INVESTIGATION OF HELICOBACTER PYLORI INFECTION IN JORDANIAN PATIENTS USING SIX ENZYME IMMUNOASSAYS FOR IMMUNOGLOBULIN G (IGG) AND IGA 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-*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 *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 *H. pylori* (Fig.1). A positive result was considered indicative of active *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% CO_2, 5% O_2, and 98% humidity) for 10 days. Isolates were identified as *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.

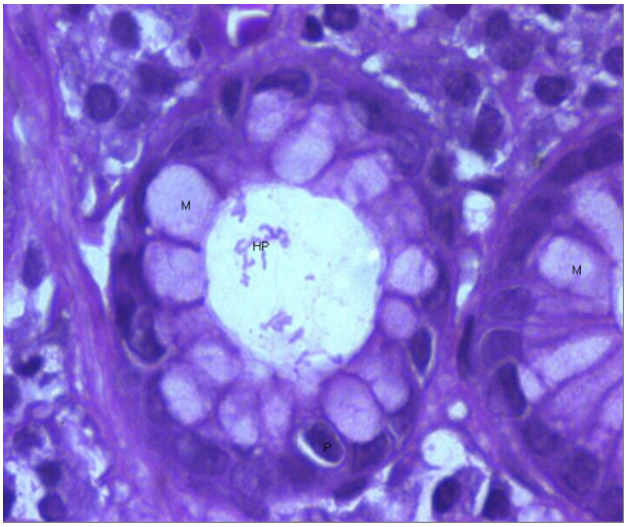

Figure 1: Shows a section from the corpus of an infected patient. M: Mucus secreting cells. HP: *H. pylori* (X 1000).

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- *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].

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 retreat 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% 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).

Media	*H. Pylori*	Antrum		Corpus		Total*	
		No	%	No	%	No	%
Brucella Agar							
	Negative	10	14	14	19.7		
	Positive	61	86	57	80.3	70	98.6
Columbia Agar							
	Negative	17	24	21	29.6		
	Positive	54	76	50	70.4	63	88.7
BHI Agar							
	Negative	24	33.8	23	33.4		
	Positive	47	66.2	48	67.6	58	81.7
Total **		68	95.8	60	84.5	71	100

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

gave a high specificity (100%) while it is found lower for the other two kits EuroImmun IgA and Biotest IgA yielded a specificity of 48% and 80% respectively. The results obtained in this study were similar to [26-28]. A large number of false positive results were observed in all IgG tests and the specificity was 52%, 28% and 44% for Biotest IgG, Biocheck IgG and Euroimmun IgG respectively. These results are lower than those obtained by others [8, 25]. Positive serology results are evidence of contact with *H. pylori* but do not necessarily indicate current infection, also persistent antibodies after clearance of *H. pylori* infection are the main reason for false positive results [26, 28& 29], another cause of false positive results is the cross reactions that could have occurred between *H. pylori* antigens and other patient's sera [30]. In a study by Maciorkowska *et al,* [31] showed that IgG antibodies were present in 31.1% of children and in 16.1% of adults with normal antrum and corpus gastric mucosa.

Finally, the imperfect relation between the presence of *H. pylori* infection and the antibody response could be explained either because of the unsatisfactory sensitivities and specificities of the commercial

Table 2: Sensitivities, specificities and accuracies of the EIAs.

EIA	Sensitivity	Specificity	Accuracy
Biocheck IgG	98.6	44	81.3
Euroimmun IgG	100	28	85.4
Biotest IgG	88.7	52	79.2
Biocheck IgA	21.1	100	43.8
Euroimmun IgA	60.6	48	56.3
Biotest IgA	48	80	56.3

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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