Relationship between *Helicobacter pylori* vacA genotypes status and risk of peptic ulcer in Saudi patients

Aiman M. Momenah, PhD, Mohammed T. Tayeb, PhD.

**ABSTRACT**

**Objectives:** To determine if there is a significant correlation between different *Helicobacter pylori* (H. pylori) vacA genotypes strains and severe gastric clinical outcomes.

**Methods:** A total of 1104 gastric biopsies from 368 patients who presented with symptoms suggestive of chronic gastritis or peptic ulcer were taken from the main hospitals in the western region of Saudi Arabia from July 2004 to July 2005. These samples were cultured for *H. pylori*, and a polymerase chain reaction (PCR) was carried out to determine vacA genotypes status.

**Results:** One hundred and three (28%) patients were positive for *H. pylori* using culture technique. The distribution of vacA genotypes was 13 for vacAs1m1, 47 for vacAs1m2 and 43 for vacAs2m2. None of the clinical isolates were vacAs2m1 positive. The study showed a significant correlation between the vacAs1m2 genotype and gastritis cases, and a significant correlation between vacAs1m1 genotype and ulcer cases.

**Conclusion:** The results of this study might be used for the identification of high-risk patients who are infected by vacAs1m1 genotype *H. pylori* strains.

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*Helicobacter pylori* (H. pylori) is a gram-negative curved rod with a tuft of 4-7 polar flagella. The organism inhabits the gastric mucosa of the human stomach in approximately half of the world’s population for a lifetime.1-3 *Helicobacter pylori* induces gastric mucosal inflammation, causing gastritis which may progress into peptic ulcers.1,4,5 *Helicobacter pylori* is the major environmental factor in the development of gastric cancer increasing from 4-6 folds the risk of its development.5-9 One of the possibilities to explain the variation in clinical outcome caused by infection with *H. pylori* is that a considerable genetic variation exists between different strains of *H. pylori*.10-12 *Helicobacter pylori* strains that having variant genes can causing a considerable inflammatory response in the host more severely than other strains.10-12 VacA, the gene encoding the vacuolating cytotoxin, has a mosaic structure consisting of one of 3 signal sequence types (s1a, s1b, s2), and one of 2 mid-region types (m1 and m2).13,14 Type s1 strains were significantly more common than type s2 strains in patients with a past or present history of ulcer.15-18 The vacA genotype of strain is closely associated with cagA gene to induce peptic ulceration.15,18,19 All vacA positive isolates included were identified among the subjects with *H. pylori* associated gastritis.18,19 Type
s1m1 and s1m2 strains produce high and moderate level of toxin. Whereas the s2m2 strain produces little or no toxin. 10,19,20 The aim of this study was to determine the most common vacA genotype among H. pylori isolates from a group of Saudi patients with gastric complaints using polymerase chain reaction (PCR) as a typing system.

**Methods. Patients’ samples.** From July 2004 to July 2005, 1104 gastric biopsies from 368 patients who presented with symptoms suggestive of chronic gastritis or peptic ulcer were taken from gastric antrum and corpus from the main hospitals in the western region of Saudi Arabia (Al-Noor Specialist Hospital [560 bed], King Abdul-Aziz Hospital [272 bed], General King Fahad Hospital [710 bed], King Abdul-Aziz Hospital and Oncology Centre [425 bed] and King Faisal Hospital [221 bed]). Patients were excluded if they had a history of previous H. pylori treatment.

**Gastric biopsies transportation and culture.** Gastric biopsies were transported in a 0.5 ml Brucella broth media (Oxoid, UK). Three Gastric biopsies were obtained from each patient, one was used for rapid CLO test (for determination of urease activity) (Oxoid, UK) and the others were cultured on H. pylori selective agar (Oxoid, UK) and incubated at 37°C in a BBL GasPak (Becton-Dickinson, USA) containing a Campy-Pak Plus microaerophilic system generator (Becton-Dickinson, USA) and incubated for 7 days. The identity of H. pylori clinical isolates were confirmed by colonial morphology,Gram-stain (curved Gram-negative bacilli) and positive reaction for oxidase, catalase and urease tests.

**Deoxyribonucleic acid methods.** Chromosomal staphylococcal DNA was extracted and purified according to previous descriptions. 21 Primers were designed to amplify 259bp, 286bp, 570bp and 645bp products within the vacAs1, vacAs2, vacAm1 and vacAm2, and 203bp product within the Ure gene (positive control) (Table 1). A 5 µl of each PCR primer (0.025 µM final concentration; TIB Molbiol, Germany; Table 1) plus 5 µl of the extracted DNA were added to a PCR master mix (100 mM Tris-HCl, and 500 mM KCl at pH 8.3 at 20°C, 1.5 mM MgCl2, 200 µM each deoxyribonucleoside triphosphate, 0.025U Taq polymerase [Qiagen, UK]). This mixture was then heated to 94°C for 5 minutes and then subjected to 35 cycles each of 95°C for one minute, 52°C for one minute, and 72°C for one minute with a final extension of 72°C for 7 minutes using the Perkin Elmer Geneamp PCR system 2400. A 10 µl aliquot of each PCR product was loaded onto 1% agarose (Sigma, USA) and run at 90V for one hour prior to viewing under UVP BioDoct-It digital imaging system (UVP, Inc, Cambridge, UK) to determine vacA genotypes.

**Statistical analysis.** Distribution of vacA genotypes among clinical isolates, and its correlations with endoscopic findings were recorded and analyzed using SPSS (version 10). Chi-squared test was used to compare genotype frequencies using SPSS (version 10).

**Results.** Among the 368 suspected patients to be infected with H. pylori by means of clinical features and endoscopic findings, 103 (28%) were positive using culture technique. Among these positive cases, PCR distribution of vacA genotypes were 13 (12.6%) for vacAs1m1, 47 (45.6%) for vacAs1m2, and 43 (41.8%) for vacAs2m2. None of the clinical isolates was vacAs2m1 positive. The results revealed that all 103 cases were positive for Ure gene. The study showed that positive samples from gastric ulcer, gastritis, normal cases according to endoscopic findings were correlated with 6.6%, 70.8%, and 22.6% with the presence of H. pylori (Table 2). The results showed a high percentage of vacAs1m2 with a distribution of 56.8% in gastritis cases (p=0.0001). In case of ulcer, the highest rates were among vacAs1m1 with a frequency of 71.4% (p=0.001), while in normal cases the highest rates were among vacAs2m2 with a percentage of 86.4%.

**Discussion.** Helicobacter pylori is a microaerophilic, gram-negative bacterium that colonizes the gastric mucosa of approximately 50% of the world’s population, and is a primary pathogenic factor in benign and malignant gastroduodenal disease. 19 Recent studies have shown that different vacA genotypes can lead to different clinical outcome consequences in certain populations. 13-20 In this study, 28% of patients tested were H. pylori positive. This finding is much less than what has been reported elsewhere in the Kingdom with rates including 87% in Eastern, 61.6% in Central and 85% in Western regions. This variability in the incidence rates between different studies might be attributable to: 1) the differences in the methods used for identification this organism, 2) different demographic distribution of the bacteria among various regions, and 3) previous antibiotic consumption.24 These reasons might also explain the different distribution of this organism among different international studies. 12,24,25 The vacA s1m1, s1m2 and s2m2 genotypes were detected in all 103 H. pylori samples PCR tested. In this study, the prevalence of the vacA genotypes s1m1 was detected.
Table 1 - Properties of oligonucleotides primers bp - base pair, F - forward, R - reverse.

<table>
<thead>
<tr>
<th>Primer designation</th>
<th>Target gene</th>
<th>Nucleotide sequence, 5’ to 3’</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ure1</td>
<td>Urease1</td>
<td>TAA CAA ACC GAT AAT GGC GC</td>
<td>203</td>
<td>22</td>
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<tr>
<td>Ure2</td>
<td>Urease2</td>
<td>CAT CTT GTT AGA GGG ATT GG</td>
<td>259</td>
<td>23</td>
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<tr>
<td>vacA s1F</td>
<td>Vaccumulating</td>
<td>ATG GAA ATA CAA CAA ACA CAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacA s1R</td>
<td>Vaccumulating</td>
<td>CTG CTT GAA TCGGC AAA C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacA s2F</td>
<td>Vaccumulating</td>
<td>ATG GAA ATA CAA CAA ACA CAC</td>
<td>286</td>
<td>23</td>
</tr>
<tr>
<td>vacA S2R</td>
<td>Vaccumulating</td>
<td>CTG CTT GAA TGC GCC AAA C</td>
<td></td>
<td></td>
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<tr>
<td>vacA m1F</td>
<td>Vaccumulating</td>
<td>CAA TCT GTC CAA TCA AGC GAG</td>
<td>570</td>
<td>23</td>
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<tr>
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<td>GCG TCT AAA TAA TTC CAA GG</td>
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<td></td>
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<tr>
<td>vacA m2F</td>
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<td>CAA TCT GTC CAA TCA AGC GAG</td>
<td>645</td>
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<tr>
<td>vacA m2R</td>
<td>Vaccumulating</td>
<td>GCG TCT AAA TAA TTC CAA GG</td>
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</table>

Table 2 - VacA genotype frequencies according to endoscopic findings.

<table>
<thead>
<tr>
<th>Endoscopic findings</th>
<th>No. of vacA genotypes (%)</th>
<th>vacAs1m1</th>
<th>vacAs1m2</th>
<th>vacAs2m1</th>
<th>vacAs2m2</th>
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</thead>
<tbody>
<tr>
<td>Gastritis</td>
<td></td>
<td>8 (10.8)</td>
<td>42 (56.8)</td>
<td>0 (0)</td>
<td>24 (32.4)</td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
<td>5 (71.4)</td>
<td>2 (28.6)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>0</td>
<td>3 (13.6)</td>
<td>0 (0)</td>
<td>19 (86.4)</td>
</tr>
</tbody>
</table>

In 12.6%, s1m2 in 45.6% and s2m2 in 41.8%. No single case for vacAs2m1 genotype was detected in this study. This finding is in agreement with previous studies as this genotype was reported to be rare. In this study, the most pathogenic vacA genotype which is vacAs1m1 was present in 71% of sampled studied, these are in agreement with previous studies where they found association between this genotype and severe gastric outcomes. In addition, this genotype was not detected in individuals with normal endoscopic finding. These findings support the role of vacAs1m1 genotype in severe clinical outcomes.

In conclusion, the results of this study might be used for the identification of high-risk patients who are infected by vacAs1m1 genotype H. pylori strains. These patients infected with such strains should have more tension regarding anti-Helicobacter treatment to prevent reoccurrence and prevent severe clinical outcome such as peptic ulcer and gastric carcinoma later on in their life.

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References