The Frequency of *Helicobacter pylori* vacA producing genotypes in patients with gastroduodenitis and peptic ulcer from Khartoum, Sudan

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Abstract:
**Background:** *Helicobacter pylori* is involved in the pathogenesis of peptic ulcer disease and associated with gastric carcinoma. It has been classified as a definite class I carcinogen by the World Health Organization. The prevalence of *H. pylori* infection may exceed 70% in some developing countries. An important virulence determinant *H. pylori* is vacuolating cytotoxin (VacA), which induces cytoplasmic vacuolation and produces epithelial cell damage and mucosal ulceration. This study was conducted to determine the frequency of *Helicobacter pylori* vacA genotypes in patients with gastroduodenitis and peptic ulcer from Khartoum, Sudan.

**Methods:** A total of fifty seven (n=57) patients with gastroduodenitis and peptic ulcer were enrolled in this study, they were collected during endoscopy by the gastroenterologists. Twelve (n=12)
Specimens positive for H pylori by presence of 16sRNA. Were analyzed using specific sets of primers for genotyping of vacA gene by PCR.

Results: The vacA gene was present in 91.7% of the H. pylori-positive specimens. The vacA s1/m1 (33.3%) and s1/m2 (33.3%) types were the most common. The vacA s2/m2 was found in (25%), and the variant vacA s2/m1 was not detected in this study.

Key words: Helicobacter pylori, VacA, gastric biopsies, PCR, Khartoum.

Introduction

Helicobacter pylori (H pylori) infects about half of the world population [1].

It is the causative agent of chronic gastritis (CG) and peptic ulcer disease (PUD) and a major risk factor for the development of gastric cancer (GC) and mucosal associated lymphoid tissue (MALT) lymphoma.

The virulence markers of the infecting strains such as urease, flagella, adhesins, oxidase, catalase, and vacuolating cytotoxin (VacA), allow H pylori to persist for years [2].

The vacuolating cytotoxin gene (vacA) encodes a vacuolating toxin that is released by H. pylori and that injures epithelial cells via its pore-forming ability. It contains at least two variable regions. The s region encoding the signal peptide) exists as s1 (including s1a, s1b, and s1c) or s2 allelic types. The m region (middle) occurs as m1 or m2 allelic types. [3,4].

Geographic differences have been detected within several of these vacA s1, s2, m1 and m2 regions. In general, strains containing vacA alleles classified as s1 or m1 have been associated with an increased risk of ulcer disease or gastric cancer compared to strains containing vacA alleles classified as s2 or m2[5]. In North America and Western Europe infection with H.pylori strains containing the s1 vacA allele is associated with peptic ulcer disease (PUD). However in Japan, South
Korea, China and India, where s1 alleles predominate, vacA genotype have not been associated with a more sever clinical outcome [6].

All possible combinations of these vacA regions have been identified, with the exception of s2/m1. The mosaic combination of the s and m region allelic types correlates with the production of the cytotoxin and is thereby associated with the virulence of the strain[3].

Specific cagA/vacA genotypes correlate significantly with cytotoxin activity and peptic ulceration. Thus, the typing of H.pylori strains may become useful in the molecular diagnosis of gastric H. pylori infection [7].

In Germany, Miehlke etal found a significant association between the H.pylori vacA s1/ m1 genotype, cytotoxic activity and gastric cancer [2].

The aim of this study was to determine the frequency of Helicobacter pylori vacA alleles (s1, s2, m1, m2) in patients with gastroduodenitis and peptic ulcer from Khartoum, Sudan.

Methods

Type and duration of the study
A cross-sectional study was conducted to determine the vacuolating cytotoxin gene (vacA), which is the virulence factor of Helicobacter pylori among patients with gastroduodenitis and peptic ulcer in Khartoum state, Sudan. The study was carried out during April_November 2013, at the Military Teaching Hospital (MTH), Aneelain Diagnostic Center (ADS) and Omdurman Teaching Hospital (OTH).

Samples
A total of fifty seven (n=57) antral gastric biopsy specimens were collected during upper endoscopy from patients suffering from gastroduodenitis symptoms.
Ethical consideration was obtained from AL Neelain University-Faculty of medical lab science Research ethical Board. Data was collected using interviewing questionnaire that cover name, age and sex.

Patient’s consent to participate in this study was obtained prior to enrollment. The samples were collected by gastroenterologists and were put in normal saline for further processing.

DNA Extraction
The DNA was extracted directly from biopsies with The GF-1 Tissue DNA Extraction Kit (Vivantis), using protocol of DNA Extraction from Animal Tissue, The extracted DNA Store at 4°C or -20°C.

Polymerase Chain Reaction (PCR)
For the vacA gene alleles analysis 2 primers specific to the particular s/m regions were used. The VA1-F/VA1-R set of primers to amplify the 259-bp (s1) or the 286-bp (s2) region of the conserved portion of vacA[8]. The second set of primers, VAG-R/VAG-F was used to amplify the 567-bp (m1) or the 642-bp (m2) region of the conserved portion of vacA [9]. And the primers sequence used were:

\[
\begin{align*}
VacA & \quad VA1-F & 5'-ATGGAAATACAACAAACACAC-3' \\
s1/s2 & \quad VA1-R & 5'-CTGCTTGAATGCGCCAAAC-3' \\
VacA & \quad VAG-F & 5'-CAATCTGTCCATCAAGCGAG-3' \\
\quad & \quad VAG-R & 5'-GCGTCAAAAATAATTCCAAGG-3' \\
\end{align*}
\]

Total reaction mixture volume was 20 μl. Using Maxime PCR Premix Kit (i-Tag) which content of (2.5 U i-Taq™ DNA polymerase, 2.5mM dNTPs),4μl of template DNA and 2 μl of primers (1 μl F primer, 1 μl R primer) were added into Maxime PCR Premix tubes (i-Tag).then distilled water added to total volume of 20 μl.
The amplification was carried out in thermal cycler according to the following conditions: initial denaturation at 95°C for 2 min, followed by 35 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min.

The final amplification products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide, and observed under UV light.

Data was analyzed by Statistical package of social sciences (SPSS) software.

Results

In this study (57) gastric biopsies were analyzed, Twelve (12) of them were positive for *H. pylori* by presence of 16 sRNA.

Out of the (12) cases positive for *H. pylori*, 11(91.7%) were positive for vacA gene, and 1(8.3%) was negative, Figure (1).

Among the total of positive vacA gene 9 (75%) were males and 2(16.6%) were females. Table (1)

Regarding age most of positive vacA gene was observed among 20-30 age with rang 5 (41.7%) as shown in Table (2).

Four different vacA alleles were amplified by PCR. For the alleles, vacA s1/s2 and vacA m1/m2, amplification bands were observed at 259/286 bp and 567/642 bp respectively, Figure(2).

The results revealed that all possible combinations of these vacA regions were found except s2/m1. The vacA s1m1 (33.3%) and s1m2 (33.3%) types were the most common. The vacA s2/m2 was found in (25%). (Table3). Only one sample was not amplified by both VacA s and m regions,
Table (1): show the presence of \textit{vacA} gene according to sex:

<table>
<thead>
<tr>
<th>Results</th>
<th>\textit{vacA} gene positive</th>
<th>\textit{vacA} gene negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>9 (75%)</td>
<td>1 (8.3%)</td>
<td>10 (83.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (16.6%)</td>
<td>0 (0%)</td>
<td>2 (16.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (91.7%)</td>
<td>1 (8.3%)</td>
<td>12 (100%)</td>
</tr>
</tbody>
</table>

Table (2): Show the distribution of \textit{vacA} gene according to age:

<table>
<thead>
<tr>
<th>Age:</th>
<th>\textit{vacA} gene positive</th>
<th>\textit{vacA} gene negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30 years</td>
<td>5 (41.7%)</td>
<td>0 (0%)</td>
<td>5 (41.7%)</td>
</tr>
<tr>
<td>31-40 years</td>
<td>2 (16.7%)</td>
<td>1 (8.3%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>More than 40 years</td>
<td>4 (33.3%)</td>
<td>0 (0%)</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (91.7%)</td>
<td>4 (8.3%)</td>
<td>12 (100%)</td>
</tr>
</tbody>
</table>

Table (3): Show the frequency of \textit{vacA} allele combinations:

<table>
<thead>
<tr>
<th>\textit{vacA} allele combinations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 12 (100%)</td>
<td></td>
</tr>
<tr>
<td>s1/m1</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>s2/m2</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>s1/m2</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>s2/m1</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (91.7%)</td>
</tr>
</tbody>
</table>
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Discussion

*H. pylori* has been classified as a definite class I carcinogen by the World Health Organization [10].

The 91.7% prevalence of the vacA gene slightly lower than study report by Paniagua. (2009) which found 100% prevalence in clinical isolates of *H. pylori* [11]. but higher than study in a population from Northeastern Mexico which found 79.2% by Estrada .( 2013) [12].

Four different vacA alleles amplified from samples, we found all possible combinations of these vacA region except s2/m1,this finding is in accordance with other studies performed in Cuban and Venezuelan population by Diana Ortiz-Princz .( 2010) [3].and different from result of study in Iranian population by Fereshteh J.( 2008) , which reported that s2/m1 was 8% of vacA alleles combinations[13].

The predominant combination of vacA alleles were s1/m1and s1/m2, this findings were similar to that reported by Diana Ortiz-Princz. (2010), De Gusmão. (2000), Figueiredo. (2001), Chen. 2005, Martins. (2005), Garcia.( 2006), who found that s1/m1 was the most predominant combination of vacA alleles[3] [14] [15] [16] [17] [18].and another study in Tehran

Figure (2): Electrophoresis for detection of the vacA gene alleles: s1, s2, m1 and m2 on 2% agarose gel. Lane M: molecular marker 100bp DNA Ladder; Lane 7: s2 allele (286bp); Lanes 4, 5, 6:s1 allele (259bp); Lanes 2, 3:m1allele (567bp); Lane1: m2 allele (642bp)
conducted by Mohamadi. (2003) reported that s1/m2 was the predominant genotype which our finding is similar[19].

**Conclusion**

Infected by *H. pylori* strain with the genotype *vacA* s1/m1 and s1/m2 may increase the risk of acquiring gastroduodenitis and peptic ulcer thus it is important to diagnose the presence of these genotypes in the patients.

**Competing interests**
None declared

**Authors’ contributions**
LSA, designed and conducted the study and drafted the paper. WIE, designed the laboratory procedures, and contributed in drafting the paper., MEY coordinated the laboratory quality control.

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