Study of Virulence Genes Cag A and Vac A in *Helicobacter pylori* Isolated from Mansoura University Hospital Patients by Multiplex PCR

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**Abstract**

*Helicobacter pylori* (*H. pylori*) is associated with various upper gastrointestinal tract disorders. Virulence genes are cofactors for the pathogenicity of *H. pylori*. The aims of the present study were to study the prevalence of cagA and vacA genes among *H. pylori* strains isolated from patients with upper gastrointestinal disorders requiring endoscopic examinations and to relate the presence of these virulence genes to the clinical presentations of those patients. The study included eighty two patients complaining of upper gastrointestinal disorders requiring endoscopic examinations. Biopsies were obtained from each subject and specific culture for *H. pylori* were performed. Multiplex polymerase chain reaction was performed for isolated *H. pylori* strains to identify the presence of cagA and vacA genes. Their complaints were mainly gastric ulcer (40.2%), simple gastritis (32.9%) and duodenal ulcer (26.8%). Culture of *H. pylori* was positive in 60.9% of samples. Virulence gene cagA was identified in 62% and VacA in 58% of *H. pylori* isolates. All strains that harbor vacA had also cagA with two isolates with cagA gene alone. *H. pylori* was isolated in significant higher percentage (P=0007) from gastric ulcer (93.9%) than duodenal ulcer (45.5%) than simple gastritis (22.2%). Both cagA and vacA were significantly (P=0.0001) associated with gastric ulcer (51.5% & 60.6% respectively) compared to other clinical finding. From this study we can conclude that *H. pylori* is a common pathogen associated with upper gastrointestinal tract mainly with gastric ulcer. *H. pylori* strains responsible for gastric ulcer were significantly harboring the caga and vaca virulence genes. These genes may predispose to severe gastric disorders. Extended large scale studies are required to find the pathogenesis of these genes in Egyptian population.

**Keywords**

Virulence Genes
CagA and
VacA,
*Helicobacter pylori*,
Multiplex PCR

**Introduction**

*Helicobacter pylori* (*H. pylori*) is a fastidious gram negative bacillus that grows under microaerophilic conditions. It is a widely identified pathogen around the world and defined as an etiological agent of various gastrointestinal disorders that range from simple dyspepsia, gastric ulcer and even gastric carcinoma. WHO has classified *H. pylori* as a class 1 carcinogen (1). The prevalence of *H. pylori* infection varies according to the difference in sanitary conditions and it may exceed 70% in...
Various factors are claimed to be associated with the degree of the pathogenicity of *H. pylori*, such as host factors, bacterial virulence genotypes and environmental factors (2). The *cagA* gene, and the vacuolating cytotoxin gene (*vacua*) are thought to be implicated in the pathogenicity of *H. pylori*. CagA is encoded by the *cagA* gene located at one end of the *cag* PAI (3). The *cagA* gene encodes for a protein that leads to a number of cellular changes. It has been reported that *cagA* gene is an indicator for the presence of all genes of the *cag*PAI including *cagT*, *cagM* and *cagE*.. The *cag*PAI family gene is responsible for type IV secretion system, which delivers CagA positive strains into the cytosol of gastric epithelial cells (4). The *cagA* gene is found in high percentage of virulent *H. pylori* strains associated with duodenal ulcer and gastric cancer(4). The profile of *cag*PAI genes of *H. pylori* shows great variability worldwide due to insertions or deletions within the *cag* gene which make its study is necessary in different geographic locations.

The other virulent gene, *vacA* gene encodes for the vacuolating cytotoxin, the pore forming toxin which leads to gastric epithelial cell injury. The vacuolating activity in host cells varies due to mosaicism of the vacA gene in signal (s) and median (m) regions (5-7).

*H. pylori* clinical isolates are categorized according to the presence or absence of *cag*PAI, affecting the degree of pathogenicity, into type I, associated with severe disease pathology, expresses functional *vacA* (vacuolating cytotoxin A) and contains the *cag* (cytotoxin-associated gene) pathogenicity island (*cag*PAI) and type II lacks *cag*PAI and had a nontoxic form of *vacA* and is regarded as less virulent (8,9).

Currently, *cagA* genotyping is used as markers for genomic diversity among populations (10).

The aims of the present study were to study the prevalence of *cagA* and vacA genes among *H. pylori* strains isolated from patients with upper gastrointestinal disorders requiring endoscopic examinations and to relate the presence of these virulence genes to the clinical presentations of those patients.

**Materials and Methods**

The study included eighty two patients complaining of upper gastrointestinal disorders requiring endoscopic examinations. The patients were recruited from Gastroenterology Surgical Center, Mansoura University, Egypt from March 2014 till October 2015. The study was approved by Mansoura Faculty of medicine medical ethical committee. Each patient signed a written approval to participate in the study.

Each patient was subjected to full medical history taking and clinical examination. Upper gastroduodenal endoscopy was performed under standard recommended precautions. Biopsies were obtained from the greater curvature of the stomach about 2 cm from pylorus and from duodenal ulcer. Each biopsy was transported to the laboratory in sterile container. Biopsies were homogenized and spread over Columbia blood agar supplied with 5% sheep blood (Oxoid Columbia agar base). Plates were incubated in a microaerophilic atmosphere (oxygen tensions 5-19% and carbon dioxide tensions 5-10%, by use of gas packs in anaerobic jar (Campy Pak; Becton
Dickinson) at 37°C for 10 days. Identification of *H. pylori* was made by Gram staining of the colonies, lack of aerobic growth on blood agar plates, and testing for the presence of urease, oxidase and catalase (11).

**DNA Extraction**

Colonies identified as *H.pylori* were subjected to DNA extraction by QIAamp DNA Mini Kit (Qiagen, Germany) as manufacturer protocol. The extracted DNA was stored at -20°C until amplification.

**Multiples -Polymerase chain Reaction for cagA and VanA genes**

Multiplex PCR was performed to simultaneously detect cagA and VasA genes. Primers sequences used amplifications and the produced bp were summarized in table 1 (12-14).

For amplification process 3 µl was added in 25-µl volumes containing 2.5 pmol of each primers of VAG and 10 pmol of primers of cag5c-F and cag3c-R, 0.25 mM of each deoxynucleoside triphosphate, 0.9 U of Taq DNA polymerase and 1.5 mM of MgCl₂ in standard PCR buffer (Qiagen).

Products were amplified using Perkin-Elmer 9700 thermal cycler with the following program denaturation for 3 min at 94°C, 35 cycles of sequential 1 min at 94°C-1 min at 55°C-1 min at 72°C, and finally 10 min at 72°C.

Detection was performed by gel electrophoresis 2% for 20 minutes.

**Results and Discussion**

The study included eighty two patients. 47.6% males and 52.4% females with mean age± SD 40.6±9.5. Their complaints were mainly gastric ulcer (40.2%), simple gastritis (32.9%) and duodenal ulcer (26.8%). Culture of *H.pylori* was positive in 60.9% of samples. Virulence gene cagA was identified in 62% and VacA in 58% of *H.pylori* isolates, table 2

All strains that harbor vacA had also cagA with two isolates with cagA gene alone, data not shown.

*H.pylori* was isolated in significant higher percentage (P=0007) from gastric ulcer (93.9%) then duodenal ulcer (45.5%) than simple gastritis (22.2%), table 3

Both cagA and vacA were significantly (P=0.0001) associated with gastric ulcer (51.5% & 60.6% respectively) compared to other clinical finding, table 4.

In the present study *H.pylori* was isolated from 60.9% of the patients. The patients mean age± SD was 40.6±9.5 years. This finding is online with previous reports which defined *H.pylori* prevalence to be from 60% up to 90% in adults patients in developing countries. This high infectious rates were attributed to low socioeconomic standards and bad sanitary conditions (15-17)

*H.pylori* was isolated in significant higher percentage (P=0007) from gastric ulcer (93.9%) then duodenal ulcer (45.5%) than simple gastritis (22.2%). Though colonization with *H.pylori* is widely reported the association of this pathogen with clinical disorders vary according to multiple factors. Firstly, it remains linked to the distribution of diseases according to age as gastric ulcer usually associated with age over 40 years, duodenal ulcer appears in young age group. Secondly, simple gastritis though associated with *H.pylori* other conditions could lead to this common
disorder such as other infectious agents like cytomegalovirus, and autoimmune disorders such as Crohn's disease, pernicious anemia, and chronic idiopathic inflammatory chemical damage due to alcohol abuse or nonsteroidal anti-inflammatory drug (NSAID) use. Third factor that is associated with pathogenic pattern of *H. pylori* is the presence of virulence genes that predispose to its pathogenicity (18-20).

**Table 1** Primers Sequences used

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>bp</th>
</tr>
</thead>
</table>
| vacA s1/vacA s2 | 5′-ATGGAAATACAACAAACACACAC-3′  
5′-CTGCTTGAATGCGCCAAAC-3′ | 259 |
| vacA m1/vacA m2 | 5′-CAATCTGTCCATCAAGCGAG-3′  
5′-GCGTCAAAAATAATTCCAAGG-3′ | 567 |
| cagA    | 5′-GTGTGATAACGCTGTCCCT-3′  
5′-GGGGTGATGATATTTCCCTAATA-3′′ | 350 |

**Table 2** Demographic, Clinical and Laboratory Data of Patients

<table>
<thead>
<tr>
<th>Data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean± SD)</td>
<td>40.6± 9.5</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39 (47.6%)</td>
</tr>
<tr>
<td>female</td>
<td>43 (52.4%)</td>
</tr>
<tr>
<td>Finding</td>
<td></td>
</tr>
<tr>
<td>Simple gastritis</td>
<td>27 (32.9%)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>33 (40.2%)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>22 (26.8%)</td>
</tr>
<tr>
<td>Culture positive</td>
<td>50 (60.9%)</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
</tr>
<tr>
<td>CagA</td>
<td>31 (62%)</td>
</tr>
<tr>
<td>VacA</td>
<td>29 (58%)</td>
</tr>
</tbody>
</table>

**Table 3** Relation of *H. pylori* Culture to Clinical Finding

<table>
<thead>
<tr>
<th>Culture</th>
<th>Simple Gastritis (n=27)</th>
<th>Gastric ulcer (n=33)</th>
<th>Duodenal ulcer (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Culture</td>
<td>6 (22.2%)</td>
<td>31 (93.9%)</td>
<td>10 (45.5%)</td>
</tr>
<tr>
<td>Negative Culture</td>
<td>21 (77.8%)</td>
<td>2 (6.1%)</td>
<td>12 (54.5%)</td>
</tr>
</tbody>
</table>

P=0.0007
Table.4 Distribution of CagA and VacA in Relation to Clinical Finding

<table>
<thead>
<tr>
<th></th>
<th>Dyspsria (n=27)</th>
<th>gastric ulcer (n=33)</th>
<th>Dodenal ulcer (n=22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA</td>
<td>6 (22.2%)</td>
<td>17 (51.5%)</td>
<td>8 (36.4%)</td>
<td>.000</td>
</tr>
<tr>
<td>vacA</td>
<td>4 (14.8%)</td>
<td>20 (60.6%)</td>
<td>5 (22.7%)</td>
<td>.000</td>
</tr>
</tbody>
</table>

Virulence genes of *H. pylori* are implicated in epithelium damage of gastric mucosa leading to gastric atrophy that can progress later on to gastric carcinoma. So, it is important to identify patients who harbor these pathogenic strains to properly interfere to prevent this progression (20).

We studied two virulent genes in *H. pylori* strains isolated from patients namely vacA and cagA genes. CagA gene was identified in 62% and vacA in 58% of *H. pylori* isolates. Similar results were obtained from different localities in Egypt (7, amen et al., 2013). The range of distribution of cagA genes varies between 17% up to 100% in different geographical regions (21,22).

As regards the presence of virulence genes association with clinical finding, both cagA and vacA were significantly (P=0.0001) associated with gastric ulcer (51.5% & 60.6% respectively) compared to other clinical findings. This finding is contradictory to that reported previously by Amer et al., (2013) (21) from one Egyptian study. However, other studies demonstrating a higher risk for development of peptic ulcer disease upon gastric infection with *H. pylori* strains containing virulence genes (23).

Virulence genes vacA and cagA genes are well known virulence factors in *H. pylori* being responsible for production of two different toxins that affect the epithelium of gastric mucosa and alter immune response resulting in severe gastric disease (24). In vitro study has demonstrated that complete absence cagPAI was associated with lower production of interleukin 8. IL-8 is multifunctional. In addition to its potent chemotactic activity, it can induce proliferation and migration of cancer cells. In this review, we focus on recent insights into the mechanisms of IL-8 signaling associated with gastric cancer. So, gastric colonization with cagA Istrains is claimed to be a risk factor for severe gastric and duodenal diseases (25).

From this study we can conclude that *H. pylori* is a common pathogen associated with upper gastrointestinal tract mainly with gastric ulcer. *H. pylori* strains responsible for gastric ulcer were significantly harboring the caga and vaca virulence genes. These genes may predispose to severe gastric disorders. Extended large scale studies are required to find the pathogenesis of these genes in Egyptian population.

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