**Frequency of *Helicobacter pylori* infection in dyspeptic patients in Libya**

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**ABSTRACT**

**Objectives:** *Helicobacter pylori* infection is a very common infection worldwide particularly in the developing countries. The organism plays an important role in peptic ulcer diseases. The aim of the study was to detect and correlate the prevalence of *Helicobacter pylori* with such diseases and to evaluate the histopathological grading of gastritis.

**Methods:** One hundred thirty two patients of 15-83 years of age (average 38) attending the endoscopy unit at the El-Jamahiria Hospital, Benghazi, Libya, mainly complaining from epigastric pain were randomly selected. Three antral biopsies for rapid urease test, direct smear stain and histology together with serum samples were obtained from each patient. Grading of gastritis and the presence of *Helicobacter pylori* was scored according to the Sydney system.

**Results:** Patients were considered infected when 2 of the biopsy-based tests were positive. *Helicobacter pylori* was detected in 108 (82%) of 132 patients examined (86% by rapid urease test, 77% by direct smear stain, 95% by histology). The endoscopic findings revealed that 77 (77%) of 100 patients with non-ulcer dyspepsia, 26 (96%) of 27 with duodenal ulcer, 4 (100%) of 4 with gastric ulcer and in one patient with gastric cancer were *Helicobacter pylori* positive. The enzyme-linked immunosorbent assay test showed 94% sensitivity and 88% specificity. Histopathological sections from the majority of duodenal ulcer and gastric ulcer patients revealed higher grade (II and III) of gastritis than non-ulcer dyspepsia patients.

**Conclusion:** *Helicobacter pylori* infection is significantly correlated with peptic ulcer diseases than with non-ulcer dyspepsia. Patients with duodenal ulcer and gastric ulcer had a significantly higher grade of gastritis than patients with non-ulcer dyspepsia.

Saudi Med J 2002; Vol. 23 (10): 1261-1265

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*Helicobacter pylori* (*H. pylori*) infection is now recognized as one of the most common bacterial infections in humans. It is estimated that more than half of the world populations are currently infected with this organism. The prevalence of such infection ranges from 25% in developed countries to >90% in developing countries. Reports on the prevalence of *H. pylori* infection in dyspeptic patients from other developing countries in the region support these figures. Eighty-one percent infection rate was reported in a study conducted in Kuwait. In Jordan, 86% was detected in one study and in another, 82% infection rate was reported. In the Kingdom of Saudi Arabia (KSA), 145 (74%) of 196 dyspeptic patients were found infected with *H. pylori*. The prevalence of *H. pylori* in Sudanese subjects with gastroduodenal inflammation was 80% in patients with gastritis and 56% in patients with duodenal ulcers (DU). In Libya, no previous study on *H. pylori* infection in dyspeptic patients was recorded. *Helicobacter pylori* plays an important role in the pathogenesis of DU, gastric ulcer (GU) and gastric carcinoma. It is well established that *H. pylori* infection is a persistent long-term condition that is
related to the occurrence and relapse of peptic ulcer diseases and to the increased risk of developing gastric cancer.\textsuperscript{1,11} Most infected patients continue their life with chronic superficial gastritis, some develop either DU or GU, or both and still very small percentage will progress to develop gastric cancer.\textsuperscript{11} Less than 10% of \textit{H. pylori} infected persons develop ulceration during the course of infection.\textsuperscript{12} Similarly, <5% of those develop gastric cancer in which \textit{H. pylori} was found to be responsible for 60-80% of these tumors.\textsuperscript{13} The severity of inflammation and the increased risk of ulcers was associated with increasing \textit{H. pylori} density.\textsuperscript{13} A correlation was found between \textit{H. pylori} density and the severity of gastritis. The severity of gastritis was greater than the antrum of DU subjects compared with non-ulcer subjects.\textsuperscript{14} On the other hand, half of the patients with chronic or recurrent dyspepsia with no peptic ulcer, esophagitis or cancer and have unexplainable symptoms are considered to have non-ulcer dyspepsia (NUD).\textsuperscript{15} Between 30-60% of patients with NUD in western countries are \textit{H. pylori} positive.\textsuperscript{15} The role of the organism in NUD is still controversial. Infection with \textit{H. pylori} was diagnosed by biopsy-based tests (urease test, direct gram stain, culture, histology) or non biopsy-based tests (serology, urea breath test).\textsuperscript{16} Serological tests are non-invasive and was used to detect immunoglobulin G (IgG) antibodies in sera of \textit{H. pylori} infected subjects. The enzyme-linked immunosorbent assay (ELISA) test is very widely used in epidemiological and post-treatment studies.\textsuperscript{4,5,12} This study was undertaken to determine the prevalence of \textit{H. pylori} infection among dyspeptic patients in Libya, correlate such prevalence with peptic ulcer diseases and to evaluate the histopathological grading of gastritis among DU, GU and NUD patients.

\textbf{Methods. Patients.} One hundred thirty two patients (67 males, 65 females) of 15-83 years of age (average 38) attending the endoscopy unit at the El-Jamahiria Hospital,\textsuperscript{17} Benghazi, Libya, mainly complaining from epigastric pain were selected randomly on a consecutive basis. At the presentation, symptoms such as vomiting, nausea, heartburn and bleeding were encountered. In addition none of the patients selected were on antibiotic treatment for the last 3 months before endoscopy. Physical examination and clinical evaluation was performed and a questionnaire form was filled-up by each patient in regard to age, sex, marital status, residency, education, socioeconomic status, smoking, non-steroidal anti-inflammatory drugs (NSAID’s) intake and history of abdominal pain. The endoscopy, laboratory and histological findings were all recorded. Serum samples were also obtained from each patient and stored at -20°C until used.

\textbf{Endoscopy.} Upper gastroendoscopy was performed on each patient for the evaluation of gastroduodenal changes and biopsy collection. Three antral biopsies were obtained for rapid urease test, direct smear stain and histology.

\textbf{Rapid urease test.} One antral biopsy is placed immediately into one ml of urease test solution containing 6% urea and 0.01% phenol red and incubated at 37°C as described previously.\textsuperscript{17} Changes in color from yellow to red that occur within minutes and up to 6 hours was considered positive for urease activity and the presence of \textit{H. pylori}. The solution is always prepared freshly at the day of biopsy collection.

\textbf{Direct smear stain.} One biopsy is placed in a drop of saline and sent to the Microbiology Laboratory. On a sterile glass slide the biopsy was dissected into a smaller pieces with a sterile forceps, smeared, air-dried, heat fixed and stained with gram stain as indicated earlier.\textsuperscript{17} \textit{Helicobacter pylori} appears as a gram-negative curved rods.

\textbf{Histology.} One biopsy is placed in 10% formalin and then processed for histological examination. Sections were stained with hematoxylin and eosin modified Giemsa stains and periodic acid-Schiff (PAS) and examined by an experienced histopathologist who was not informed regarding the clinical outcome. The histopathological grading of gastritis and the presence of \textit{H. pylori} was scored according to the Sydney system as previously described;\textsuperscript{18} grade 0 - very few mononuclear cells, no polymorphnuclear leukocytes (PMN) in the lamina propria (LP) and no \textit{H. pylori}, grade I - few mononuclear cells and PMNs in LP and foveolar epithelium with few \textit{H. pylori} mainly in the superficial mucosal layer, grade II - moderate number of mononuclear cells and PMNs in LP and foveolar epithelium with moderate number of \textit{H. pylori} focally or evenly distributed in the superficial mucosal layer or colonizing the gastric pits, or both and glands, and grade III - marked infiltration of mononuclear cells and PMNs in LP, foveolar epithelium and glandular involvement with marked \textit{H. pylori} colonization of the mucosal layer and gastric pits. Other histopathological parameters included were intestinal metaplasia and glandular atrophy.

\textbf{Serology.} The ELISA kit, Cobas core anti-\textit{H. pylori} enzyme immunoassay (Roche SA, Basel, Switzerland) was used for qualitative and quantitative determination of IgG anti-\textit{H. pylori} as specified by the instruction manual.

\textbf{Statistical analysis.} Data was analyzed using Statistical Package for Social Sciences (SPSS) 10.0. A P value of <0.05 was considered to be statistically significant.

\textbf{Results.} Patients were considered infected with \textit{H. pylori} when 2 of the biopsy-based tests were positive. \textit{Helicobacter pylori} was detected in 108 (82%) of the 132 patients examined (86% were
Table 1 - Correlation of H. pylori infection of 132 dyspeptic patients with endoscopic findings.

<table>
<thead>
<tr>
<th>Disease</th>
<th>n of patients examined</th>
<th>H. pylori +ve patients n (%)</th>
<th>H. pylori -ve patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ulcer dyspepsia</td>
<td>100</td>
<td>77 (77)</td>
<td>23 (23)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>27</td>
<td>26 (96)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>4</td>
<td>4 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>1</td>
<td>1 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>108 (82)</td>
<td>24 (18)</td>
</tr>
</tbody>
</table>

P<0.05, +ve - positive, -ve - negative, n - number, H. pylori - Helicobacter pylori

Table 2 - Correlation of H. pylori infection of 132 dyspeptic patients according to their age.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n of patients examined</th>
<th>H. pylori +ve patients n (%)</th>
<th>H. pylori -ve patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤45</td>
<td>97</td>
<td>80 (82)</td>
<td>17 (18)</td>
</tr>
<tr>
<td>&gt;45</td>
<td>35</td>
<td>28 (80)</td>
<td>7 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>108 (82)</td>
<td>24 (18)</td>
</tr>
</tbody>
</table>

P<0.05, +ve - positive, -ve - negative, n - number, H. pylori - Helicobacter pylori

Table 3 - Histopathological grading of gastritis in 132 dyspeptic patients with duodenal ulcer, gastric ulcer and non-ulcer dyspepsia.

<table>
<thead>
<tr>
<th>Grading</th>
<th>DU n (%)</th>
<th>GU n (%)</th>
<th>NUD n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>23 (23)</td>
</tr>
<tr>
<td>Grade I</td>
<td>1 (4)</td>
<td>1 (25)</td>
<td>67 (67)</td>
</tr>
<tr>
<td>Grade II</td>
<td>14 (52)</td>
<td>3 (75)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Grade III</td>
<td>11 (40)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100)</td>
<td>4 (100)</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

P<0.05, n - number
DU - duodenal ulcer, GU - gastric ulcer, NUD - non-ulcer dyspepsia

Discussion. Although biopsy-based tests (campylobacter [CLO], culture, histology) are considered the gold standard for the diagnosis of H. pylori infection, the results can still vary among each one of them. Since the CLO test measures the urease activity of H. pylori, the number of bacteria present in the gastric biopsy and their total level of urease production were found to influence the sensitivity of the test. Similarly, culturing the organism depends on the amount and viability of the bacteria, thus culture depends on the time between collection of the biopsy and inoculation of the media since H. pylori is sensitive to oxygen. Direct smear staining is a sensitive technique that gives the investigator an immediate results but, still subject to sampling error. Histology on the other hand, relies on visual observation of the bacteria in stained sections, which means it does not rely on any activity or viability of the bacteria, but can be affected by patchy distribution of the organism and sampling error. Thus, as recommended by other investigators,
a combination of 2 tests was found to increase the sensitivity.\textsuperscript{16,17} We have found that 82% of the patients examined were \textit{H. pylori} positive and that histology is being the most sensitive among the other tests. This is in agreement with earlier reports from developing countries regarding the prevalence of \textit{H. pylori}.\textsuperscript{1-9} Previous report\textsuperscript{18} indicated that smear examination showed 75% sensitivity in one study and 84% in another study. In our study, smear examination showed 77% sensitivity. Prevalence of \textit{H. pylori} infection has been found in 95-99% of patients with DU. \textit{Helicobacter pylori} being a risk factor for the development of DU was further documented. It was reported that 15% of \textit{H. pylori} seropositive patients develop DU compared to 3% of seronegative subjects of 40-66 years of age.\textsuperscript{19} We have found that 96% of our DU patients were infected with \textit{H. pylori}, a percentage that is similar to other findings as reported earlier.\textsuperscript{18} In comparison with the biopsy-based tests, serology proved to be useful for the detection of \textit{H. pylori} infection.\textsuperscript{20} In a previous study,\textsuperscript{21} no statistically significant difference was found between the invasive tests (culture, urease test, histology, polymerase chain reaction) and the non-invasive tests (urea breath test, serology, immunoblot, antigen stool detection) in the diagnosis of \textit{H. pylori} infection. Most tests had an over all sensitivity, specificity, positive and negative predictive values of >90%. They concluded that non-invasive tests are accurate for the diagnosis of \textit{H. pylori} infection. A review of the overall performance of the commercially available serology kits that measures IgG antibodies also showed that serology is an accurate mean of diagnosing \textit{H. pylori} infection.\textsuperscript{22} In an earlier study, comparison between serology and combination of CLO, histology and culture revealed 94% sensitivity and 88% specificity.\textsuperscript{20} Our ELISA results appeared to show similar sensitivity and specificity in detecting \textit{H. pylori} infection in dyspeptic patients as compared to the biopsy-based tests. This was also in agreement with a previous report.\textsuperscript{21} Quantitative analysis of the serum IgG levels revealed that patients with DU and GU had higher IgG concentrations than those with NUD. This might suggest that the severity of the inflammatory changes leads to the augmentation of the antibody responses. Histopathological grading in this study showed that DU and GU patients had a higher grade of gastritis and \textit{H. pylori} densities than NUD patients. Similar findings were also reported recently.\textsuperscript{23} Previous studies also indicated that DU are associated with the severity of inflammation and increasing \textit{H. pylori} density in which 6% were found to be of grade 0, 16% were grade I, 19% were grade II and 27% were grade III. Only a weak association between \textit{H. pylori} and GU was detected.\textsuperscript{13} Khulusi et al\textsuperscript{14} indicated that DU subjects had a greater antral infection density and that both severity and activity of gastritis were greater in the antrum of DU compared with non-DU subjects. It was concluded that antral \textit{H. pylori} infection density is probably an important determinant of DU development and there is a baseline of infection density that is necessary for ulcer formation.\textsuperscript{14} Although 50% of patients with non ulcer dyspepsia were reported to harbor \textit{H. pylori} gastritis, yet there is no convincing evidence that \textit{H. pylori} is causally linked to chronic dyspepsia.\textsuperscript{24} The evidence linking the infection to chronic upper abdominal pain or discomfort in the absence of peptic ulceration is still uncertain. It is now accepted that \textit{H. pylori} is not associated with a specific symptom profile.\textsuperscript{24} The majority of our patients who had NUD were \textit{H. pylori} positive. In a recent study on patients with upper digestive tract (UDT) and non-UDT symptoms, \textit{H. pylori} infection was found to be more prevalent, in both groups, for characteristics such as being born in a developing country, overcrowding during childhood, and primary educational level.\textsuperscript{25} Education and socio-economic status appear to be an important factor in the acquisition of \textit{H. pylori} by our patients.

In conclusion, we found that \textit{H. pylori} is highly prevalent in the Libyan dyspeptic patients. Duodenal ulcers and GU patients had higher grade of gastritis and \textit{H. pylori} densities than NUD patients. The ELISA test is useful for the diagnosis of \textit{H. pylori} infection.

References


