



Original Article

## Frequency of *Helicobacter pylori* blood-group antigen-binding adhesion 2 and sialic acid binding adhesion genes among dyspeptic patients in Tabriz, Iran

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### Abstract

**Introduction:** The purpose of this research was to analyze blood-group antigen-binding adhesion (babA2) and sialic acid binding adhesion (sabA) genotypes status in *Helicobacter pylori* (H. pylori) isolates and their relationship with clinical outcomes.

**Methods:** Gastric biopsy specimens were homogenized and placed in Brucella agar medium supplemented with 5% sheep blood and 3 antibiotics and were cultured at 37 °C under microaerophilic conditions and incubated for 4-7 days. H. pylori was identified by typical morphology, gram-staining and urease tests, and babA2 and sabA genes were detected by polymerase chain reaction (PCR).

**Results:** From a total of 100 H. pylori isolates; babA2 and sabA genes were detected in 23.0 and 26.4%, respectively. There was a significant relationship between these genes and clinical outcomes ( $P < 0.050$ ).

**Conclusion:** We found that the babA2 status was not related to clinical outcomes in Tabriz, Iran. However, sabA was a promoting determinant for disease, and multivariate analysis disclosed sabA to be an independent marker of non-ulcer diseases in our subjects.

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### Introduction

*Helicobacter pylori* (H. pylori) is a gram-negative, curved, and highly motile bacterium.<sup>1</sup> This bacterium has special tropism to human's stomach and causes gastritis, peptic ulcer and gastric carcinoma and lymphoma.<sup>2</sup> These diseases are the result of interaction between the bacterium and the host. However,

environmental situations, host factors, as well as bacterial virulence factors may be essential for the differential development of diseases.<sup>3</sup>

Bacterium adherence to gastric epithelial cells is necessary for disease development.<sup>3</sup> H. pylori has several adherence proteins such as blood-group antigen-binding adhesion (babA) and sialic acid binding adhesion

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(sabA).<sup>4</sup> Recently, the gene encoding babA has been cloned (and termed babA2), which allows identification of H. pylori isolates harboring the babA2 genotype by polymerase chain reaction (PCR). babA2 is responsible for binding of H. pylori to blood-group antigen which binds to Lewis b and related antigens.<sup>5</sup> Lewis b antigen and related fucosylated ABO blood group antigens are recognized by babA, and babA believed to be the main molecule involved in adherence to the gastric mucosa and implicated the presence of this factor, possibly specific to H. pylori-infected inflamed tissue, which binds to a receptor distinct from that of babA.<sup>6</sup>

SabA binds to carbohydrates and has an important role in initial colonization of H. pylori.<sup>7</sup> Previous studies reported that presence of these factors is associated with the development of gastric cancer,<sup>7</sup> so these molecules influence the severity of inflammation and disease outcome.<sup>8</sup>

However, the association between these factors and clinical outcomes has not exactly been determined, and the prevalence of the H. pylori babA2 and sabA genotypes has not yet been determined in Tabriz, Iran. In this study, we investigated the presence of babA2 and sabA genes in clinical H. pylori isolates with different clinical outcomes in Tabriz.

## Methods

Gastric biopsies were transmitted to microbiology laboratory into Stuart medium (Merck, Germany). They were homogenized between two sterile slides, and were placed in Brucella agar (6 µg/ml vancomycin, 2.5 µg/ml amphotericin B and 20 µg/ml trimethoprim), and were cultured at 37 °C under microaerophilic conditions (6% O<sub>2</sub>, 7% CO<sub>2</sub>, and 78% N<sub>2</sub>), and incubated for 4-7 days. H. pylori was identified by typical morphology, gram-staining and urease tests. At last, 100 bacteria were isolated, and their Deoxyribonucleic acid (DNA) were extracted by sodium dodecyl sulphate (SDS), proteinase K and cetyltrimethylammonium bromide (CTAB) method,<sup>9</sup> and stored at -20 °C.

The conditions for the primers used in this study are shown in table 1. We used the

primers as previously reported,<sup>5</sup> with modified PCR condition. Each PCR reaction was done in a final volume of 20 µl as follows: babA2 and sabA: 13.35 µl molecular grade water, 2 µl of × 10 PCR buffer, 0.6 µl mgCl<sub>2</sub> (1.5 mM), 0.25 µl deoxynucleotide triphosphates (dNTP) (100 mM), 0.4 µl of primers (0.5 µM), 1 µl of Taq DNA polymerase (1.25 U) and 2 µl of DNA (~50 ng). DNA amplification was done using thermocycler (Eppendorf). DNA fragments were separated electrophoretically on 1% agarose gel in × 0.5 Tris-borate-EDTA (TBE) buffer [40 ml Edetic Acid (EDTA), 55 g boric acid and 108 g Tris, pH = 8], at 75 v for 75 min and stained with 0.5 µg/ml ethidium bromide solution.

Data were entered into the SPSS software (version 19, SPSS Inc., Chicago, IL, USA) and were evaluated by Fisher's exact test and logistic regression, and P < 0.050 was regarded statistically significant.

## Results

From 362 gastric biopsies, 100 H. pylori isolates were obtained. Patients (including 100 H. pylori positive) attended in endoscopy ward, suffering from dyspepsia (53.6%), epigastria pain (28.6%), anemia (7.1%), gastro-esophageal reflux disease (7.1%), and gastrointestinal bleeding (3.6%), in Imam Reza Hospital, Tabriz. This study was approved by the local ethics community (No: 5/4/3202, Date: 2013/07/07).

H. pylori isolates were obtained from gastric biopsy specimens of 100 patients (42 males and 58 females), with a mean age of 42.6 years, who underwent endoscopy from January 2013 to February 2014. Endoscopy findings of 22 patients were reported normal. H. pylori isolates were recovered from 18 patients with peptic ulcer diseases (14 duodenal ulcers, 4 gastric ulcer) and 60 patients with non-ulcer diseases.

From all of 100 isolates, 23.0% was positive for babA2 gene, and sabA gene was present in 26.4% (Figure 1). There is a significant relationship between these genes and dyspepsia (P = 0.010).

Table 1. PCR primers and conditions for detection of babA2 and sabA genes

| Primers | Nucleotide sequence (5'- 3') | Product size (bp) | Initial denaturation temperature [°C (min)] | Denaturation temperature [°C (s)] | Annealing temperature [°C (s)] | Extension temperature [°C (s)] | Cycles | Final extension temperature [°C (min)] | Reference |
|---------|------------------------------|-------------------|---------------------------------------------|-----------------------------------|--------------------------------|--------------------------------|--------|----------------------------------------|-----------|
| babA-F  | AATCCAAAAAGGAGAAAAAGTATGAAA  | 832 bp            | 94 (7)                                      | 94 (55)                           | 58 (55)                        | 72 (65)                        | 35     | 72 (7)                                 | 10        |
| babA-R  | TGTTAGTGATTTCGGTGTAGGACA     |                   |                                             |                                   |                                |                                |        |                                        |           |
| sabA-F  | CTTTAAGGAACATTTTATGAAAA      | 785 bp            | 94 (7)                                      | 94 (55)                           | 57 (55)                        | 72 (62)                        | 35     | 72 (7)                                 | 6         |
| sabA-R  | CACCGCGTATTGCGTTGGGTA        |                   |                                             |                                   |                                |                                |        |                                        |           |

PCR: Polymerase chain reaction; babA: Blood-group antigen-binding adhesion; sabA: Sialic acid binding adhesion



Figure 1. Identification of *H. pylori* (*Helicobacter pylori*) isolated from gastric biopsy samples and genotyping of babA2 and sabA genes by PCR (polymerase chain reaction)

Lane 1, 100 bp DNA-ladder; Lane 2, negative control without DNA of babA2; Lane 3 positive control of babA2; Lane 4 and 5, babA2-negative isolates; Lane 6, 7 and 8, babA2-positive *H. pylori* isolates; Lane 9, negative control without DNA of sabA gene; Lane 10 and 11, sabA-negative *H. pylori*; Lane12, positive control of sabA; Lane, 13, 14 and 15, sabA-positive *H. pylori* isolates.

Binary logistic regression analysis indicated that possession of babA2 ( $P = 0.100$ ) was not a promoting determinant for the disease categories in our subjects, but sabA ( $P = 0.005$ ) was a promoting determinant for disease. However, multivariate analysis disclosed sabA to be an independent marker of non-ulcer diseases.

### Discussion

H. pylori cause the most prevalent bacterial infection globally.<sup>10</sup> This bacteria permanently colonize gastric epithelial cells in approximately 25.0% of the population in developed countries, and 70-90% in developing countries.<sup>11</sup> Chronic infection due to H. pylori in susceptible individuals is associated with a variable degree of mucosal damage. Colonization with this bacteria is usually without clinical consequences but increases the risk of developing peptic ulcer disease, gastric adenocarcinoma, and lymphoma.<sup>11</sup>

H. pylori has an important role in the development of various gastroduodenal diseases; however, only a small proportion of infected people with this bacterium develop diseases. There is ongoing interest in identifying H. pylori virulence factors that may predict the risk of clinical presentation.<sup>12</sup> The clinical development of H. pylori infection is the result of interaction between several factors of the host and the bacterium. Among the bacterial factors, there are evidences of the influences of certain H. pylori adherence genotypes in clinical outcomes.<sup>13</sup>

This study was designed to characterize the genotypes of H. pylori from gastric biopsy specimens from dyspeptic patients in Northwest of Iran, a developing country where the prevalence of the infection can be as high as 90.0%.<sup>11</sup> Two genes have been proposed by many to be associated with the virulence of H. pylori and to our knowledge; this is first time to be characterized in H. pylori isolates from Azerbaijani population. The relationship of these genes with the development of peptic pathology might have a relation with the treatment success or failure.<sup>13</sup>

Major factors in the exact tropism and pathogenicity of H. pylori in the stomach include adherence and secretion of bacterial toxins. Specific tropism of H. pylori in the stomach depends on adhesions as babA2 and sabA that bind to epithelial cells on the gastric surface.<sup>14</sup> In this study, we investigated the frequency of two adhesion factors including babA2 and sabA in 100 patients with different clinical outcomes in Tabriz.

In this study, the babA2 gene was present in 23.0% of isolates. We found an association between babA2 with dyspepsia. However, multivariate logistic regression analysis indicated that possession of babA2 was not a promoting determinant for the disease categories in our subjects. Thus, our present clinical and experimental data do not confirm previous experimental studies that represent a key role of babA2 in the pathogenesis of ulcer disease.<sup>14,15</sup> It is important to note that there is substantial allelic variation in babA2 gene and additional adherence gens. The association of babA2 gene with different clinical outcomes has been shown in several studies.<sup>13-15</sup> Our findings are not in agreement with the study conducted by Arevalo-Galvis et al., who reported 57.0% of isolates were babA2 positive and more often found in patients with duodenal ulcer. Furthermore, other South American studies report that this gene frequency range from 46.0 to 82.3%.<sup>13</sup>

Erzin et al., from Turkey reported that babA2 gene is present in 23.3 and 46.6% of isolates from patients with the non-ulcer disease, and duodenal ulcer, respectively.<sup>16</sup> From Germany, babA2 genotype distribution identified in various gastric diseases.<sup>14</sup> Garcia et al., showed babA2 gene frequency in the Brazilian population is high, and is in association with various upper gastrointestinal diseases.<sup>15</sup> In Costa Rica, the prevalence of babA2 was 73.7%, which was higher than in Western studies (38.0-43.0%), but lower than that of Asian studies, also in Japanese patients (96.8%).<sup>5</sup> In Mexico, similar to our study, babA2 frequency is low (21.7%).<sup>17</sup>

The present study showed that sabA gene frequency is moderate. The sabA prevalence



was significantly different in each country. Yamaoka from Taiwan reported that *sabA* frequency in duodenal ulcer, gastric ulcer, and non-ulcer dyspepsia is 33.0, 22.0, and 29.0 percent, respectively.<sup>6</sup> In France and Germany, the frequency of *sabA* gene has been reported high, (86.0%).<sup>6</sup> A study in Tehran, Iran, showed *sabA* presences in 86.7% with gastric ulcer, and in 83.3% with gastritis and duodenal ulcer.<sup>7</sup> It has been proposed that *sabA*-positive status is negatively associated with ulcer diseases. We also found that the *sabA* status was related to clinical outcome in this area and the *sabA* gene was significantly associated with the presence of non-ulcer diseases ( $P = 0.005$ ). The current study confirms the distinctive difference in *H. pylori* genotypes in Iranian groups residing within Tehran and Tabriz.

In this study, because the isolates often were collected from symptomatic patients, the results reproduce the findings in these groups of patients rather than in whole population. We could not confirm a relationship between *babA2* genotype and clinical status, suggesting that this gene is not helpful for the general prediction of particular disease risk in this area.

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The clinical importance of the considered virulence-associated genes of *H. pylori* and geographical area is still a subject of controversy. The difference between the reports may be due to patient selection, sample size, diverse geographic, PCR condition, gene expression, and unknown adherence factors independent of the *sabA* and *babA2*.

## Conclusion

We found that the *babA2* status was not related to clinical outcomes in this area. However, *sabA* was a promoting determinant for disease, and multivariate analysis disclosed *sabA* to be an independent marker of non-ulcer diseases in our subjects.

## Conflict of Interests

Authors have no conflict of interest.

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