The Prevalence of Helicobacter Pylori babA, homB, aspA, and sabA Genes and Its Relationship with Clinical Outcomes in Turkey

Nimet Yılmaz and Meltem Koruk Özer

1. Introduction

Helicobacter pylori (H. pylori) is a gram-negative, spiral-shaped, 4-6-flagellated mobile bacterium that grows in the digestive tract and microaerophilic environment at 37°C in culture. The coccoïd form of H. pylori is also called sleeping form. H. pylori produces urease that catalyzes the hydrolysis of urea to yield ammonia and carbonic acid. Flagella, urease, and adhesins are all essential factors for H. pylori to colonize the gastric mucosa [1].

H. pylori may be present in almost half of the world population. The incidence of H. pylori infection varies according to gender, race, and social and socioeconomic status of the population. The people who are living in developing countries are very commonly infected with H. pylori whereas the frequency of H. pylori infection is rare in Australia, Canada, and the USA [2]. The occurrences of new gastric cancer cases were variable in developing countries (8.4%) and developed countries (4.5%) [3]. Gastrointestinal cancer-related death rate is the third most common cause of all cancer-related deaths. H. pylori are correlated with the development of duodenal ulcer and gastric cancer. H. pylori infected individuals are having risk of developing peptic ulcer in 15-20%, gastric cancer in 1%, and primary gastric lymphoma in 0.1%. H. pylori infection is a high risk factor for the development of peptic ulcer, gastric malformation, and adenocarcinoma [4]. Therefore, it may cause significant health problems. The transmission way of H. pylori has not been fully clear, yet [5].

H. pylori are adapted and colonize harsh, acidic environment of the stomach and survive in acidic environment that
causes induction of gastritis, peptic ulcer, or gastric cancer. *H. pylori* is actually an opportunistic pathogen. Some virulence factors of *H. pylori*, such as *cagA* and *vacA*, are the most pathogenic factors among all virulence factors [6]. There are also some other genes of *H. pylori* such as *babA*, *homB*, *aspA*, and *sabA* the significance for pathogenicity of which is not clear yet.

*H. pylori* adhesions such as the Lewis blood group antigen-binding adhesion (*babA*) and the sialic acid-binding adhesion (*sabA*) are considered to have a significant function on initial colonization of *H. pylori* [7, 8].

*H. pylori* outer-membrane proteins (*hom*) family is a small protein family including the C-terminal hydrophobic motif and signal sequences of outer membrane proteins. The *hom* family is one of the outer-membranes coding gene family that is divided into two families: *homA* and *homB* which are 90% identical; the difference is related to central domain [9].

Recent studies on adherence features of *H. pylori* have reported that *babA* promotes attachment of *H. pylori* to the gastric epithelial cells. The *babA* facilitates entry of *cagA* and *vacA* virulence factors into host cells [10, 11].

The second adhesion is *sabA* first identified in the *babA*-mutant *H. pylori* strain [8]. The *sabA* binds to sialylated carbohydrates on the surface of neutrophils. From this perspective, *sabA* is thought to promote immune response [11].

The aim of this study was to determine *H. pylori babA*, *homB*, *aspA*, and *sabA* genes and to identify the rate of these virulence genes in the biopsy samples by PCR analysis.

2. Materials and Methods

2.1. Collection of Biopsy Samples. A total of 214 patients were included in this study: 115 nonulcer dyspepsia and 99 peptic ulcer. The patients were from south east part of Turkey undergoing upper gastrointestinal endoscopy at the endoscopy unit of the Department of Gastroenterology, University of Gaziantep. During endoscopy, biopsy samples were taken and the obtained tissues were placed into 0.8% saline physiologic solution and then cultured immediately.

Informed consent was taken from all patients and The Ethics Committee of Medical School of University of Gaziantep approved the study. Results were confirmed both clinically and microbiologically.

2.2. Microbiologic Analysis

2.2.1. Culturing. In order to prevent contamination, aseptic conditions were provided. The obtained tissues were immediately placed into a liquid 0.8% serum physiologic solution and inoculated into Columbia agar with 5% sheep blood (BD, Heidelberg, Germany), containing *H. pylori* selective supplement (OXOID LTD, Basingstoke, Hampshire, England) to eliminate another bacterial contamination, and then incubated under anaerobic conditions, 5% CO₂ at 37°C for 4-6 days.

2.2.2. Urease, Catalase, and Oxidase Tests. To prove existence of *H. pylori*, catalase (Merck, Darmstadt, Germany), urease (Merck, Darmstadt, Germany), and oxidase (Merck, Darmstadt, Germany) tests were performed and also *H. pylori* morphology was identified.

2.2.3. Gram-Staining. To observe *H. pylori* under the light microscope, gram staining method was performed. Crystal violet (Merck, Darmstadt, Germany) was applied to heat-fixed smears of bacterial culture. Lugol (Merck, Darmstadt, Germany) that binds crystal violet was added. To decolorize it, ethanol (Merck, Darmstadt, Germany) was added and then stained with safranin (Merck, Darmstadt, Germany).

2.3. Genotyping of *H. pylori*

2.3.1. DNA Isolation. Genomic DNA was extracted from histopathologically confirmed cases of nonulcer dyspepsia and peptic ulcer using Qiagen DNA isolation kit Qiagen, QiAmp DNA Mini Kit (Hilden, Germany) according to manufacturer’s instructions. The DNA was stored at -20°C until used for molecular studies.

2.3.2. PCR Analysis. Touchdown PCR protocols were performed using Dream Taq DNA Polymerase (Thermo Scientific, Lithuania, EU) kit. PCR amplifications were performed on 50 µl master mixture that contained 100 ng of genomic DNA, 10 pmole each of primers, 10X buffer, 2 mM each of nucleotides (Deoxynucleotide Triphosphate, Thermo Scientific, Lithuania, EU), and 0.5 units of Taq Polymerase. PCR annealing temperatures for primers of *aspA*, *babA*, *cagA*, *homB*, *sabA*, and *vacA* were 59°C, 55°C, 58°C, 56°C, and 55°C, respectively. PCR products were then electrophoresed for 45 min at 130 Volt on 1% agarose gel in the presence of 0.5g/mL of ethidium bromide (Sigma, Steinheim, Germany) and illuminated under UV light (UVP EC3 imaging system, Upland, CA, USA).

2.3.3. Statistical Analysis. Comparisons of variables were performed with the chi-square test, One-way ANOVA test, and Tukey’s Multiple Comparison Test (GraphPad Prism 5) to compare the differences among nonulcer dyspepsia and peptic ulcer patients. *p* values <0.05 were considered significant.

3. Results

Gastric biopsies from all patients included in the study were cultured and initially assessed for the presence of *H. pylori* by urease, catalase, and oxidase tests. As a result of these tests, *H. pylori* were detected in 82 patients (38.32%), whereas bacteria could not be detected in 132 patients (61.68%). All *H. pylori*-positive patients (82) were further analyzed for the presence of *H. pylori* virulence factors by PCR using *babA*, *homB*, *aspA*, and *sabA*-specific primers encoding (Table 1(a)) *babA*, *homB*, *aspA*, and *sabA* genes (Figure 1).

A total of 82 *H. pylori*-positive patients (46 nonulcer dyspepsia (25 females, 21 males) and 36 peptic ulcer (16 females, 20 males)) were enrolled in this study (Table 1(b)). The mean age of the overall population was 45.7±16.5 years. There were significant relationships between gender and
Table 1: Oligonucleotide primers and sizes of the PCR products for virulence factors of H. pylori.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
<th>Annealing Temperature (°C)</th>
<th>Primer Length</th>
<th>Product Size (bp)</th>
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<tr>
<td>AspA-F</td>
<td>ATGCGTATGGAGCATGATTTCATT</td>
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<tr>
<td>AspA-R</td>
<td>TTTATGCTTTTTGAAAGCGTGTTT</td>
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<tr>
<td>BabA-F</td>
<td>ATGAAAAACACATCTTTTCATTA</td>
<td>52.5</td>
<td>24</td>
<td>2192</td>
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<tr>
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<td>57.4</td>
<td>27</td>
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<tr>
<td>CagA-F</td>
<td>AATACACCAACGGCTTCAAGG</td>
<td>57.3</td>
<td>20</td>
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<tr>
<td>CagA-R</td>
<td>GCTGACAAAGGAGCACTTCCC</td>
<td>59.4</td>
<td>20</td>
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<tr>
<td>HomB-F</td>
<td>TACAGACGCTCAAGGCAATG</td>
<td>57.3</td>
<td>20</td>
<td>1005</td>
</tr>
<tr>
<td>HomB-R</td>
<td>CTCTTGGTGGGCCGTTT</td>
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<td>20</td>
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</tr>
<tr>
<td>SabA-F</td>
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<td>59.4</td>
<td>20</td>
<td>187</td>
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(b) Gender Kind of disease Number of patients AspA+ BabA+ CagA+ HomB+ SabA+ VacA+ Mean Age

<table>
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<tr>
<th>Gender</th>
<th>Kind of disease</th>
<th>Number of patients</th>
<th>AspA+</th>
<th>BabA+</th>
<th>CagA+</th>
<th>HomB+</th>
<th>SabA+</th>
<th>VacA+</th>
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<td>16</td>
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<td>2</td>
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<td>2</td>
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<tr>
<td></td>
<td>GU</td>
<td>20</td>
<td>15</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>49.4±17.82</td>
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<tr>
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<td>NS</td>
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<td>18</td>
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<td>1</td>
<td>3</td>
<td>4</td>
<td>47.52±17.01</td>
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<tr>
<td></td>
<td>GU</td>
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<td>9</td>
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<td>1</td>
<td>0</td>
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<td>45.93±12.28</td>
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(c) Number of patients NS GU Total p value

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<th>GU</th>
<th>Total</th>
<th>p value</th>
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<td>36</td>
<td>82</td>
<td>&lt;0.05</td>
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<tr>
<td>BabA</td>
<td>7</td>
<td>9</td>
<td>16</td>
<td>ns</td>
</tr>
<tr>
<td>CagA</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>ns</td>
</tr>
<tr>
<td>HomB</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>ns</td>
</tr>
<tr>
<td>SabA</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>ns</td>
</tr>
<tr>
<td>VacA</td>
<td>12</td>
<td>8</td>
<td>20</td>
<td>ns</td>
</tr>
</tbody>
</table>

3.1. Virulence Factors

(i) Blood Group Antigen-Binding Adhesin, babA. The babA gene of H. pylori was determined in 16 patients (19.51%), whereas 66 patients (80.49%) were classified as babA-negative (Table 1(c)). Out of 16 babA gene positive patients, 7 of them (43.75%) were from nonulcer dyspepsia patients and 9 of them (56.25%) were from peptic ulcer patients (Table 1(b)). The presence of babA was statistically significant in nonulcer dyspepsia (p < 0.001) and peptic ulcer (p < 0.001) (Table 2(b)) (Figures 2(a)–2(g)).

(ii) Helicobacter Outer Membrane Family Member, homB. The 1005-bp PCR product indicating the presence of homB gene was detected in 3 patients (3.66%), whereas 79 patients (96.34%) were negative for homB gene (Table 2(a)). Out of 3 homB-positive strains, 2 isolates (66.6%) were from nonulcer dyspepsia patient and 1 isolate (33.3%) was from patient diagnosed with peptic ulcer disease. The presence of homB was associated with the presence of aspA (p < 0.001) and babA (p < 0.05) (Table 2(a)). A statistically significant correlation between homB and aspA and babA gene was

Figure 1: Characterization of virulence factors of H. pylori by PCR. Each lane shows different virulence factors of H. pylori strain isolated from human samples. M: Marker, A: babA (2192 bp), B: cagA (1741 bp), C: VacA (1624 bp), D: AspA (1401 bp), E: HomB (1005 bp), and F: sabA (187 bp).

the nonulcer dyspepsia and peptic ulcer diseases related to these virulence factors (p<0.001). However, there were no significant differences in mean age (p>0.05).
Table 2: Comparison and statistical analysis of _H. pylori_ virulence factors.

(a) | Comparison | p value | 95% CI |
--- | --- | --- | --- |
_CagA vs HomB_ | ns | -0.18 to 0.13 |
_CagA vs SabA_ | ns | -0.14 to 0.16 |
_CagA vs AspA_ | < 0.0001 | 0.50 to 0.81 |
_CagA vs BabA_ | < 0.05 | 0.002 to 0.31 |
_CagA vs VacA_ | < 0.05 | 0.002 to 0.314 |
_HomB vs SabA_ | ns | -0.11 to 0.19 |
_HomB vs AspA_ | < 0.0001 | 0.52 to 0.83 |
_HomB vs BabA_ | < 0.05 | 0.02 to 0.33 |
_HomB vs VacA_ | < 0.05 | 0.02 to 0.33 |
_SabA vs AspA_ | < 0.0001 | 0.49 to 0.80 |
_SabA vs BabA_ | ns | -0.009 to 0.302 |
_SabA vs VacA_ | ns | -0.009 to 0.302 |
_AspA vs BabA_ | < 0.0001 | -0.65 to -0.34 |
_AspA vs VacA_ | < 0.0001 | -0.65 to -0.34 |
_BabA vs VacA_ | ns | -0.15 to 0.15 |

(b) | NS | GU |
--- | --- | --- |
_AspA_ | < 0.0001 | < 0.0001 |
_BabA_ | < 0.001 | < 0.001 |
_CagA_ | < 0.001 | > 0.05 |
_HomB_ | < 0.001 | < 0.001 |
_SabA_ | < 0.001 | < 0.001 |
_VacA_ | < 0.001 | < 0.001 |

_detected_. Moreover, the status of _homB_ had significant effect on nonulcer dyspepsia (_p_ < 0.001) and peptic ulcer patients (_p_ < 0.001) (Figure 2(d)) (Figures 2(a)–2(g)).

(iii) Aspartate Ammonia-Lyase, _aspA_. Fifty-nine biopsies that were obtained from different patients (71.94%) were positive for the _aspA_ gene, with the remaining 23 (28.04%) being _aspA_-negative as a result of the 1401-bp PCR product (Table 1(c)). Out of 59 _aspA_-positive strains, 34 isolates (57.62%) were from nonulcer dyspepsia patients, and 25 isolates (42.38%) were from patients diagnosed with peptic ulcer disease. The frequency of _aspA_ (71.95%) (_p_ < 0.0001) was significantly higher compared to the frequency of _babA_ (19.51%), _homB_ (3.65%), and _sabA_ (7.31%) in nonulcer dyspepsia and peptic ulcer patients. The presence of _aspA_ was associated with the presence of _babA_, _homB_, and _sabA_ (_p_ < 0.0001) (Table 2(a)). There was a positive correlation between _aspA_ and _babA_, _homB_, _sabA_, and _vacA_ genes. The presence of _aspA_ had statistically significant impact on nonulcer dyspepsia (_p_ < 0.001) and peptic ulcer (_p_ < 0.001) (Table 2(b)) (Figures 2(a)–2(g)).

(iv) Sialic Acid-Binding Adhesin, _SabA_. The 187-bp PCR product indicating the presence of _sabA_ gene of _H. pylori_ was determined in 6 patients (7.31%), whereas 76 patients (92.69%) were classified as _sabA_-negative (Table 1(c)). Out of 6 _sabA_-positive patients, 5 of them (83.33%) were from nonulcer dyspepsia patients and 1 of them (16.66%) was from peptic ulcer patient. The presence of _sabA_ was just associated with the presence of _aspA_ (_p_ < 0.001). Furthermore, the presence of _sabA_ gene had significant effect on nonulcer dyspepsia (_p_ < 0.001) and peptic ulcer (_p_ < 0.001) (Table 2(b)) (Figure 2(f)).

Possible combinations of all of these virulence factors were determined in Turkish population (Figures 2(a)–2(g)).

4. Discussion

_H. pylori_ is a gram-negative bacillus which causes gastritis, peptic ulcer, and gastric cancer [12]. The prevalence of _H. pylori_ depends on geographic regions, age, social and economic status, occupation, and living environment [6, 13]. _H. pylori_ have genetically diverse strains, and the strains differ in virulence [14].

In this study, the distribution of _aspA_, _babA_, _homB_, and _sabA_ genes in _H. pylori_ isolated from patients suffering from gastroduodenal diseases in Turkey determined using PCR analysis and the relationship between these virulence factors was assessed.

Studies have reported that there is a relationship between _babA_-positive _H. pylori_ and gastric inflammation in humans. Furthermore, the _babA_-positive _H. pylori_ increased risk of peptic ulcer and gastric cancer in humans [15, 16].

The _babA_ gene has been detected on the outer membrane of the _H. pylori_ strain. It has been shown that the _babA_ is able to induce DNA double-strand breaks (DSBs) in the cells, but DSBs are the strictest type of DNA destruction and can cause chromosomal aberrations, such as deletions, insertions, and translocations resulting in loss of heterozygosity which are hallmarks of gastric cancer [17].

_H. pylori_ strains that were isolated from East Asia expressed _babA_ gene but _H. pylori_ strains from 24 western countries did not express _babA_ gene. These bacterial strains caused mild gastric problem. A meta-analysis review revealed that the existence of _babA_ is correlated with high risk of peptic ulcer (OR = 2.069), especially the duodenal ulcer (OR = 1.588). This type of association was observed only in Western countries and not in Asian countries [18].

During the first 2-12 weeks during the experimental _H. pylori_ infection, the _babA_ expression disappeared in the experimental animals [19, 20].

_H. pylori_ that was isolated from patient samples showed incredible variety at the _babA_ locus, which can translate distinct adhesin that binds only blood group (O/Le^b^, or A/ALe^b^ and B/BLe^b^) [21].

We have also observed the same results as documented in literatures. The _babA_ expression is a dynamic process. The vigorous and variety nature of host glycosylation adds extra complexity. It has been shown that loss of _babA_ expression associated with gender. For this conclusion, the mice model has been used [22]. The _oorA_, _scoD_, _aroQ_, _fldA_, and _aspA_ of _H. pylori_ proteins are thought to hypothetically interact. It is deduced that these proteins also play a role in oxidation reduction [23].
Figure 2: PCR application of the biopsy samples taken from H. pylori positive patients with nonulcer and gastric ulcer samples. The presence of AspA (a), BabA (b), VacA (c), HomB (d), CagA (e), and SabA (f) genes in nonulcer and gastric ulcer samples by using PCR. (g) The comparison of H. pylori virulence factors in normal and gastric ulcer samples.
Significant increase in protein with antioxidant activity (aroQ, aspA, fldA, icd, OorA, and scoB) and high acid environment adaptation proteins (katA and napA) in *H. pylori* has been shown to be high [23]. It has been shown that an increase in the expression of three genes encoding enzymes involved in intrabacterial ammonia production was observed. The genes are amidase *amiE* and *amiF* and aspA. These enzymes can help neutralize the protons entering under acidic environmental conditions by producing intrabacterial ammonia. This study revealed that aspA is taking part in intrabacterial ammonia production [24].

The cytotoxin-associated gene (*cagA*) and vacuolating cytotoxin (*vacA*) are *H. pylori* virulence factors and associated with gastric ulcer, gastric cancer [25, 26]. However, it has been documented that there is no difference between the presence of the *homB* gene and severe gastric diseases [27]. Besides, the disease reason has been correlated with many outer membrane proteins (OMPs). Particularly, the outer membrane proteins of *H. pylori* such as *alpA*, *alpB*, *babA*, *homB*, *hopZ*, *ospA*, and *sabA* are all correlated with variable disease outcomes [28]. *H. pylori* has a high content of simple sequence repeats, mainly in genes encoding outer membrane proteins [29]. The *cagA* and *vacA* are polymorphic genes. The outer membrane proteins families are strictly correlated paralogs. For instance, the bab- genes family consists of three paralogs babA, babB, and babC. These paralogs *H. pylori* genes can be located at three different chromosomal loci [30].

During infection and an increase of *H. pylori* colonization, *homB*, outer membrane protein, is very crucial for adherence of the *H. pylori* to the gastric epithelium. A statistically significant correlation between *homB* and *aspA* (*p < 0.0001*) and *babA* (*p < 0.05*) gene was detected in this study. On the other hand, there was no significant relationship between *homB* and *sabA* gene according to this study [31].

The *hom* gene family is a small paralogous protein. The *homB* and *homA* genes are almost the same which are 90% [24]. The *homB* was observed more often than *homA* in East Asia. The *homB* was correlated with an enlarged risk of peptic ulcer disease in East Asia. The *homB* has a role in proinflammation. The *homB* was important for bacterial attachment to host cell surface [9].

There are many different adhesion components existing on *H. pylori* to bind carbohydrates. The *sabA* has vital and important role in the primary colonization of *H. pylori*. *H. pylori* *sabA* proteins are also taking part in stable infections and development of chronic inflammation which directs to tissue damage [32].

*H. pylori* *sabA* is a diversity gene. The *sabA* gene has been associated with different stomach diseases such as 100% in gastric cancer, 86.7% in gastric ulcer, and 83.3% in gastritis and duodenal ulcer [33].

In conclusion, virulence factors of *H. pylori* gene sequences might differ markedly from other regions or other countries. These differences are detectable by PCR analysis and sequencing. These data suggest that virulence factor variants may present new markers for other factors involved in gastric carcinogenesis or probably influencing the result of *H. pylori* infection [34].

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Disclosure

The authors have no relevant financial or nonfinancial relationships to disclose.

### Conflicts of Interest

The authors declare that there are no actual or potential conflicts of interest related to this article.

### Acknowledgments

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### References


