Original Article

The Role of Elisa test in the diagnosis of *helicobacter pylori* infection

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Summary:

Background: *Helicobacter pylori* represents the major etiologic agent of gastritis, gastric and duodenal ulcer disease and can cause gastric cancer. Diagnostic testing for *Helicobacter pylori* can be divided into invasive and non-invasive techniques based upon the need for endoscopy. Serological test is one of the non – invasive tests although measuring these antibodies is not reliable method of diagnosis but may be used in certain condition.

Objectives: To evaluate serum IgG antibodies against *Helicobacter pylori* by ELISA technique.

Patients and Methods: The current study consisted of 115 patients (74 males, 41 females) attending The Gastrointestinal tract Center and Gastroscopy department in Baghdad Medical City and was subjected to gastroscopy, during the period from November 2004 to May 2005. Those how were examined for serum IgG against *Helicobacter pylori* by ELISA technique were compared with 10 apparently healthy individuals representing the control group.

Results: By using the serological method (ELISA) 85 patients out of the 115 showed positive results (73.91%), however 7 out of the 10 individuals representing the control group were serologically positive (70%).

Conclusion: Positive IgG antibody test for *Helicobacter pylori* indicates a marker for infection rather than an indicator for active infection.

Key words: *Helicobacter pylori*, Gastritis, Elisa test for *helicobacter pylori.*

Introduction:

Helicobacter pylori is a bacterial pathogen related to a number of intestinal disorders, range from non ulcer dyspepsia and peptic ulcer to gastric tumor (1, 2). *Helicobacter pylori* causes more than half of peptic ulcers worldwide by damaging the mucous coating allows powerful stomach acid to get through to the sensitive lining beneath. Together the stomach acid and *Helicobacter pylori* irritate the lining of the stomach or duodenum and cause an ulcer (3). The host immune reaction to *Helicobacter pylori* may be an important cause of mucous incompetence because large number of neutrophils and lymphocytes are attracted to the bacterium. The attraction is related to the presence of chemotactic protein that is liberated by *Helicobacter pylori* (4). Chronic gastritis is characterized by an increase in mucosal plasma cell density and increased epithelial expression of serology component (5). Early studies, following the identification of *Helicobacter pylori* as an etiological agent of chronic gastritis, demonstrated that infection was associated with a specific gastric immunoglobulin G (IgG) and immunoglobulin A (IgA) response to the bacterium (5). A specific IgA and IgG response to *Helicobacter pylori* also occurs in duodenal bulb mucosa of patients with duodenitis (6). IgA response at mucosal site are thought to be functional in decreasing bacterial motility, inhibiting adherence, neutralizing biologically active bacterial products and preventing antigen uptake (7). Almost all infected patients have IgG antibodies in the serum and a systemic IgA response may be found in some people, but measuring these antibodies is not reliable method of diagnosis (8).

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Patients and Methods:

This study was conducted in the period from November 2004 to May 2005 including 125 individuals, 115 were patients with dyspepsia, (74 males and 41 females), with a mean age of (40.8) attending the Gastrointestinal Tract Center and Gastroscopy Department in Baghdad Medical City and were subjected to diagnostic upper gastrointestinal gastroscopy. The control group consisted of 10 apparently healthy volunteers, 4 females and 6 males their age range from 35 to 50 years with a mean age of 42.5 years with no history of dyspepsia, gastric or duodenal ulcer or acute gastritis or duodenitis. Venous blood samples were taken from both patients and controls groups. Serum was stored at -20°C. These blood samples were subjected to enzyme linked immunosorbant assay for IgG antibody to *Helicobacter pylori*. Five ml venous blood was taken from 115 patients and 10 controls collected in dry tube, after clotting the sera were obtained by centrifugation for (10 minutes at 3000rpm) and stored at (-20) until used. The principle of ELISA test is using horseradish

peroxidase conjugate for the indication reaction as described by Frei et al in 1995(9).

Statistical analysis:

The efficacy of the tests was determined by calculating the sensitivity and specificity of each test.

Sensitivity was defined as the proportion of number of *Helicobacter pylori* infected who had a positive test and was calculated as (10):

$$\frac{\text{No. of True positive}}{\text{No. of True positive + No. of False negative}} \times 100$$

Specificity was defined as the proportion of individual free of *Helicobacter pylori* that had a negative test and was calculated as:

$$\frac{\text{No. of True negative}}{\text{No. of False positive + No. of True negative}} \times 100$$

Results:

Endoscopically, gastritis was diagnosed in (48.59%) of the total patient group, duodenal ulcer was seen in (29.56%), however (6.9%) of the patients had duodenitis and (3.4%) were diagnosed as having abnormal mass (gastric adenocarcinoma).On the other hand, (1.7%)had gastric ulcer and (11.3%) showed non ulcer dyspepsia. The Elisa testing results revealed that of the (115) collected serum specimens, (85) were positive for anti *Helicobacter pylori* IgG antibodies (73.91%). It was positive in 7 individuals out of the 10 (70%) who represented the control group, as shown in table (1).

Table (1): Serological test (ELISA) in patient and control groups

Serological test IgG	Patients			Control		
	Total no. examined	No. of positive cases	%	Total no. examined	No. of positive cases	%
	115	85	73.91	10	7	70

Table (2) Shows the sensitivity and specificity of the non invasive serological test (ELISA) in comparison to other invasive tests done for the same patients.

Table (2): Percentage sensitivity and specificity of serological test in detecting *Helicobacter pylori* compared to other invasive tests

Tests	Positive cases	Sensitivity	Specificity
Urease	41	87.23	100
Culture	5	10.63	100
Histopathology	43	91.5	100
Serology (Elisa test)	85	100	55.3
Leukostix	53	95.8	88.23

***Total Helicobacter pylori positive cases =47**

Table (3) shows different diagnostic tests used for dyspeptic patients to detect *Helicobacter pylori* infection.

Table (3): Diagnostic tests for dyspeptic patients to detect *Helicobacter pylori* infection

Tests		No. of positive cases	Percentage of positive cases	No. of negative
Rapid urease test	P	41	87.2	6
	C	1		
Histopathology examination	P	43	91.5	4
	C	1		
Culture of *H.pylori*	P	5	10.6	42
	C	0		
IgG antibody test	P	47	100	0
	C	4		
Rapid leukocyte strip test	P	45	95.8	2
	C	0		

P: Patients
C: Control

Discussion:

Diagnostic testing for *Helicobacter pylori* can be divided into invasive and non-invasive techniques based upon the need for endoscopy. The techniques may be direct (culture, microscopic demonstration of the organism) or indirect (using urease or an antibody response as a marker of the disease). The choice of tests depends upon issues such as cost, availability, clinical situation, population prevalence of infection, pre-test probability of infection and factors such as the use of proton of pump inhibitors and antibiotics which may influence certain test results, large studies have found uniformly high sensitivity (90-100 percent), but variable specificity (76-96 percent); the accuracy has ranged from 83 to 98 %. (11).During our study the sensitivity of ELISA test found to be 100% compared to other tests (urease, culture, histopathology and leukostix) which showed sensitivity of (87.23%,10.63%, 91.5% and 95.8%) respectively. While the specificity of ELISA was 55.3%,compared to other tests (urease, culture and histopathology which showed specificity of 100%) ,and in leukostix test the specificity was 88.23%,these results may agree with other investigators abroad who found that in biopsy – based method the sensitivity was low but specificity was high. (12). Different studies in Iraq studied the prevalence of *H.pylori* either in normal population or in diseased people using ELISA technique to detect IgG but they didn't measure the sensitivity and specificity of the tests (13, 14 and 15). A study done by Zalabska in the period from(2009-2010) showed a sensitivity of 82-96% and specificity of 67-92% regarding the detection of IgG , IgM, and IgA which were confirmed with

western blot technique to allow more precise specification of the infection (16).Another study denoted a sensitivity ,specificity, positive predictive value, negative predictive value and accuracy of ELISA of 93.5%,94.4% ,95.6%,91.9%and 93.9%, respectively in patients aged lower than 45 years, and 100%, 81.3%, 100% and 95.6%, respectively in patients aged more or equal to 45 years, respectively. Thus they considered the quantitative ELISA test as a good non-invasive test even in old age groups(17). However, serology does not distinguish reliably between active and past infection. Furthermore, the positive predictive value of serology is poor in areas where the prevalence of *Helicobacter pylori* infection is low. Thus, the stool or breath testing are better alternatives to serology.

Conclusion:

Laboratory – based serologic testing using ELISA technology to detect IgG antibodies against *Helicobacter pylori* is inexpensive, non invasive, and well –suited to primary care practice. However, concerns over its accuracy have limited its use. Thus a positive IgG antibody test for *Helicobacter pylori* indicates a marker for infection rather than an indicator for active infection.

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