gastric diseases. The goal of this study was to assess the frequency of babA, cagE and cagA genotypes among H. pylori strains isolated from gastric biopsies of endoscopic patients in the north of Iran.

**Methods:** The present study was performed on 90 strains of H. pylori isolated from patients with gastric diseases (Gastric ulcer (GU), Duodenal ulcer (DU), Gastritis (G), Non-ulcer dyspepsia (NUD) and Gastric adenocarcinoma (GC)). DNA was extracted from all isolated strains and PCR method was performed to detect the prevalence of babA2, cagE and cagA genes using specific primers.

**Results:** Among 90 samples of H. pylori, babA2, cagE, and cagA genes were detected in 42.2%, 30% and 82.2% of strains respectively. The statistical analysis showed that the prevalence of cagA gene in GU, G, DU, and NUD was significantly higher than other genes. Moreover, cagA, and babA2 genes were significantly more prevalent in GC patients compared to cagE gene. Our isolates exhibited 8 distinct arrangements of virulence patterns. The occurrence of cagA (35.6%) was the most prevalent pattern followed by cagA/babA2 (20%) and cagA/babA2/cagE (14.4%).

**Conclusion:** In summary, as first report from Guilan province in the north of Iran, we showed significant association between the presence of babA2, cagE, and cagA genes in different types of gastric disorders.

Keywords: Helicobacter pylori, babA, cagE, cagA, Gastric disorders.

1. **INTRODUCTION**

*Helicobacter pylori* is a micro-aerophilic, spiral-shaped, Gram-negative, urease and catalase positive pathogenic microorganism which has the ability to colonize in the mucus layer of the gastric epithelium [1-3]. The adherence of *H. pylori* to the mucus layer of the gastric epithelium has a significant impact on the initial colonization and persistence of the bacteria in the human stomach during decades or for an entire lifetime [1, 4].

Epidemiological studies have shown that *H. pylori* is considered to be the most remarkable cause of...
chronic gastritis and plays a crucial role in the development of other gastroduodenal diseases, for instance, duodenal ulceration, gastric lymphoma, and gastric cancer [2, 5]. This bacterium is able to perform its roles by several mechanisms. It can survive in the highly acidic gastric environment by metabolizing urea to ammonia via urease that creates a neutral environment enveloping the bacterium [6]. In addition, H. pylori changes its position across gastric mucus and can adhere to epithelial cells using a sort of adhesin-like proteins [7]. Once adhered to epithelial cells, H. pylori induces a powerful immune system response that leads to the development of chronic inflammation [7]. There are genetic differences among different H. pylori strains and the presence of high level of genetic variation confers an ability to this bacterium to have a high adaptation to the host gastric epithelium [8].

The most pivotal genes related to virulence in H. pylori are the cytotoxin-associated gene A (cagA). Blood group antigen-binding adhesin A (babA), induced by contact with epithelium (iceA), cytotoxin-associated gene E (cagE) [9, 10]. BabA mediates binding activity between bacterial adhesin and human Lewis b blood group antigens present on the surface of gastric epithelial cells [9]. The presence of interaction processes between BabA and Lewis b related antigens are the best defined adhesin-receptor interactions in H. pylori [11, 12]. Three main allelic variants of babA gene have been identified (babB, babA1, babA2). Some studies have shown a significant relation between babA2 positive genotypes and occurrence of peptic ulcer diseases [13]. Additionally, cagE and cagA genes have been introduced as two candidate virulence markers and are likely to play a fundamental role in the pathogenicity of the bacterium [10, 14]. Recent studies showed a pathogenicity island within the H. pylori genome that includes 31 genes [15]. The presence of cag-PAI has a considerable impact on the inflammatory state of the gastric mucosa by polymorphonuclear cell infiltration and enhances the interleukin-8 (IL-8) secretion after infection [14]. CagE is involved in NF-KB activation and interleukin-8 (IL-8) secretion in host epithelial cells [16, 17]. cagA gene plays an important role in gastric pathologies [14]. After the establishment of infection by H. pylori into human gastric epithelial cells, CagA acts as a critical carcinogenic and virulent factor via sequential CagA signal transduction pathway [18].

Since there was no report on the prevalence of H. pylori genotypes in the north of Iran, the present study focused on the frequency of babA2, cagE and cagA genes among 90 H. pylori strains isolated from gastric biopsies of endoscopic patients which were equal in the number of genders in each disease in the north of Iran and their association with gender and age of patients.

2. MATERIAL AND METHODS

2.1 Patients and H. pylori Isolation

In this cross-sectional study, 90 H. pylori isolates were included from biopsies of 300 patients referred for endoscopy at the teaching hospitals of Rasht city in the north of Iran during two years, from March 2013 to February 2015. To avoid any bias in the results, the equal number of patients in both gender and number of samples in each group of disease was considered. Gastric biopsies of patients with Gastric ulcer (GU), Duodenal ulcer (DU), Gastritis (G), Non-ulcer dyspepsia (NUD) and Gastric adenocarcinoma (GC) who had an indication for endoscopy were taken by a specialist after obtaining informed consent. The study was approved by regional Ethics Committee and was in accordance with the declaration of Helsinki. Biopsies were cultured on Columbia agar supplemented with 5% sheep blood, 10% FCS (Fetal calf serum) and 6 g/ml cefsulodin, 10 g/ml trimethoprim and 5g/ml vancomycin and incubated at 37°C for 5-7 days under microaerophilic condition, colonies with Gram-negative bacilli in microscopic examination and catalase, oxidase, and urease positive were confirmed as H. pylori [19].

2.2. Identification of H. pylori by PCR

DNA was extracted from fresh isolates of H. pylori before storage at -80°C using DNA extraction kit (Roche Co., Germany) according to the manufacturer. Then, DNA density was assessed by Nanodrop. All extracted DNA amplified for ureC (glmM) gene [20] by PCR using automatic thermo cycler (Eppendorf Personal 5332, Germany). PCR reactions were performed in a final volume of 25 μL containing 2.5 μL 10x buffer, 0.75 μL MgCl2 (50 mM), 2.5 unit Taq DNA polymerase (Cinnagen CO., Iran), 0.5 μLdNTP (10mM) (Cinnagen Co., Iran), 20 Pico mole from each primers and 1 μL from genomic DNA as a template. These primers (Table 1) were used to amplify a fragment of
294 bp from the *ureC* gene. Amplification was performed with 35 cycles of pre-incubation (94º for 3 min), denaturation (94º for 1 min), annealing (55ºC for 1 min), extension (72ºC for 1 min) and a final extension (72ºC for 5 min). PCR products were electrophoresed in 1.5% agarose gel (Roche, Germany) containing Sybrsafe. DNA ladder (Roche Co, Germany) was used to detect the molecular weights of observed bands under UV lamp.

2.3. Amplification of cagA, cagE and babA Genes

Extracted DNA from all isolated *H. pylori* was used as a template for amplification of babA2, cagE and cagA genes using specific primers (Table 1) according to the aforementioned materials which used to amplifying *ureC* gene [21-23]. All PCR products were subjected to electrophoresis on 1.5% agarose gel containing sybrsafe (Fig. 1). babA2, cagE and cagA amplicons (one positive sample from each gene) were sequenced to verify that they represented the studied virulence genes and were used as positive controls and DNase-free water was used as negative control. The sequencing was performed by Bioneer Company (Munpyeongseoro, Daedeok-gu, Daejeon, South Korea), and sequences were compared using online BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

2.4. Statistical Analyses

Chi-square and Fisher’s exact test were used to evaluating the relation between the genotypes and qualitative variables. Additionally, independent t-test was used for the relation of genes with quantitative variables.

### 3. RESULTS

In the present study, 90 endoscopic patients were assessed in 5 dissimilar disorders groups consisting of NUD, DU, GC, G, and GU in which each group contained 18 patients who were completely the same in sex distribution (p=0.999). Also, 5 different patients groups had no statistically

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers</th>
<th>Sequences (5'→3')</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>glmM</td>
<td>glmM-F</td>
<td>AAGCTTTTAGGGGTGTTAGGGTTT</td>
<td>294</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>glmM-R</td>
<td>AAGCTTACTTCTAACACTAACGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>babA2</td>
<td>babA-F</td>
<td>AATCCAAAAAGGAGAAAAAGTATGAAA</td>
<td>832</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>babA-R</td>
<td>TGTTAGTGATTCGATTGGAGACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cagE</td>
<td>cagE-F</td>
<td>AGACATGCAAAAAGGTAT</td>
<td>900</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>cagE-R</td>
<td>CAATCTAGTGTTGGTGGTGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cagA</td>
<td>cagA-F</td>
<td>ATATGCTAAATTAGCAACTTGAGCGA</td>
<td>298</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>cagA-R</td>
<td>TTGAATAATCAACAAACATCAGCCAT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. (1). Agarose gel electrophoresis of PCR products for A: cagA gene; B: cagE gene; C: babA2 gene. In figures: M: 100bp DNA size marker, column C-: negative control, C+: positive control, numbers: results of clinical samples.
differences with a view to age mean (p=0.051).

The frequency of babA2, cagE, and cagA genes associated with each type of diseases is shown in Table 2. The statistical analysis showed that the prevalence of cagA gene in GU, G, DU, and NUD was significantly higher than other genes (P <0.05). Moreover, cagA and babA2 genes were significantly more prevalent in GC patients compared to cagE gene (P <0.05). Data analysis demonstrated that there was no significant association between the existence of babA2, cagE, and cagA genes in different kind of diseases and gender. Additionally, according to the independent t-test, in the comparative survey between the age of patients with 5 different types of gastric disorders and investigated genes, there was no significant association.

Table 2. The frequency of babA2, cagE, and cagA genes in H. pylori isolates.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Age Mean ± SD</th>
<th>babA2 No. (%)</th>
<th>cagE No. (%)</th>
<th>cagA No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric ulcer</td>
<td>48.4 ± 15.1</td>
<td>7 (38.9)</td>
<td>3 (16.7)</td>
<td>14 (77.8)</td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>65.9 ± 16.3</td>
<td>13 (72.2)</td>
<td>7 (38.9)</td>
<td>16 (88.9)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>49.9 ± 17.8</td>
<td>3 (16.7)</td>
<td>7 (38.9)</td>
<td>16 (88.9)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>51.3 ± 15.5</td>
<td>8 (44.4)</td>
<td>6 (33.3)</td>
<td>15 (83.3)</td>
</tr>
<tr>
<td>Non-Ulcer dyspepsia</td>
<td>55.1 ± 19.6</td>
<td>7 (38.9)</td>
<td>4 (22.2)</td>
<td>13 (72.2)</td>
</tr>
<tr>
<td>Total</td>
<td>53.9 ± 17.7</td>
<td>38 (42.2)</td>
<td>27 (30)</td>
<td>74 (82.2)</td>
</tr>
</tbody>
</table>

With regard to the distribution of the virulence genes, our isolates exhibited 8 distinct arrangements of virulence patterns (Table 3). The occurrence of cagA (35.6%) was the most prevalent pattern followed by cagA/babA2 (20%), and cagA/babA2/cagE (14.4%). Meanwhile, the highest co-occurrence of investigated genes, cagA/babA2/cagE was seen in GC patients (6/13, 46.2%).

4. DISCUSSION

*Helicobacter pylori* is a successful human pathogen that grows in the human gastric epithelium and has a crucial role in the development of gastric diseases such as peptic ulcer, duodenal ulcer and gastric cancer [24, 25]. This common bacterium seriously infects half of the world’s population [26]. Several reports suggested that *H. pylori* colonization rate is at the highest level in developing countries [26]. Many different kinds of factors could be effective in the bacterium pathogenicity, for instance, genetic, environmental and bacterial virulence factors [21]. Considerable evidence has admitted that several important virulence genes play a pivotal role in *H. pylori* infection such as babA2, cagA and cagE genes. These gene products confer the ability of a bacterium to adhere to gastric cells which can induce pathogenicity of the bacterium [21]. In the present study, the prevalence of babA2, cagE and cagA genes in 90 *H. pylori* strains isolated from a gastric biopsy of endoscopic patients in the north of Iran and their relation to mentioned diseases were determined. Furthermore, the association between the presence of babA, cagE and cagA genes and age and sex of patients were identified. In this regard, cagA gene was found in 82.2% of isolates. The results revealed that there was no significant relationship between the individual existence of cagA gene and any specific gastric disorder. Additionally, cagE gene has been identified in 30% of strains. As a result, statistical analyses indicated that there were no significant differences between the presence of this gene and 5 different kinds of disorders. In contrast, babA2 has been detected in 42.2% of iso-

Table 3. Virulence patterns identified among *H. pylori* isolates.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>NUD No. (%)</th>
<th>DU No. (%)</th>
<th>G No. (%)</th>
<th>GC No. (%)</th>
<th>GU No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No gene</td>
<td>3 (16.7)</td>
<td>1 (5.6)</td>
<td>1 (5.6)</td>
<td>1 (5.6)</td>
<td>1 (5.6)</td>
<td>7 (7.8)</td>
</tr>
<tr>
<td>cagA</td>
<td>5 (27.8)</td>
<td>7 (38.9)</td>
<td>9 (50)</td>
<td>3 (16.7)</td>
<td>8 (44.4)</td>
<td>32 (35.6)</td>
</tr>
<tr>
<td>babA2</td>
<td>2 (11.1)</td>
<td>1 (5.6)</td>
<td>0</td>
<td>1 (5.6)</td>
<td>2 (11.1)</td>
<td>6 (6.7)</td>
</tr>
<tr>
<td>cagE</td>
<td>0</td>
<td>0</td>
<td>1 (5.6)</td>
<td>0</td>
<td>1 (5.6)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>cagA, babA2</td>
<td>4 (22.2)</td>
<td>3 (16.7)</td>
<td>1 (5.6)</td>
<td>6 (33.3)</td>
<td>4 (22.2)</td>
<td>18 (20)</td>
</tr>
<tr>
<td>cagA, cagE</td>
<td>3 (16.7)</td>
<td>2 (11.1)</td>
<td>4 (22.2)</td>
<td>1 (5.6)</td>
<td>1 (5.6)</td>
<td>11 (12.2)</td>
</tr>
<tr>
<td>babA2, cagE</td>
<td>0</td>
<td>1 (5.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>cagA, babA2, cagE</td>
<td>1 (5.6)</td>
<td>3 (16.7)</td>
<td>2 (11.1)</td>
<td>6 (33.3)</td>
<td>1 (5.6)</td>
<td>13 (14.4)</td>
</tr>
</tbody>
</table>
lated strains and there was a significant association between the presence of \( \text{babA2} \) and GC disease. Hussein Ali Osman and colleagues analyzed gastric biopsy samples of 105 \( H. \text{pylori} \)-positive patients and detected the prevalence of \( H. \text{pylori} \) virulence genes (\( \text{cagA, babA2, and dupA} \)) and their correlation with clinical dyspepsia outcomes. Their results demonstrated that there was no significant association between \( \text{babA, cagA and dupA} \) genes and clinical outcomes (\( P > 0.05 \)) [27]. Additionally, Chomvarin and colleagues analyzed 112 patients’ biopsies and showed no statistically significant relation between \( \text{babA, cageE and cagA} \) genes and clinical outcomes in Thai population (\( P > 0.05 \)) [23]. Several crucial factors have a noticeable effect on the differences between our results and other reports on patient’s selection and different geographic areas. Our study investigated the correlation between the existence of \( \text{babA, cagE and cagA} \) genes and 5 different types of upper gastric diseases; other studies searched about one or two diseases [28, 29].

In summary, as the first report from Guilan province in the north of Iran, we showed the association of \( \text{babA2, cagA, and cagE} \) genes with different types of gastric disorders. In this regard, we found a significant association between the presence of specific genotype and gastric diseases. This finding can provide good epidemiological background contributing to the international data of \( H. \text{pylori} \) virulence genes distribution.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by regional Ethics Committee and was in accordance with the declaration of Helsinki.

HUMAN AND ANIMAL RIGHTS

No Animals were used for studies. All humans research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGMENT

This work was supported by the Guilan University of Medical Sciences Fund.

REFERENCES


