Prevalence of *Helicobacter pylori* cagA, babA2, and dupA genotypes and correlation with clinical outcome in Malaysian patients with dyspepsia

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Background/aim: The severity of disease outcome in dyspepsia has been attributed to *Helicobacter pylori* virulence genes. The aim of this study was to determine the distribution of *H. pylori* virulence genes (cagA, babA2, and dupA) and to determine whether or not there arises a significant correlation with clinical dyspepsia outcomes.

Materials and methods: *H. pylori* genotypes cagA, babA2, and dupA were identified by polymerase chain reactions from gastric biopsy samples in 105 *H. pylori*-positive patients.

Results: The positive rates for cagA, babA2, and dupA genes in *H. pylori* dyspeptic patients were 69.5%, 41.0%, and 22.9%, respectively. cagA was more prevalent in Indians (39.7%), babA2 was more prevalent in Malays (39.5%), and dupA detection occurred more frequently in both Indians and Malays and at the same rate (37.5%). The Chinese inhabitants had the lowest prevalence of the three genes. Nonulcer disease patients had a significantly higher distribution of cagA (76.7%), babA2 (74.4%), and dupA (75.0%). There was no apparent association between these virulence genes and the clinical outcomes.

Conclusion: The lower prevalence of these genes and variations among different ethnicities implies that the strains are geographically and ethnically dependent. None of the virulence genes were knowingly beneficial in predicting the clinical outcome of *H. pylori* infection in our subjects.

Key words: *Helicobacter pylori*, cagA, babA2, dupA, ethnicity, virulence genes

1. Introduction

*Helicobacter pylori* affects more than half of the world’s population and over 70% of those inflicted reside in developing countries (1). *H. pylori* colonizes the gastric mucosa, causing chronic gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (2,3). The clinical outcome linked to these diseases has been associated with host genetic factors, environmental factors, and pathogen virulence factors (4). A number of proteins, including vacA, cagA, babA, dupA, SabA, and iceA, have been inferred to play a vital role in the virulence of *H. pylori* by increasing the severity of the disease outcome (5–8).

The cytotoxin-associated gene (cagA) is most commonly associated with cytotoxin production and the induction of interleukin 8 (IL-8) by gastric epithelial cells (9). The cag pathogenicity island (PAI), in which the cagA gene is localized at one end, is involved in the induction of gastric IL-8 production, though most reports have demonstrated that the cagA protein is not involved in IL-8 induction (10,11). However, one study has verified that cagA participates in IL-8 induction in a strain-dependent and time-dependent manner (12). cagA is deemed to be one of the most imperative virulence factors in the pathogenesis of *H. pylori*. cagA belongs to a cag PAI that codes a type IV secretion system and this secretion system is responsible for the translocation of cagA into host cells (13). In Western countries, cagA-positive strains are reported to be linked with severe clinical outcomes, but in East Asian countries, it remains abstruse when trying to find this link because almost all *H. pylori* strains possess cagA (14).
babA is a blood-group antigen-binding adhesin encoded by the babA2 gene, which has been shown to bind H. pylori to human Lewis b blood-group (Leb) antigens on gastric epithelial cells (15). Although three bab alleles have been identified (babA1, babA2, and babB), only the babA2 gene product is functional for Leb binding activity (16). Some studies discovered a significant relation between babA2 positive genotypes and the occurrence of peptic ulcer diseases (12), while others failed to find these relationships (17,18).

The duodenal ulcer (DU)-promoting gene (dupA) was initially described by Lu et al. in a study examining 14 vir gene homologs and their association with gastroduodenal disease, and especially with DU; hence, the gene was named dupA (6). dupA has been linked to an increased risk of DU and protection against gastric atrophy, intestinal metaplasia, and gastric cancer in Japan and Korea (6). Although some researchers have supported Lu et al.’s theory, others have found no such association. A study conducted within the Iraqi population reported that dupA is associated with peptic ulcers (19). In comparison, Argent et al. did not discover any correlation between dupA and DU in populations from Belgium, South Africa, China, and the United States (20).

The Malaysian population is divided into three ethnic groups (Malay, Chinese, and Indian) and these groups reflect differences in H. pylori infection. There are variations regarding the association between H. pylori virulence markers and H. pylori-associated diseases from one geographic area to another. Therefore, the aim of the present study was to assess the distribution of cagA, babA2, and dupA in H. pylori strains from Malaysia’s multiethnic population and to determine its association with clinical outcomes.

2. Materials and methods

This was a prospective study conducted on 226 patients who underwent routine endoscopies from July 2012 to January 2014 in the endoscopy units of Hospital University Sains Malaysia and Hospital Kuala Lumpur. Patients were excluded from the study if they had received treatment with antibiotics, proton pump inhibitors, H2 receptor antagonists, or bismuth compounds within the 4 weeks prior to the study. After the endoscopic examination, the gastric biopsy specimens from the antrum were examined for the presence of H. pylori by rapid urease tests, culture, and histology.

This study was approved by the Human Research Ethics Committee, University Sains Malaysia, Kubang Kerian, Kelantan, Malaysia, and the National Medical Research Registry. Written informed consent was obtained from each patient prior to enrollment in the study.

2.1. Rapid urease test

Gastric antral biopsies were collected for the rapid urease test (RUT). The diagnosis of infection was based on the RUT, culture, and histology. All 105 biopsy samples tested positive by RUT. Culture was performed on 81 samples, out of which 33 samples tested positive for H. pylori. Out of 30 samples diagnosed by histology, only 20 samples proved positive for H. pylori. RUT was performed with a solution of 1 mL of distilled water, one drop 1% of phenol red, and 100 mg of urea. One antral biopsy sample was placed in the solution immediately after endoscopy and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 h (21).

2.2. Transport of samples

Biopsy samples for polymerase chain reaction (PCR) were placed in 500 µL of Brucella broth with 20% (v/v) glycerol and kept at –80 °C until processing (22).

2.3. Culture and identification of H. pylori

Gastric biopsy specimens were inoculated onto Columbia agar base (Oxoid, UK) supplemented with 7% laked horse blood and H. pylori Dent’s selective (containing 5.0 mg/mL vancomycin, 2.5 mg/mL trimethoprim, 2.5 mg/mL cefsulodin, and 2.5 mg/mL amphotericin B), and the plates were incubated for 5–7 days at 37 °C under microaerophilic conditions. Organisms were identified as H. pylori by Gram stain and oxidase, catalase, and urease tests.

2.4. DNA extraction

Genomic DNA was extracted from a gastric biopsy using the QIAamp DNA tissue extraction kit (QIAGEN, Germany) according to the manufacturer’s instructions and the DNA was stored at –20 °C until analysis.

2.5. PCR amplifications and conditions

PCR amplifications of cagA, babA2, and dupA were carried out with the use of the primers listed in Table 1 (23–26). The PCR reaction mixtures were prepared using the TopTaq Master Mix Kit (QIAGEN, Germany) in a final volume of 25 µL containing 1.25 U of TopTaq DNA polymerase, 1X PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTP, 0.2 µM of each primer, 10 µL of molecular grade water, and 2.5 µL of DNA. The mixtures were placed in a PCR thermocycler (Eppendorf, Germany).

The PCR conditions for cagA included an initial denaturation of target DNA at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, with final extension at 72 °C for 15 min. As for the babA2 and dupA genes, the PCR conditions were: 35 cycles of denaturation at 94 °C for 45 s, primer annealing at 52 °C for 45 s, and extension at 72 °C for 45 s. PCR products were run on 1.5% agarose gels containing red gel in the TBE buffer according to the manufacturer’s instructions.
2.6. Data analyses
Stata Version 11 (StataCorp, USA) was used for analysis. The chi-square test or Fisher’s exact test was applied in order to analyze variances in H. pylori virulence genes among gastric ulcer (GU), DU, and gastritis. The statistical significance was set at P < 0.05.

3. Results
3.1. Patients and H. pylori
Out of 226 patients, 105 (46.5%) were confirmed to be infected with H. pylori by RUT. The infected patients (57 males and 48 females) ranged from between 26 to 86 years old (mean age: 54.48 ± 12.94 years). Based on the endoscopic findings, 77 patients were diagnosed with nonulcer dyspepsia (NUD) or gastritis, 9 had GUs, 5 had DUs, and 13 were normal.

3.2. Frequency of cagA, babA2, and dupA
cagA was detected in 73 (69.5%) of the biopsy samples. The distribution of cagA among the three groups (Indian, Malay and Chinese) was 29 (39.7%), 26 (35.6%), and 18 (24.7%), respectively (Table 2). The Indian population exhibited the highest distribution of cagA compared to the others.

The babA2 gene was observed in 43 (41.0%) patients derived from biopsy samples. The distribution of the babA2 gene among Indian, Malay, and Chinese populations was 14 (32.6%), 17 (39.5%), and 12 (27.9%), respectively (Table 2).

The dupA gene was also found in 24 (22.9%) biopsy samples. The distribution of the dupA gene among Indian, Malay, and Chinese inhabitants was 9 (37.5%), 9 (37.5%), and 6 (25.0%), respectively (Table 2).

3.3. cagA, babA2, and dupA genes and clinical outcome
The prevalence of cagA was higher in the NUD group (76.7%) than in the GU (11.0%), DU (4.1%), and normal groups (8.2%). Similarly, babA2 was most prevalent in NUD (77.1%) patients. dupA was more frequent in the NUD group (75.0%) than in the others (Table 3). The endoscopic findings (NUD, DU, GU, and normal group) were higher in male patients (54.3%) than in females (45.7%). Overall, there was no significant difference between cagA, babA2, and dupA genes and clinical outcomes (Table 3).

A combination of cagA, babA2, and dupA was detected in 15 biopsy samples and a combination of cagA and babA2 was noted in 38 biopsy samples. Twenty-one patients had a combination of both cagA and dupA. A total of 16 biopsy samples tested positive for babA2 and dupA, as indicated in Table 4. There was no significant variance observed between the combinations and clinical outcomes.

4. Discussion
The clinical development of H. pylori infection depends on a combination of many factors pertaining to both the host and the bacteria. Among the bacterial factors, studies have revealed that certain H. pylori genotypes cause more severe pathologies (27).

Table 1. Primers used for PCR amplification of cagA, babA2, and dupA genes.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5′-3′)</th>
<th>Size base pairs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA-D008</td>
<td>GGTCAAAATGCGGTCATGG</td>
<td>297</td>
<td>(23,24)</td>
</tr>
<tr>
<td>cagA-R008</td>
<td>TTGAATAATCAAAAAACATCACGCCAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>babA2</td>
<td>CCAAACGAAAACAAAAACGT</td>
<td>271</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>GCTTGTCAAAAACCGGTCGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dupA1</td>
<td>CGTATCAATATGGATTGCTT</td>
<td>197</td>
<td>(26)</td>
</tr>
<tr>
<td>dupA2</td>
<td>TCTTTCTAGCTTGACGA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Distribution of cagA, babA2, and dupA gene by ethnicity.

<table>
<thead>
<tr>
<th>Ethnic group (n)</th>
<th>cagA</th>
<th>babA2</th>
<th>dupA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Indian (37)</td>
<td>29 (39.7)</td>
<td>8 (25.0)</td>
<td>14 (32.6)</td>
</tr>
<tr>
<td>Malay (42)</td>
<td>26 (35.6)</td>
<td>16 (50.0)</td>
<td>17 (39.5)</td>
</tr>
<tr>
<td>Chinese (26)</td>
<td>18 (24.7)</td>
<td>8 (25)</td>
<td>12 (27.9)</td>
</tr>
</tbody>
</table>
In this study, we determined the frequency of \textit{cagA}, \textit{babA}2, and \textit{dupA} in dyspeptic patients. The prevalence of \textit{cagA} differs in every part of the world. The prevalence of \textit{cagA} is lower in Western countries (28,29) when compared to East Asian countries, where \textit{cagA} is present in more than 90% of cases irrespective of clinical presentation (30).

The prevalence of \textit{cagA} in this study was 69.5%; this is slightly lower than reports from East Asian countries. Studies conducted locally have reported differing percentages. According to Ramelah et al., the prevalence of \textit{cagA} was 94% (31), while Amjad et al. reported 43% (32). This divergence within the same country may well be due to differences in sample size, primer sets, or the variety of strains within the same country. In addition, the results of this study did not elaborate on conclusive evidence linking \textit{cagA} with NUD patients; our results are in agreement with supplementary studies in Asian countries (31,33) that failed to find any association. However, various studies have reported that \textit{cagA} was statistically concomitant with peptic ulcers (34,35).

\textit{babA}2 attaches \textit{H. pylori} to these cells, enabling delivery of vacA and \textit{cagA} toxins near the gastric epithelium and therefore increasing gastric tissue damage (15). The prevalence of the \textit{babA}2 genotype in our study was 41.0%.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Sex & NUD n (%) & GU n (%) & DU n (%) & Normal n (%) & \text{*P-value} \\
\hline
Male & 44 (56.4) & 4 (44.4) & 4 (80.0) & 5 (38.5) & 0.371 \\
Female & 34 (43.6) & 5 (55.6) & 1 (20.0) & 8 (61.5) & \\
\hline
Virulence genes & & & & & \\
\hline
\textit{cagA}+ & 56 (76.7) & 8 (11.0) & 3 (4.1) & 6 (8.2) & 0.146 \\
\textit{cagA}- & 22 (68.8) & 1 (3.1) & 2 (6.2) & 7 (21.9) & \\
\textit{babA}2+ & 32 (74.4) & 5 (11.6) & 3 (7.0) & 3 (7.0) & 0.290 \\
\textit{babA}2- & 46 (74.2) & 4 (6.5) & 2 (3.2) & 10 (16.1) & \\
\textit{dupA}+ & 18 (75.0) & 1 (4.2) & 2 (8.3) & 3 (12.5) & 0.700 \\
\textit{dupA}- & 60 (74.1) & 8 (9.9) & 3 (3.7) & 10 (12.3) & \\
\hline
\end{tabular}
\caption{Distribution of \textit{cagA}, \textit{babA}2, and \textit{dupA} and clinical outcome in \textit{H. pylori}-infected patients.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Virulence genes & NUD n (%) & GU n (%) & DU n (%) & Normal n (%) & \text{*P-value} \\
\hline
\textit{cagA}+/\textit{babA}2+/\textit{dupA}+ & 11 (32.4) & 1 (20.0) & 1 (33.3) & 2 (66.7) & 0.678 \\
\textit{cagA}-/\textit{babA}2-/\textit{dupA}- & 23 (67.6) & 4 (80.0) & 2 (66.7) & 1 (33.3) & \\
\textit{cagA}+/\textit{babA}2+ & 28 (82.4) & 5 (100) & 2 (66.7) & 3 (100) & 0.549 \\
\textit{cagA}-/\textit{babA}2- & 6 (17.6) & 0 (0.0) & 1 (33.3) & 0 (0.0) & \\
\textit{cagA}+/\textit{dupA}+ & 16 (47.1) & 1 (20.0) & 2 (66.7) & 2 (66.7) & 0.565 \\
\textit{cagA}-/\textit{dupA}- & 18 (52.9) & 4 (80.0) & 1 (33.3) & 1 (33.3) & \\
\textit{babA}2+/\textit{dupA}+ & 12 (35.3) & 1 (20.0) & 1 (33.3) & 2 (66.7) & 0.654 \\
\textit{babA}2-/\textit{dupA}- & 22 (64.7) & 4 (80.0) & 2 (66.7) & 1 (33.3) & \\
\hline
\end{tabular}
\caption{Combined \textit{cagA}, \textit{babA}2, and \textit{dupA} genotypes and clinical outcome.}
\end{table}
Our results are consistent with a study conducted in China, which reported a prevalence rate of 38.9% in dyspeptic patients (36), but slightly lower than studies from Turkey (53.8%) (34). Oliveira et al. discovered that babA2 was more frequently found in patients with DU and gastric cancer (35). The current study did not include a gastric cancer case and DU accounts only for 4.8% of the studied population, so this might have contributed to the low prevalence of babA2 in our study. Gerhad et al. discovered that babA2 was associated with peptic ulcer disease in Western populations (15). Our study failed to find any substantial associations between babA2 and the clinical outcome. This is in agreement with a previous conducted study (18).

During the present study, 24 (22.9%) dupA-positive H. pylori strains were observed in the patients’ biopsy samples. Our data are in line with a study of Japanese patients (21.3%) (6) and slightly lower than the findings of a study conducted in Malaysia also unearthed a comparable prevalence of 21.3% (38). During the present study, 24 (22.9%) dupA-positive H. pylori strains were observed in the patients’ biopsy samples. Our data are in line with a study of Japanese patients (21.3%) (6) and slightly lower than the findings of a study conducted in Malaysia also unearthed a comparable prevalence of 21.3% (38). dupA has been linked to an increased risk of DU and a decreased risk of gastric cancer (6). In contrast, Lu et al. (6) did not observe an association between dupA and DU in East Asia. These differences in results might be due to strain variations moving from one region to another. Furthermore, the DU patients used in our study account for only 4.8% of the study group, which prevents us from drawing a definitive conclusion.

Combinations of two or three of the virulence genes were not noticeably diverse among the NUD, GU, DU, and normal groups in our study, although a study conducted in Cuba reported a significant association between a combination of cagA and babA2 genotypes (39).

In our study, NUD patients tended to have a higher distribution of cagA (76.7%), babA2 (74.4%), and dupA (75.0%) compared to peptic ulcer disease. Our result is in agreement with a study from Iran, which found an obviously much higher prevalence of the cagA gene (73%) in NUD patients (40). Others have found a higher presence of cagA in peptic ulcer disease patients than in NUD (31,41). This dissimilarity might be due to an imbalance in NUD and peptic ulcer disease cases.

In conclusion, although there is no association between virulence genotypes and clinical outcomes in our study, the lower prevalence of these genotypes in H. pylori-positive patients and variations among different ethnicities indicates that there is strain variation among countries and ethnic groups.

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