INVESTIGATION OF HELICOBACTER PYLORI INFECTION IN JORDANIAN PATIENTS USING SIX ENZYME IMMUNOASSAYS FOR IMMUNOGLOBULIN G (IGG) AND IG A TESTING

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ABSTRACT

Helicobacter pylori (H. pylori) is the etiologic agent of a variety of gastrointestinal disorders. The rationale of the current study is to evaluate six enzyme immunoassays for detection of anti-H. pylori immunoglobulin G (IgG) and IgA in Jordanian patients. Biopsy specimens and blood samples were obtained from patients underwent the endoscopy unit at Al-Bashir hospital in Jordan. The serum samples were investigated for the presence of anti-H. pylori IgG and IgA antibodies in patients with positive H. pylori biopsy samples. The results showed that IgG utilizing kits are more sensitive than of IgA kits and the IgA kits are more specific than of that IgG kits. Moreover, the biopsy is seemingly the gold standard for diagnosis of H. pylori is followed by H. pylori culture on brucella agar medium. An imperfect relation between the presence of H. pylori infection and the antibody response was existed that could be explained either because of the unsatisfactory sensitivities and specificities of the commercial kits used or because of weak immunological response in our patients to H. pylori antigens. Collectively, the H. pylori diagnosis that depends on the detection of anti-H. pylori antibodies in the hospital setting and in the screening programs should consider another test for confirmation the initial diagnosis.

Key words: Helicobacter pylori, IgA, IgG, Jordan

Conflict of interest: Non Declared

INTRODUCTION

Helicobacter pylori is fastidious, small, curved, microaerophilic and highly motile Gram negative bacteria. It is about 2.5 - 5.0 μm long and 0.5 - 1.0 μm wide with 4-6 unipolar sheathed flagella [1]. H. pylori is identified on the basis of colony morphology (translucent colonies varying in size from barely detectable with the naked eye to approximately 3 mm); H. pylori are Gram-negative, curved rods that are urease, catalase and oxidase positive. The addition of tetrazolium salts aids in the identification of H. pylori colonies cultured on agar media [2]. H. pylori is the etiologic agent of a variety of gastrointestinal disorders including chronic active gastritis, peptic ulcer and gastric cancer [3]. It is clear that virtually all H. pylori infected subjects develop local and systemic immune response against this organism [4], as with most bacterial infections, H. pylori stimulates an immune response and circulating antibodies appear in the peripheral blood stream. This is the basis for serology tests to diagnose H. pylori [5]. It has been shown that the immune response to H. pylori at the mucosal level is predominantly of the IgA type, while the systemic immune antibody response essentially consists of the IgG class [6]. Immunoglobulins of the IgM class do not appear to differ between H. pylori positive and negative patients [7].

Performance varies significantly between different commercial serologic kits, with the highest exceeding 90% in sensitivity and specificity and the lo-
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west having less than 70% in sensitivity and less than 50% in specificity [8]. The purpose of this study was to evaluate six commercially available ELISA kits for detection of anti-\textit{H. pylori} IgG and IgA in patients attending the Gastroenterology unit at Al-Bashir hospital in Jordan. Moreover, to assess the culture on different media for detecting \textit{H. pylori} isolates.

**MATERIALS AND METHODS**

**Patients**

The study group included 71 individuals aged 13 to 83 years (37.7 ± 1.5 years) who underwent endoscopy at the Gastroenterology unit. The study protocol respected the most recent Declaration of Helsinki [9], and all of the patients gave consent to the use of their sera and clinical data for research purposes after being informed about the nature of the study.

**Media**

Brucella medium (HiMedia Laboratories, Mumbai, India), Columbia medium (Biomark, India) and Brain Heart Infusion (BHI) medium (Liofil, Italy) were prepared in accordance with the manufacturer’s instructions. The antibiotic supplement in each media are identical; each medium contains 10 mg of vancomycin per liter, 2.5 IU of polymyxin B per liter, 5 mg of trimethoprim per liter, 2 mg of amphotericin B per liter and 5 mg of cefsulodin per liter. All media were further supplemented with whole blood (5%) and 40 mg of 2,3,5-triphenyltetrazolium chloride (Sigma, USA) per liter.

**Biopsy Specimens**

During upper endoscopy, gastric mucosal biopsy was taken for histopathology, formalin fixed, cut into five μm sections, stained with hematoxylin and eosin and viewed for the presence of \textit{H. pylori} (Fig.1). A positive result was considered indicative of active \textit{H. pylori} infection as reported by us earlier [10]. Additional two biopsies one from the antrum and the other from the corpus were taken for microbiological studies as shown below.

The biopsy specimens were collected into tubes containing three ml of brucella broth supplemented with 0.5% (w/v) bovine serum albumin. The tubes were transported to the laboratory on an ice box in less than three hours.

**Isolation and Identification**

The biopsy specimens were finely minced with a sterile scalpel blade in one ml of brucella broth in sterile conditions. One drop of the tissue homogenate was inoculated onto each of the three selective media plates. After incubation at 37°C under microaerophilic conditions (10% CO₂, 5% O₂, and 98% humidity) for 10 days. Isolates were identified as \textit{H. pylori} on the basis of colonial morphology, in addition to the negativity for Gram staining, nitrate reduction, glucose and sucrose fermentation and indole production, moreover, the positivity for catalase, oxidase, and urease tests.

**Enzyme Immunoassays:**

Venous blood samples (5ml) were obtained from each patient at the time of endoscopy. The serum was separated, divided into aliquots, and stored at -20°C. All sera were tested with six ELISA kits: Anti- \textit{Helicobacter pylori} (Anti-Hp) IgG ELISA (BioCheck Inc. USA), Anti-Hp IgA ELISA (BioCheck Inc. USA), Anti-Hp IgG ELISA (Biotest, Germany), Anti-Hp IgA ELISA (Biotest, Germany), Anti-Hp ELISA IgG (Euroimmun, Germany), and Anti-Hp ELISA IgA (Euroimmun, Germany). The assays were performed in accordance with the manufacturers’ instructions.

**Statistical Analysis**

Nominal data were analyzed using chi-square test and \(P\) values < 0.05 were considered statistically significant. Data were tabulated and analyzed using the SPSS 17 statistical package (SPSS Inc. Chicago, IL). The sensitivity, specificity and accuracy were calculated as described by us earlier [11].

Figure 1: Shows a section from the corpus of an infected patient. M: Mucus secreting cells. HP: \textit{H. pylori} (X 1000).
RESULTS

Culture
Brucella agar medium gave the highest isolation rate. Out of 71 positive biopsy specimens; isolation of \( H. \) pylori was achieved in 61 antrum specimens (86%). On the other hand, 57 out of 71 corpus biopsy specimens (80.3%) were culture positive. The number of patients from whom \( H. \) pylori was isolated from either the corpus or antrum specimens was 70 out of 71 infected, thus \( H. \) pylori was recovered in 98.6% of the patients infected. Using Columbia agar the organism was recovered from 54 antral specimens (76%). On the other hand, when corpus specimens were cultured the recovery was achieved from 50 specimens (70.4%). The total number of patients from whom \( H. \) pylori was isolated either from corpus or antrum specimens was 63, thus \( H. \) pylori was recovered in 88.7% of the patients infected. BHI gave the lowest isolation rate for \( H. \) pylori. Out of 71 positive antral biopsy specimens, the organism was isolated only from 47 specimens (66.2%). In the opposite direction when corpus specimens were cultured, the number of specimens treat the organism was 48 specimens (67.6%) (table 1).

Enzyme Linked Immunosorbent Assay (ELISA)
The serum samples were investigated for the presence of anti-\( H. \) pylori IgG and IgA antibodies in patients with positive \( H. \) pylori biopsy samples. The results showed that IgG utilizing kits are more sensitive than of IgA kits and the IgA kits are more specific than of that IgG kits. Euroimmun IgG kit is the most sensitive one, on the hand, Biocheck IgA is the most specific kit. The sensitivities, specificities and accuracies of the six kits are illustrated in table (2).

DISCUSSION

\( H. \) pylori infection is prevalent in Jordan as evidenced by several investigators [12-14]. In the current study, we evaluated seventy one positive \( H. \) pylori infected Jordanian patients with three different culture media and six EIA assays for anti-\( H. \) pylori antibodies (IgG and IgA). It is well known that gastric mucosal biopsy for \( H. \) pylori is the standard gold for diagnosing that infection. Several histological stains, one of them hematoxylin and eosin, was compared to culture and the results revealed that all stains showed high specificity ranging from 97 to 100% [15].

A wide variety of solid media have been used by many investigators for the isolation of \( H. \) pylori [16-19]. In this study, different results were obtained by using three selective media for isolation of \( H. \) pylori. Brucella agar was considered the best medium and showed the highest \( H. \) pylori recovery rate (98.6%), while Columbia and BHI agar produce a lower recovery rate, 88.7% and 81.7% respectively. The use of selective media has been recommended in order to improve the culture isolation of \( H. \) pylori from gastric biopsy specimens and to prevent bacterial overgrowth by contaminants [20, 21].

These media (Brucella, Columbia and BHI Agar) were used successfully by many investigators [18, 22]. In a study by Hachem et al., [23] proved that BHI agar was the best one among the media tested and gave 96% recovery rate. While the recovery rate was 78% with trypticase soy agar, 64% with egg yolk agar and 32% with Columbia blood agar-cyclodextrin. In this study, Brucella agar was the most accurate of the media evaluated for culture isolation of \( H. \) pylori from gastric biopsy specimens. The inclusion of 2,3,5-triphenyltetrazolium chloride gives \( H. \) pylori colonies a specific golden yellow pigment which helps in their identification, therefore reducing the false identification of \( H. \) pylori. It is worth to mention that all plates were incubated in an atmosphere of 10% \( \text{CO}_2 \) and 98% relative humidity for up to 10 days to provide excellent growth conditions. Tee et al., [20] stated that selective medium provides heavier growth than non selective one. Their results shows that Skirrow’s medium gave the highest isolation rate while growth on Dent’s and modified Glupezyniski media were equal and less better than the first one. Chocolate agar yielded a 76% positivity rate. The addition of blood not only enhances the growth of \( H. \) pylori, but also facilitated to distinguish \( H. \) pylori colonies from those of other bacteria; colonies of \( H. \) pylori appear distinctly clear [17].

In the present study, 100% of \( H. \) pylori infected patients had a positive test result with Euroimmun IgG. On the other hand the other two kits, Biocheck IgG and Biotest IgG identified 98.6% and 88.7% respectively. On the other hand 60.6% of \( H. \) pylori infected patients had a positive test result with Euroimmun IgA. Only 21.1% and 48% of the infected patients were identified by Biocheck IgA and Biotest IgA kits respectively. The sensitivities of the three IgA based tests in comparison to IgG antibodies confirm the results of previous studies. Best et al, [24] report a sensitivity of only 50% for IgA, while a sensitivity of 100% was found for IgG in the same cohort in the same study. Also Kindermann et al, [25] support these results and found that the sensitivities were <50% for the IgA kits and varied markedly between the IgG kits between 70.7-92.7%. Biocheck IgA kit
Table 1: Relative Isolation Results of *H. pylori* Organism on the Three Culture Media (BA, CA, and BHI).

<table>
<thead>
<tr>
<th>Media</th>
<th><em>H. Pylori</em></th>
<th>Antrum</th>
<th></th>
<th>Corpus</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Brucella Agar</td>
<td>Negative</td>
<td>10</td>
<td>14</td>
<td>14</td>
<td>19.7</td>
<td>70</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>61</td>
<td>86</td>
<td>57</td>
<td>80.3</td>
<td>118</td>
<td>98.6</td>
</tr>
<tr>
<td>Columbia Agar</td>
<td>Negative</td>
<td>17</td>
<td>24</td>
<td>21</td>
<td>29.6</td>
<td>38</td>
<td>44.6</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>54</td>
<td>76</td>
<td>50</td>
<td>70.4</td>
<td>104</td>
<td>79.4</td>
</tr>
<tr>
<td>BHI Agar</td>
<td>Negative</td>
<td>24</td>
<td>33.8</td>
<td>23</td>
<td>33.4</td>
<td>47</td>
<td>52.2</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>47</td>
<td>66.2</td>
<td>48</td>
<td>67.6</td>
<td>95</td>
<td>77.8</td>
</tr>
<tr>
<td>Total **</td>
<td></td>
<td>68</td>
<td>95.8</td>
<td>60</td>
<td>84.5</td>
<td>128</td>
<td>100</td>
</tr>
</tbody>
</table>

* Positive results from antrum and/or corpus
** Positive results on the three media

The results showed that the Euroimmun EIA IgG is more sensitive than other kits, while Biocheck EIA IgA is more specific than others.
kits used or because of weak immunological response in our patients to H. pylori antigens. Moreover, H. pylori diagnosis that depends on the detection of anti-Helicobacter pylori antibodies in the hospital setting and in the screening programs should consider another test for confirmation the initial diagnosis. Thus, it is important to submit that the combination between the non-invasive (such as serology) and invasive (such as culture and biopsy) methods may markedly improve the detection of H. pylori.

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REFERENCES

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