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The Prevalence of *Helicobacter pylori* Virulence Related Genes (*hpa* and *babA2*) in Iranian Patients with Gastrointestinal Disorders

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Abstract

Background: The clinical result severity of *Helicobacter pylori* infection is determined by a combination of environmental factors, host genetic background, and *H. pylori* virulence factors. A number of genes containing vacA, *iceA*, *babA2*, *cagA*, *cagE*, *hsp60-70*, and hpa have been identified to enhance *H. pylori* pathogenicity by encoding virulent proteins. The *babA* and hpa proteins are considered 2 major adhesion molecules, and thus they are key agents in the initial step of *H. pylori* invasion.
Objectives: This study aimed at investigating the existence of *babA2* and *hpa* virulence factors of *H. pylori* in Iranian patients with gastrointestinal complications. The relationship between these agents and clinical results was also investigated.
Methods: A total of 80 positive biopsies out of 156 samples were studied to determine *babA2* and *hpa* gene frequency by PCR. The positive biopsies were collected from patients suffering from gastric cancer (n =18), peptic ulcer (n = 26), and gastritis (n =36).
Results: The *babA2*+ strains were found in 51 (64%) patients and the *hpa*+ strains in 57 (71%) patients were associated with sex (P = 0.02). However, the frequency of these factors was not significant between gastric disease groups (P > 0.05).
Conclusions: Our results revealed different frequency of these virulence factors in Iran, which emphasized the effects of geographical influences. Also, it was found that male patients had higher rate of *hpa* than females, highlighting the gender specific factors.

Keywords: Gastrointestinal, *BabA2*, *Helicobacter pylori*, *Hpa*

1. Background

Helicobacter pylori is a Gram-negative bacterium in the digestive tract, which is mainly found in human gastric mucosa (1). *Helicobacter pylori* is an important etiological factor in multiple gastraduodenal disorders including functional peptic ulcers, dyspepsia, gastric cancer, and mucosa-related lymphoid tissue lymphoma (2). Some of these virulence effects of *H. pylori* are encoded by *babA2*, *VacA*, *iceA*, *cagA*, *cagE*, *Hsp60-70*, and Hpa genes, which act differently and have meager functions in pathogenesis (3). *Helicobacter pylori* reaches the gastric mucosa and it makes use of several adhesions such as *hpa* and *baba*; baba is one of the most studied adhesions and is encoded by the gene *babA2* and binds to Lewis-b blood group antigens (4). Based on the serological document, the frequency of this agent is approximately 60% to 80% in Iran (5).

BabA is the blood group antigen-binding adhesion, which is encoded by the *babA2* gene and binds to Lewis-b blood-group antigen that exists in gastric epithelial cells. There are 3 *bab* alleles (*babA1*, *babA2*, and *babB*) of *H. pylori*, but only the *babA2* gene indicates Lewis-b binding function (6). The *BabA* adhesion of *H. pylori* is an external membrane protein that is attached to the fucosylated histo-blood group antigens on the outer surface of gastric epithelial receptors (6, 7). Duodenal users and adenocarcinoma *H. pylori* agglutinin (*hpa*) is a binding protein of *H. pylori* that is coded by the *hpa* gene and binds *H. pylori* to gastric mucosal cells. Weighting 29 *Kda*, *hpa* is the main flagellar sheath protein and is considered an *H. pylori* adhesion (8, 9); *hpa* is a colonization factor for *H. pylori* and it only gives rise to low immune responses after infection (8). *Hpa* was originally defined as a haemagglutinin found on the surface of the bacteria and not noticeable on the flagella (10).

2. Objectives

The present study aimed at identifying the frequency of *babA2* and *hpa* virulence genes of *H. pylori* isolates in gastric biopsy samples, establishing their associations with clinical disease, and correlating these data to the presence of gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma.

3. Methods

3.1. Ethics Statement

This study was approved by the ethical committee of Golestan University of Medical Sciences (ethic code: 35619112278).

3.2. Sampling

A total of 156 antral biopsy samples were obtained from patients who referred to endoscopy section of Shahid Sayad Shriazi hospital and 5 Azar hospital (Gorgan, Iran) from January 2014 to April 2015. Patients underwent endoscopy because of gastritis, peptic ulcer, gastric adenocarcinoma, and MALT lymphoma. Gorgan is located in the middle of Golestan province in north of republic of Iran and southeast of the Caspian Sea (11). We excluded those patients who received antibiotics. All patients were asked to sign a written informed consent. The present study was conducted based on the codes of the Helsinki's Declaration.

3.3. Identification Methods

Rapid urease test (RUT) was performed by RUT kit (Bahar Afshan, Iran) according to manufacturer's instruction. Further hematoxylin-eosin staining and Giemsa staining were used for histologic diagnosis. The histological slides were examined by 2 independent experts in histopathology to confirm the diagnosis. DNA was extracted from *H. pylori* positive biopsy samples using the Genomic DNA filtration Kit (Fermentas, Germany) based on the manufacture's protocol. Purified DNA was kept at -20°C until used. The sequence of *babA* and *hpa* genes were amplified by PCR using the correspond primers (Table 1). The amplification reaction (50 μL) contained 5 μL 10X PCR buffer, 2.5 mM $Mgcl_2$, 0.2 mM of each dNTP, 1 μL forward and reverse primer, 5 μL DNA template, and 2U Taq-Pol (Genetbio, South Korea). The PCR cycling situations were as follow: original denaturation stage at 95 for 5 minute, 35 cycles of 95°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute, and last extension phase at 72°C for 5 minutes. PCR outcomes were analyzed by 1.5% agarose gel electrophoresis premixed with ethidium bromide and visualized under a UV transilluminator.

3.4. Statistical Analysis

Statistical analysis was performed by statistical software package SPSS for Windows Version 18.00 (SPSS Inc. Illinois, USA). Chi-square test or Fisher's exact test were used to measure the relationship between *H. pylori* virulence indicators and clinical effects. P values less than 0.05 were considered statistically significant.

4. Results

The infection of *H. pylori* was confirmed by histologic analysis and RUT. From 156 samples, a total of 80 samples were infected by *H. pylori* (51.28%). We identified 80 gastric cancer patients and 36 patients with gastritis, moreover, 26 peptic ulcer patients were infected by the microorganism. The *babA2* + strains were found in 51 (64%) patients and *hpa* + strains in 57 (71%) patients, which were associated with sex (P = 0.02). From 80 samples infected with *H. pylori*, 36 (45%) were female and 55% were male. The primers used in this study are demonstrated in Table 1, and the frequencies of *babA2* and *hpa* gene in the biopsies in different gastric disease are summarized in Table 2. From 51 *babA2*+ strains, 21 (41.1%) were peptic ulcers, 19 (37.2%) gastritis, and 11 (21.7%) gastric cancer. *BabA2* and *hpa* genes were common in peptic ulcer participants. Moreover, there were no statistically significant differences among the groups (P > 0.05). The frequencies of *babA2* and *hpa* genes were higher in males than in females (Table 3), and only the *hpa* frequency was statistically significant (P = 0.02, OR = 3.21, 95% CI: 1.16 - 8.85).

Table 1. The Primers Used in This Study

Gene Name	Sequence (5' - 3')	Product Reference Length
bab A2	Forward: AATCCAAAAAGGAGAAAAAGTATGAAA	832
	Reverse: TGTTAGTGTGATTTCGGTGTAGGACA	
hpa	Forward: ATAAAGCTTTCGGTGGTGGTGGAACGATG	850
	Reverse: TATCTCGAGTTGTCGGTTTCTTTGC	

5. Discussion

The clinical progress of *H. pylori* infection is determined by several virulence factors that might forecast the risk for symptomatic clinical results. The attachment of *H. pylori* to epithelial cells is an appropriate step in explicit tropism and in developing gastraduodenal pathologies and persistency of infection (12). *BabA2* protein attaches *H. pylori* to the blood group antigen Lewis-b existing on the surface of human gastric epithelial cells (3). In the present study, we determined the frequency of *babA2* and *hpa* in 80 *H. pylori* isolates from selected patients with functional dyspepsia. In the current study, the *babA2* gene was found in 40% of gastric biopsies; this frequency was higher

Table 2. Frequency of *babA2* and *hpa* Genes in Different Studied Groups[a]

Gene Name	Gasteric Cancer	Gasteritis	Peptic Ulcer	P Value
babA2	11 (61.1)	21 (58.3)	19 (73.3)	0.47
hpa	10 (55.5)	25 (69.4)	22 (84.6)	0.10

[a]Values are expressed as No. (%).

Table 3. Frequency of *babA2* and *hpa* Genes in Different Gender Groups (44 Males and 36 Females)

Gene Name	Male	Female	P Value[a]
babA2	28 (63.6)	23 (63.8)	0.58
hpa	36 (81.8)	21 (58.3)	0.02

[a]Fisher's Exact Test was applied. Bold interface indicate significant value.

than *babA2* reported by Paniagua et al. (21.7%) and lower than Boria et al. (82.3%) and Safaei et al. (71.6%) (13, 14).

Numerous documents revealed that *H. pylori* expression *babA* are related to more severe mucosal cellular inflammation and increased risk of clinical outcome diseases like active gastritis, peptic ulcer, and gastric cancer (15). The study of Cadamuro et al. revealed that occurrence of *babA* was significantly associated with duodenal ulcer and adenocarcinoma and would be a useful marker to identify patients who are at higher risk for *H. pylori*-related diseases (15). Mizushima et al. found that *babA* prevalence in Japan is higher than the Western countries. They detected the *babA* gene in 85% of their local isolates, while the rate is about 66% to 72% for the Western countries. No significant relationship was found between the *babA2* genotype and the clinical outcomes (16). However, in our study, *babA* gene was detected in 51% of the isolates, and no association was found between the *babA* gene and gastraduodenal disease.

Helicobacter pylori adhesion A (*hpaA*), which is a conserved surface lipoprotein and is essential for colonization of *H. pylori*, can induce immune responses and produce anti-bodies against *HpaA* in humans. Furthermore, this protein is a promising vaccine candidate against *H. pylori* infection (17). *Hpa* is a strong colonization factor for *H. pylori*. In this study, we determined the frequency of the *hpa* genotype in 80 *H. pylori* isolates from patients with peptic ulcer or gastric adenocarcinoma. Therefore, it is difficult to explain different clinical outcomes only from virulence factors such as *babA2*, *hpa* of *H. pylori*. The *hpa* gene was found to be present in 27.5% of *babA2*-negative strains. In our study, the prevalence of *hpa* genotype was 71.2%, it was 31.2% in gastritis, 27.5% in peptic ulcer, and 12.5% in gastric cancer. Furthermore, in this study, no relationship was found between presence of *babA* and *hpa* genes and gastric disease. However, other studies have found a strong association between the occurrence of *babA2* gene and DU disease.

6. Conclusions

The results of this study provided information about the prevalence of 2 virulent factors of *H. pylori*. However, our data did not support the hypothesis that the virulence factors of *H. pylori*, *BabA* and *Hpa* are powerfully related to gastric adenocarcinoma, peptic ulcer disease, and chronic active gastritis in Western countries. Moreover, we did not identify a statistically significant relationship between *babA* and *hpa* virulence factors.

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Footnotes

Authors' Contribution: All authors contributed to the conception and design of the study, collecting data, and analyzing and interpreting the data. Fatemeh Mehravar drafted the manuscript. All authors were involved in critically revising the article for important intellectual content and gave final approval of the version to be published. All authors read and approved the final manuscript.

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