MOLECULAR DETECTION OF HELICOBACTER PYLORI AMONG GASTRODUODENITIS AND PEPTIC ULCER PATIENTS IN KHARTOUM STATE

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INTRODUCTION:
Helicobacter pylori infection is associated with gastroduodenitis, gastric ulcer and duodenal ulcer. Many of studies have released causes of gastric ulcer and duodenal ulcer (approximately 95% of duodenal ulcers and 85% of gastric ulcers) to infection with H. pylori. The study was aimed to detect Helicobacter pylori in patients with gastroduodenitis and peptic ulcer in Khartoum State by employing Polymerase chain reaction (PCR) to detect H. pylori 16S gene, sudan.

Methods: Molecular testing for H. pylori 16-S gene was done on 57 stomach and duodenal biopsy specimens using PCR technique. Biopsy specimens were collected by gastroenterologist using endoscopy. Multiple gastric biopsy specimens were taken from the stomach antrum and the corpus and the duodenum. Transport of specimens was in normal saline and kept at -80 OC till used. Extraction was done by using Vivantis GF-1 Nucleic acid extraction kit (Vivantis, MALAYSIA). The amplification reaction was carried out in thermo cycler machine PCR system with program system consisting of (1 min at 94°C, 2 min at 55°C, 3 min at 72°C), and final extension was done at 72°C for 5 min) PCR products were separated in a 1.5% agarose gel, then stained with ethidium bromide and viewed under gel documentation system. A result was considered positive when a band of the appropriate size was visible in the gel. Standard procedures for reducing contamination were strictly followed.

Results: Twelve samples (21.1%) out of 57 were positive by PCR, while 45 samples (78.9%) were negative.

Conclusion: The frequency of 16 S rRNA genes of H. pylori among endoscopic patients was 21.1%.
carcinoma (6) H. pylori consists of a large diversity of strains, and the genomes of three have been completely sequenced. The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1,550 genes. The two sequenced strains show large genetic differences, with up to 6% of the nucleotides differing (7).

- Pathogenesis of H. pylori

To colonize the stomach, H. pylori must survive the acidic pH of the lumen and use its flagella to burrow into the mucus to reach its niche, close to the stomach's epithelial cell layer (8). Many bacteria can be found deep in the mucus, which is continuously secreted by mucus-secreting cells and removed on the luminal side. To avoid being carried into the lumen, H. pylori senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface (9).

H. pylori is also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells (10). It produces adhesins which bind to membrane-associated lipids and carbohydrates and help it adhere to epithelial cells. For example, the adhesin BabA binds to the Lewis b antigen displayed on the surface of stomach epithelial cells (11).

H. pylori produces large amounts of the enzyme urease, molecules of which are localized inside and outside of the bacterium. Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia. The ammonia is converted to ammonium by accepting a proton (H⁺), which neutralizes gastric acid. The survival of H. pylori in the acidic stomach is dependent on urease. The ammonia produced is toxic to the epithelial cells, and along with the other products of H. pylori including proteases, vacuolating cytotoxin A (VacA), and certain phospholipases, damages those cells (12).

Some strains of H. pylori produce a vacuolating cytotoxin A (VacA), and a cytotoxin (CagA). The CagA gene is a marker for strains that confer an increased risk of both peptic ulceration and gastric malignancy; although other factors play a role as strains lacking the toxin can still cause gastritis. The gene forms part of a pathogenicity island, which also encodes a secretion system capable of injecting bacterial macromolecules, including CagA, into host cells. The injecting CagA protein is phosphorylated by a host kinase and subsequently interacts with various signal transduction pathways to affect epithelial cell morphology and behaviour (13).

MATERIAL AND METHOD:

This is descriptive cross sectional study done on patients conduct in April to September 2013. Biopsy specimens was collected by gastroenterologist using endoscopy and multiple gastric biopsy specimens were taken from the stomach antrum and the corpus and the duodenum. Transport of specimens was in normal saline and kept at -80 OC till used. Extraction was done by using Vivantis GF-1 Nucleic acid extraction kit (Vivantis, MALAYSIA). This kit applies the principle of a spin mini-column technology and the use of optimized buffers ensure that only DNA and/or RNA is isolated while cellular proteins, metabolites, salts and other impurities are removed during subsequent washing steps. First, endoscopy biopsy samples were collected and extracted in plain container, followed by a lysis buffer and proteinase K. The DNA was extracted according to the manufacturer's instructions. Finally, the DNA was eluted in 200 μl elution buffer provided with the kit.

The amplification and detection of 16S rRNA gene of H. Pylorus was carried out by PCR method in a thermal cycler. For amplification, 5 μL of 16S rRNA gene of H. pylori was added to a 15-μL reaction mixture. H. pylori primers (H.pylori-F:5’-GGTAAGAGATCAGCCTATGTCC-3’, H. pylori-R: 5’-GCTAAGAGATCAGCCTATGTCC-3’) were used for the PCR reaction. The PCR reaction mixture contained in addition to DNA, 1μl of each primer, and Go Taq ready-to-use master mix (iNTRON Biotechnology) in a 20 μL total reaction volume. Thermocycled for 30 cycles (1 min at 94°C, 2 min at 55°C, 3 min at 72°C), and final extension was done at 72°C for 5 min. PCR products were separated in a 1.5% agarose gel, then stained with ethidium bromide and viewed under gel documentation system . A result was considered positive when a band of the appropriate size was visible in the gel. Standard procedures for reducing contamination were strictly followed. After that The gel was run at 50 V for 30 min. The gel was then examined in Gel documentation system INGeNius. (Figure 4.3).

ETHICAL CONSIDERATION:

Permission to conduct this study was obtained from College of Graduate Studies, Sudan University of Science and Technology and verbal consent were obtained from patients and heads of endoscopies units at MTH and OTH DATA ANALYSIS:

Collected data were analyzed using the statistical package of social science (SPSS) program. Chi-square statistical analysis were used to determine P value significance range.

EXCLUSION CRITERIA

Patients under antibiotic treatment against H. pylori were excluded.

RESULTS:

- Frequencies of H. pylori DNA in patients with gastroduodenitis and peptic ulcer
out of the 57 patients examined by conventional PCR, 12 were found positive (21.1 %) for *H. pylori* DNA.

- **The effect of age of patients on detection of** *H. pylori*

Table 4.1 displays that, high rate of infection by *H.pylori* in the age group (61-80)year. However, 8/34 (23.5%), 1/17 (5.9%), and 3/6 (50%) were found *H.pylori*-positive among age groups 21-40 year, 41-60 and 61-80 years, respectively. with no significant difference (P value = 0.064) between them.

- **The effect of gender of the study populations infected with** *H. pylori*

The results in table 4.2 revealed that 10 out of 34 males (29.4 %) were found *H.pylori* DNA- positive and 2 out of 23 females (8.6 %) were shown positive for *H.pylori* DNA. There was no significant difference between the two genders (P value = 0.060).

<table>
<thead>
<tr>
<th>Age groups</th>
<th><em>H. pylori</em> Positive</th>
<th><em>H. pylori</em> Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>21-40</td>
<td>8/34</td>
<td>23.5</td>
<td>26/34</td>
</tr>
<tr>
<td>41-60</td>
<td>1/17</td>
<td>5.9</td>
<td>16/17</td>
</tr>
<tr>
<td>61-80</td>
<td>3/6</td>
<td>50</td>
<td>3/6</td>
</tr>
<tr>
<td>Total</td>
<td>12/57</td>
<td>21.1</td>
<td>45/57</td>
</tr>
</tbody>
</table>

(P value = 0.064)

- **The effect of gender of patients on detection of** *H.pylori*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10/34</td>
<td>2/23</td>
<td>12/57</td>
</tr>
<tr>
<td>%</td>
<td>29.4</td>
<td>8.6</td>
<td>21.1</td>
</tr>
</tbody>
</table>

(P value = 0.060)

Figure 4.3: Gel electrophoresis of *H. pylori* DNA PCR product. Lane no. 1 contains 100-bp DNA ladder. Lane no. 2 contains control positive, other lanes 3, 4, 5 contains positive samples, other lanes contain negative samples (band appear at 520 bp).

**DISCUSSION:**

Fifty seven patients with gastroduodenitis and peptic ulcer (n=57) were randomly tested for the present study, 34 of them were males (59.6%), and 23 were females (40.4%), with mean age of 43 year. The published studies in Sudan related to our study were few. However, results obtained in this study were similar to those of Esfahani et al (2008) in Iran, where the rate of *H. pylori* infection was (21.7%).

The results obtained in the study weren’t similar to Annika et al (1997) in Britain, where the rate of *H. pylori* infection was reported as 90%. Also high rate of *H.pylori* infection were reported by Richard et al (1994) in USA, Ousman et al (2011) in West Africa and Weiss et al (1994) in USA. The results of the above studies were 57%, 52% and 49%, respectively. High rate of infection was reported by Al-Sulami et al, (2013) in Iraq 66.5% in comparison with our study.

This variation of results could be attributed to ethnic differences and the small sample size used in our study. Abuse of NSAID in Sudan may cause an increase in the incidence of the gastritis without involvement *H.pylori*.

**CONCLUSION:**

The frequency of *H. pylori* among endoscopic patients was 21.1 %, so the gastroduodenitis and peptic ulcer could be possible associated this infection.

**REFERENCES:**


