Study of Helicobacter pylori among a sample of Iraqi diabetic patients with peptic ulcer disease*

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Amria H. Shubber** FRCP

Abstract:
Background: - Whilst upper gastrointestinal disturbances are frequently observed in patients with diabetes mellitus. We want to know the prevalence of Helicobacter pylori infection and peptic ulcer disease in Iraqi diabetic patients.

Objective: - The present study is an attempt to determine the prevalence of Helicobacter pylori in a sample of Iraqi diabetic patients with peptic ulcer disease by applying different diagnostic criteria.

Methods: - This case-control study was carried at the gastrointestinal unit of Al-Yarmouk Teaching Hospital. The total of 80 samples (blood & antral biopsies) from patients and control groups with an age range of 20-80 years including 30 diabetics with peptic ulcer, and a control group of 50 non diabetic with peptic ulcer. Every gastric biopsy specimen was subjected to cultural, histological and rapid urease test and blood samples to measure the value of glucose level in all study groups.

Results: - Of the total studied patients 30 diabetics have peptic ulcer (P.U) H. pylori was present in 60% of patients as evaluated by culture and rapid urease test, compared to the non diabetic with peptic ulcer (control group) as evaluated by culture (48%) and in rapid urease test (52%). Histological method revealed 76.66% of patients showed positive H. pylori infection while the control group showed 48% only.

Conclusion: - The prevalence of H. pylori infection was found to be significantly higher in patients group of diabetic with peptic ulcer and dyspepsia than in control group (P<0.05). In patients group histological method showed high percentage and more accurate results for diagnosing the positive H. pylori infection when compared to the culture and RUT.

Keyword: - H. pylori, peptic ulcer disease and diabetes mellitus.

Introduction:
The researchers showed that most ulcers develop as a result of infection with bacteria called Helicobacter pylori. Although all the three factors: lifestyle, digestive fluids and H. pylori infections may play a role in ulcer development, but still H. pylori is a primary cause.

H. pylori has several highly specialized virulence factors; some factors may be related to bacterial structures, other factors may be related to bacterial productions of either enzymes (urease) or cytokines.

Both acute and chronic hyperglycemia can lead to specific gastrointestinal (GI) complications. Diabetes mellitus is a systemic disease that may affect organ system, and the GI tract is not exception. The higher seroprevalence of H. pylori infection in diabetes with poor glycemic control have been demonstrated when compared with well controlled DM.

Various methods have been employed to assess the prevalence of H. pylori as a causative agent of chronic disease affecting human stomach and duodenum through out diabetics, including the serology and urea breath tests, rapid urease testing (RUT), histological examination, bacterial culture of gastric tissue and polymerase chain reaction analysis.

Aim of the study:
Isolation and determination of H. pylori prevalence from biopsies of diabetic and non diabetic patients with peptic ulcer disease by rapid urease test, cultural and histological methods then determination which method is the most effective to diagnose H. pylori among positive three tests applied.

Patients, Materials & Methods:

● Study groups: - This study was carried out in Gastroenterology department at Al-Yarmouk Teaching hospital, during the study period (January 2003 to October 2004). The criteria for inclusion were, diabetic patients with dyspepsia referred for upper gastrointestinal endoscopy with provisional diagnosis of peptic ulcer disease (P.U.D). In addition, control groups of non-diabetic patients with dyspepsia, patients included in this study were subdivided into two groups as follows:
a-Diabetic with peptic ulcer: 30 patients (12 female and 18 male).

b-Non diabetic with peptic ulcer: 50 controls (15 female and 35 male).

- **Culture media:** Skirrow’s media, Blood agar base No.2, Brain heart infusion agar (Rashmi Diagnostic-Bangalore-India). Liquid urea medium (manual preparation) [13].

- **Kits:** Glucose Enzymatic colorimetric (SYRBIO-France) and Microaerophilic gas kit (Oxoid-England).

- **Stains:** Gram stain, Haematoxylin & Eosin stains, Strong carbol fuchs in (0.75%) (Institute of sera and vaccines-Baghdad) and Giemsa stain (BDH-England).

- **Reagents and solutions:** Oxidase reagents, Sterile normal saline (0.9%), Formalin (10%) and Phenol red indicator.

- **Growth Supplements:** Ferrous sulfate, Sodium metabisulfate and Sodium pyruvate (FBP).

- **Antibiotics:** Nalidixic acid and Cephalothin- sensitivit y test. Antibiotic pills (VNCT) (Vancomycin, Nystatin, Colistin and Trimethoprim – England).

Three biopsies were taken from the antrum of every P.U patients [14] whether diabetic or not, then suspended into:

- **a**- Liquid urea medium for rapid urease test (0.5ml).

- **b**- Sterile normal saline (0.9%) for bacteriological culture (0.5ml).

- **c**- Formalin (10%) for histological analysis (2ml) [11].

The biopsy samples were ground up by a glass grinder [15,16] then plated on Skirrow’s Campylobacter selective medium supplemented with 5% human blood, VNCT antibiotics [12] as well as growth supplement FBP [17,18].

For further identification the following techniques were applied:

- **a**- Gram method in which strong carbol fuchs in instead of safranine was used for direct smear stain [13,19].

- **b**- Oxidas, catalase, urease activity test and antimicrobial sensitivity test with Nalidixic acid and Cephalothin were applied [20,21].

For rapid urease test the biopsy was immersed with urea rich liquid medium and phenol red as pH indicator dye, then incubated at 37°C for 10-15 minutes for up to one hour [22] to see the change in color (from dark yellow to dark red) [23].

Finally, for histological examination, sections were stained with haematoxylin and Eosin (H&E) and Modified Giemsa stain [24,25]. According to Ko et al., (1999) these sections were examined by histopathologist in Histopathology unit/ Teaching Laboratories/ Al-Yarmouk Teaching Hospital [26].

- **Enzymatic method:** These methods were used to measure the level of glucose in all 80 fasting patients, blood samples were collected and analyzed according to the company instruction (manual kit of the SYRBIO-Company).

- **Statistical analysis** [27,28] was done as follows: -

Chi-squared test (P value< 0.05 was considered as positive level of significance) was calculated. Specificity, sensitivity, predictive value of positive (PPV) and negative (NPV) tests were determined as well.

- **Golden Method:** Patients were considered *H. pylori* infected when two of the biopsy (culture, RUT and histology) based tests were positive [29], and this is considered as a golden method for calculation of sensitivity and specificity of the diagnostic test.

Results:

Eighty blood samples with antral biopsies were obtained from patients (male & female) to evaluate the prevalence of *H. pylori* infection and peptic ulcer (P.U) disease in diabetes with dyspeptic symptoms.

In most cases, *H. pylori* infection was diagnosed by positive bacterial culture and rapid urease test (RUT). According to the data presented in table-1, the analysis of culture in patients group (diabetic with P.U) reveals prevalence of *H. pylori* infection (60%) while in the control group (non diabetic with P.U) prevalence was (48%). Table -2- shows the positive rapid urease test (RUT) in patients group reveals prevalence of *H. pylori* infection (60%) while in the control group a prevalence of *H. pylori* infection was 52%.

The rate of sensitivity for both culture and RUTs was 60%, while the specificity rate for culture was 52% and for RUT was 48%.
Table-1- *H. pylori* positive culture test of antral biopsy specimens in diabetic and non diabetic PU patients.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>+ve Culture</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non diabetic with P.U</td>
<td>F. 15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>M. 35</td>
<td>16</td>
</tr>
<tr>
<td>Total No.(50)</td>
<td>% 24 (48%)</td>
<td>26</td>
</tr>
<tr>
<td>Diabetic with P.U</td>
<td>F. 12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>M. 18</td>
<td>10</td>
</tr>
<tr>
<td>Total No.(30)</td>
<td>% 18 (60%)</td>
<td>12</td>
</tr>
<tr>
<td>Total (80)</td>
<td>% 42</td>
<td>38</td>
</tr>
</tbody>
</table>

Sensitivity 60%
Specificity 52%
PPV 42.9%
NPV 68.42%
P value* P>0.05

#positive predictive value, $negative predictive value

*P value ≤ 0.05

Table-2- *H. pylori* positive urease test of antral biopsy specimens in diabetic and non diabetic PU patients.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>+ve Urease</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non diabetic with P.U</td>
<td>F. 15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>M. 35</td>
<td>18</td>
</tr>
<tr>
<td>Total No. (50)</td>
<td>% 26 (52%)</td>
<td>24  (48%)</td>
</tr>
<tr>
<td>Diabetic with P.U</td>
<td>F. 12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>M. 18</td>
<td>10</td>
</tr>
<tr>
<td>Total No. (30)</td>
<td>% 18 (60%)</td>
<td>12</td>
</tr>
<tr>
<td>Total (80)</td>
<td>% 44</td>
<td>36</td>
</tr>
</tbody>
</table>

Sensitivity 60%
Specificity 48%
PPV 40.9%
NPV 66.7%
P value P>0.05

*H. pylori* infection evaluated by positive results obtained from histological sections stained with Giemsa stain. Table-3- shows the rate of *H. pylori* infection diagnosed in patient group was significantly different by histology method with a rate of 76.66% compared to the control group 48% (P<0.01). The
sensitivity and specificity rates from histology test were 76.7% and 52% respectively. Table 4 represents the distribution of duodenal and gastric ulcers in our study groups. The results were statistically significant (P<0.01) high percent of duodenal ulcer compared to gastric ulcer and mixed (duodenal & gastric) ulcers, and high incidence of duodenal ulcer in males than in females in patients group (94.44%) and control group (97.7%).

### Table 3: *H. pylori* positive histology test of antral biopsy specimens in diabetic and non diabetic PU patients.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non diabetic with P.U</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>M.</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Total No. (50)%</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>(48%)</td>
<td>(52%)</td>
</tr>
<tr>
<td><strong>Diabetic with P.U</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>M.</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Total No. (30)%</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>33</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>76.7%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>48.9%</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>78.8%</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>P&lt;0.01</td>
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</tbody>
</table>

### Table 4: Distribution of duodenal and gastric ulcers in diabetic and non diabetic peptic ulcer patients.

<table>
<thead>
<tr>
<th>Types of P.U</th>
<th>Non diabetic with P.U (50)</th>
<th>Diabetic with P.U (30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duodenal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.</td>
<td>14</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>M.</td>
<td>34</td>
<td></td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>%</td>
<td>93.33%</td>
<td>97.7%</td>
<td>66.66%</td>
</tr>
<tr>
<td>Gastric</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>%</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Duodenal &amp; gastric</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>30%</td>
<td>70%</td>
<td>40%</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>%</td>
<td>30%</td>
<td>70%</td>
<td>60%</td>
</tr>
</tbody>
</table>

### Discussion:

The study revealed the prevalence of *H. pylori* confirms the finding reported by ACG, (2004) [30] but relatively lower than that reported by Bake et al., (2002) who showed 86% positive RUT [10], followed by AL-Baldwin, (2001) who reported 99% positive culture and 82.5% positive urease activity [13], but our result are higher than that reported by Schenk et al., (1999) [31].

The sensitivity for both culture and RUTs was 60% ,while the specificity rate for culture was 52% and for RUT was 48%.This percentage is relatively lower than that reported by Isomoto et al., (2002) and Kearney, (2003)[11,22]this can be explained by, RUT measures the urease activity of *H. pylori*,the number of bacteria present in gastric biopsy and their total level of urease production were found to influence the sensitivity of the test[32], similarity, 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 [33,10].
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The rate of *H. pylori* infection diagnosed by histology method was 76.66% in patients group compared to the control group (48% (P<0.01)). This finding is in agreement with AL-Baldawi, (2001) who reported 78.6% [33], but relatively lower than that reported by Bakka et al., (2002) 95%[10]. The sensitivity and specificity rates from histology test were 76.7% and 52% respectively.

Schembri et al., (1993) and Bakka et al., (2002) suggested that histology relies on visual observation of the bacteria in stained sections, which means it does not rely on any activity or viability of the bacteria, which can be affected by patchy distribution of the organism and sampling error[33,10]. Thus as recommended by other investigators a combination of two tests was found to increase the sensitivity[34,14]. Moreover, the false negative results occur especially if patients are taking proton pump inhibitor (PPI) of urease [22].

Diabetic patients become susceptible to other infections, the gastrointestinal (GI) tract is no exception. The symptoms of diabetics masking the symptoms of GI tract unless; this infection is so severe [35].

The distribution of duodenal and gastric ulcers in different study groups was in agreement with that reported by Marshall, (1995) and NDDIC, (1998)[36,4].

Abu-Farsak, (2001) and Rosenstock et al., (2003) attributed the sex difference to the presence of more risk factors in men specially smoking which is associated with the development of delayed healing and recurrence of PU, as well as, resistance to treatment, in addition to the physiological stresses[37,38]. Han et al., (2000); Valle, (2001) and Thal, (2005) found that the prolonged un-neutralized gastric acidity altered gastric emptying, decreased proximal duodenal bicarbonate production and cigarette-induce generation of noxious mucosal free radicals may affect the incidence of PUD [39,40,41].

Conclusions:

1-Diabetics with peptic ulcer revealed higher percentage of *H. pylori* isolates (60%) by culture and rapid urease test, compared to the non diabetic with peptic ulcer as evaluated by culture (48%) and in rapid urease test (52%).

2-Histopathology study indicates the presence of (76.66%) *H. pylori* in diabetic with peptic ulcer compared to the (48%) in non diabetic with peptic ulcer, this method showed high percentage and more accurate results for diagnosing the positive *H. pylori* infection when compared to the culture and RUT.

4-In our study groups the percentage of duodenal ulcer was higher than gastric ulcer and mixed (duodenal+gastric) ulcers (P<0.01), so D.M. have no effect to determine the type of P.U.D., also the duodenal ulcer in males was higher than in females.

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