Detection of Immunoglobulin G and M Antibodies to *Helicobacter Pylori* in Serum by an Enzyme Immunoassay Method

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الخلاصة

في هذه الدراسة تم الكشف عن عدوى الملوية البوابية (*Helicobacter pylori*) باستخدام تقنية الايلايزا (ELISA) لتحديد امكانية استخدام الفحوصات المصلية في تشخيصها ، ولذلك الغرض تم الكشف عن مضادات الملوية البوابية (Anti *H.pylori*) من نوع IgG و IgM في عينات مصل الدم لـ 88مريضا تتراوح اعمارهم بين 20 الى75 سنة في مستشفيات اربيل . كانت النتائج موجبة بالنسبة الى مضادات IgG لـ 44 مريض (50%) و لـ ٧٤ مريض (84.09%) لمضادات IgM. تم استحصال عينات الخزع (Biopsy) من 40 مريض (45.45%)، وتم الكشف عن وجود او عدم وجود الاصابة ب *H.pylori* بثلاث طرق: الزرع الجرثومي ، الكشف عن انزيم اليوريس وطريقة صبغة الغرام وكانت النتائج ايجابية بنسب 37.5% و 82.5% و 20% على التوالي. عند مقارنة هذه النتائج مع نتائج الايلايزا لكل من IgG و IgM لهؤلاء المرضى تبين ان النتائج ايجابية بنسبة (85%) و (95%) لكل من IgG و IgM على التوالي . الحساسية والخصوصية والدقة لتقنية الايلايزا لمضاد IgG في المصل لـ *H.pylori* كانت 94 % و 70% و 90% على التوالي.

ABSTRACT

The aim of this study was to detect *Helicobacter pylori* infection using an enzyme-linked immunosorbent assay (ELISA) to determine whether serological tests can be used for diagnosis. Serum samples were collected from 88 patients (ages ranges between 20 to 75 years) attending for gastrointestinal endoscopy in Erbil hospitals for detection of anti-

Helicobacter pylori specific IgG and IgM antibodies in these specimens. IgG and IgM were positive in 44(50%) and 74 (84.09%), respectively. Biopsy specimens were collected from 40 (45.45%) of these 88 patients. The presence or absence of current *H. pylori* infection was determined by culture, urease and gram stain, that were positive in 37.5%, 82.5% and 20%, respectively which compared with their IgG(85%) and IgM(95%) respectively. The sensitivity, specificity and accuracy of the assay for serum IgG to *H. pylori* were 94%, 70% and 90% respectively.

Key Words: Helicobacter pylori, Immunoglobulins, ELISA

INTRODUCTION

Helicobacter pylori is a spiral-shaped microaerophilic gram-negative bacterium that is often observed in the mucous layer that coats the gastric mucosa (1). *Helicobacter pylori* is a causative agent in chronic gastritis (2), peptic ulcers (3), and gastric cancer (4). The detection of antibodies specific to *H. pylori* in serum is important in the diagnosis of these diseases (5). However, *H. pylori* infections occur in the gastric mucosa (6) Multiple invasive and noninvasive methods are available for the detection of *Helicobacter pylori* (7). Invasive methods necessitate endoscopy and require gastric tissue. They include tests for urease activity, histological evaluation, and culture of the bacterium (8-10). Noninvasive techniques to detect bacterial infection include urea breath tests (UBT) and anti-*H. pylori* antibody detection by serologic methods. Use of noninvasive methods of detecting *H. pylori* is imperative when screening an unselected population. Serologic tests offer high sensitivity and specificity (11-13); furthermore, simultaneous measurement of serum immunoglobulin G (IgG), M (IgM), and A (IgA) antibodies to *H. pylori* can be used to determine the prevalence of both acute and chronic infection (14,15). Serological tests are useful in *H. pylori* infection because virtually all patients colonized with this organism undergo a local antibody response directed against antigens covering the surface and flagella of the organisms; in the majority of cases this antibody response is also detectable in the serum (16-18). Specific immunoglobulin M(IgM) antibodies can be detected shortly after the infection is acquired, but IgA and IgG titers indicate chronic infection (19). Enzyme -linked immunosorbent assays (ELISA) have become widely used to detect *H. pylori* antibodies in serum (20- 22). Serological tests for *H. pylori* are advocated to replace endoscopy as a primary diagnostic procedure in patients under the age of 45 years (23, 24).

The object of this study was to detect IgG and IgM antibodies in the serum of *H. pylori* patient by EIA method, comparing this with culture, stain and urease detection.

MATERIALS AND METHODS

PATIENTS.

Serum samples were obtained from 88 adults (34 females and 54 males, ages 20 to 75 years [mean, 43 years] presenting with upper gastrointestinal symptoms requiring endoscopic evaluation to the Gastroenterology Department at the two General Hospitals in Erbil, (Rizgary and educational hospital). Serum was stored at -20°C until assayed. Antral biopsy specimens were obtained from 40 of these patients.

MICROBIOLOGY:

Culture: The biopsy specimens for microbiological analysis were collected into tubes containing 0.9% saline, and there were transferred to the microbiology laboratory and processed within an hour. One piece of the biopsy specimen was cut, homogenized and inoculated on Brucella agar, Brain heart infusion agar and Blood agar, all three media supplemented with 5% human blood and amphotericin B (2 mg/l), trimethoprim (10 mg/l) and vancomycin (10 mg/ml), (all three media from Accumix). Plates were incubated at 37 C^0 in a micro-aerophilic atmosphere in a humid chamber for a period ranging from 3 to 10 days. Suspicious isolates were identified *H.pylori* was identified as small translucent colonies, identification of cultures was based on Gram staining, oxidase and catalase activities, and urease positivity.

Modified Gram Staining: One piece of the biopsy specimen was cut and used for staining (In modified gram 0.2% carbolfuchsin instead of safranin for counter stain (25). Biopsies were crushed between two slides. Slides were fixed and were stained with carbolfuchsin and Gram stain to record morphology and the presence of Helicobacter- like organisms.

Urease test of biopsies: One of the biopsies was inoculated on urea agar (Oxoid urea agar base) slant tube by streaking the surface of the slant and stabbing the butt of the tube all the way to the bottom, immediately were incubated at 37º C for 24 h (the tubes were checked every 30 min). Positive reaction was indicated when tube color changed to pink Patients were considered as *H.pylori*-positive either if *H.pylori* was cultured from the biopsies or if urease test of biopsies was positive and as *H.pylori*-negative if both the culture for *H.pylori* and urease test were negative.

Serology: Blood (5 to 10 ml) was taken from the patients The serum was separated and stored at-20°C until the EIAs' were done. For serology studies. Anti- *H. pylori* IgG and IgM antibodies were tested in all samples by an enzyme immunosorbent assay (ELISA) test.

ELISA Test: *Helicobacter pylori* IgM enzyme immunoassay and *Helicobacter pylori* IgG enzyme immunoassay test kits (BioCheck, Inc) were performed according to the manufacturer's instructions. Briefly, the serum was diluted 1:40 for both kits (the EIA-G and the EIA-M). The diluted serum samples (100,ul) and four ready-to-use reference serum samples were pipetted into appropriate microtiter wells (the *H. pylori* IgM or IgG specific antibody, if present, binds to the antigen), and the plates were incubated for 30 min at room temperature. The microtiter wells were washed 4 times with diluted wash buffer (1x) and one time with distilled water, 100 µl of Enzyme conjugate was added to each well (which binds to the antibody-antigen complex.) and the plates were incubated for 30 min at room temperature. The microtiter wells were washed 4 times with diluted wash buffer (1×) and then one time with distilled water. 100 µl of TMB Reagent was added to each well, Incubated at room temperature for 20 minutes. After stopping the enzyme reaction with stopping solution (100µl) to each well. The results were recorded using a spectrophotometer within 15 minutes with a microtiter plate reader at wave length of 450 nm. The results are read by a microwell reader compared in a parallel manner with calibrator and controls. The calculated ELISA was read as negative if the ELISA value of both IgG and IgM is less than 0.90, positive if 1.00 or greater.

RESULTS AND DISCUSSION

Since infection by *H. pylori* induces the production of systemic antibodies. Serodiagnostic tests permit the rapid, noninvasive, sensitive, and specific detection of *H. pylori* (28, 29). Serological detection of *H. pylori* antibodies by well formulated and standardized tests such as ELISA has the ability to replace endoscopy as the diagnostic procedure of choice (30). ELISA is one of the most extensively employed tests, because it is relatively inexpensive, quick, easy to perform, suitable for screening large populations, and capable of detecting class-specific immunoglobulins (31, 32). Additionally, growth of the organisms is difficult, and recent evidence indicates that at best only 71% of *H. pylori* strains will grow in a specific medium (33). For best results, several selective media with various antibiotic supplements in addition to at least one nonselective medium would be required to provide the highest culture recovery rates. The presence of specific *H. pylori*-

directed IgG antibodies has shown excellent correlation with the presence of *H. pylori* enteric infection (34). A total of 88 patient consented to take part in this study (biopsy specimen collected from 40 of them only). These were 54 men and 34 women, with a mean age of 52.5 years (range 20-75); selected characteristics of the 88 patients examined in the present study are listed in Table (1).

Results of the various tests used in the present study for detection of *H. pylori* are presented in Table (2). Seventy four (84.09%) of the 88 patients

Table (1): Gender, And Age of 88 Samples of Patients That Used In This Study.

Characteristic	No. (%)
Gender	
Male	54 (61.36)
Female	34 (38.64)
Total	88 (100)
Age groups (yr)	
20-30	17 (19.32)
30-40	24 (27.27)
40-50	26 (29.55)
50-60	12 (13.63)
60-70	6 (6.82)
70-80	3 (3.41)
Total	88(100%)

were positive for IgM, (50%) of them were with positive IgG and only ten (11.36%) of the 88 samples were negative for both IgG and IgM antibodies the pattern of ELISA results were shown in table (3).

Table (2): Pattern of Results for Culture, modified Gram Stain, urease Detection IgG and IgM (40 Selected Patients).

No. of specimens	modified Gram stain	Culture	Urease Test	IgG	IgM
5	+	+	+	+	+
9	-	+	+	+	+
3	+	-	+	+	+
1	-	+	+	-	+
4	-	-	-	-	+
2	-	-	-	+	+
14	-	-	+	+	+
1	-	-	+	+	-
1	-	-	-	-	-

Table (3): No. and Percent of Patterns of ELISA Results.

Serum samples	IgG	IgM
40(45.45%)	+	+
34(38.64%)	-	+
4(4.54%)	+	-
10(11.36%)	-	-

The gastric biopsy specimens of 40 dyspeptic patients, *H. pylori* was found by urease detection, culture, gram staining, IgG, and IgM in 33 (82.5%), 15(37.5%), 8 (20%), (85%), and (95%), respectively table (4). thirty three (85%) of the 40 patients were positive *H. pylori* by one or more of the 'gold standard' tests (culture, gram stain and urease detection). The remaining 7 patients were negative for *H. pylori* by all three tests. only five of the 40 patient were positive for all obtained tests. Compared with bioptic methods (culture, staining, or urease testing), the serum ELISA was 94% sensitive, 70% specific and 90.25% accuracy for the detection of *H. pylori* infection. In comparison between male and female, the infection rate in males was higher than in females, *H. pylori* positivity were 29(65.90%), and 47(63.51%) in male and 15(34.09%) and 27(36.49%) in female for both IgG and IgM respectively as in table (5). Table (6) represent the relation between age and ELISA positivity, IgG antibodies were more detected in age (30-40) years old, 31.81%, where IgM decreased with age increasing.

Table (4): Percent pattern of Results for Culture, Modified Gram Stain, Urease Detection IgG and IgM (40 Selected Patients).

modified Gram stain	Culture	Urease Test	IgG	IgM
20%	37.5%	82.5%	85%	95%

A wide range of sensitivities and specificities has been reported for detection of *H. pylori* by ELISA (35-37).We determined the presence of anti-*H. pylori* IgG antibodies in the serum of 88 patients using a qualitative ELISA when

Table (5): Comparison Between Gender and ELISA Positivity

Gender	Positive IgG	Positive IgM
Female	15 (34.09%)	27 (36.49%)
Male	29 (65.90%)	47 (63.51%)

Table (6): Relation Between Age And ELISA Positivity

Age range(year)	Percent of ELISA positive	
	IgG	IgM
20-30	22.73%	24.32%
30-40	31.81%	29.73%
40-50	22.73%	27.03%
>50	22.73%	18.92%

compared (only 40 of them) with bioptic methods (culture, staining, or urease testing), and found this to be had a high sensitivity (94%), and accuracy of (90.25%), but low specificity (70%) these results in agreement with that found by Perez-Perez et al, 1990 (35); Lin et al, 1996 (36) and Hanvivatvong et al, 2002(37), they reported that in developing countries, commercial ELISA kits have high sensitivities but low specificities. A higher sensitivity and specificity were reported when using the antigen prepared from local *H. pylori* isolates than the commercial ELISA reagent kits ((38-40) for the diagnosis of *H. pylori* infection. In comparison between male and female, in this study the infection rate in males was higher than in females for both IgM and IgG detection and this will be in agreement with Deankanob et al, 2006, thirty-nine (60.9%) of the males and 18 (50.0%) of the females were seropositive, and disagreement with Andersen, *etal*, 1996(40). Which they reported that IgM seroprevalence indicate that women have a higher risk of primary *H. pylori* infection than do men in adult life. In this study we concluded that ELISA can be used for the detection of specific IgM and IgG to *H. pylori* and that the presence or absence of IgM and IgG antibodies to *H. pylori* may reflect whether or not an acute or chronic infection exists. We concluded that ELISA can be used for the detection of specific IgM to H. pylori and that the presence or absence of IgM antibodies to *H. pylori* may reflect whether or not an acute infection exists.

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