Detection of *Helicobacter pylori* IgG and IgM Antibodies in Iraqi Dyspeptic Patients

الكشف عن الأجسام المضادة IgG وIgM للبكتريا البوابية الحلزونية في المرضى العراقيين المصابين بسوء الهضم

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Abstract

This study is conducted to detect *H. pylori* IgG and IgM antibodies by different serological techniques (Immunochromatography test for rapid *H. pylori* IgG antibodies detection and enzyme linked immunosorbant assay test for *H. pylori* IgM antibodies detection). One hundred and twenty five dyspeptic patients are subjected to esophageal gastroduodenoscopy at the Endoscopy Unit at Baghdad Teaching Hospital from 10/1/2013 to 8/1/2014. Venous blood samples are collected from each patient. This study recorded, (89%) sensitivity for each of IgM Test by ELISA kit and Rapid IgG Test by Immunochromatography kit, but Rapid IgG Test has more specificity (85.64%) than IgM Test (80.8%).The higher prevalence 31.2% of *H. pylori* IgM antibodies observed among age group (21-40) years, whereas the IgM showed a slight rise 2.4% from the (10-20) years age group as acute *H. pylori* infection, although statistically there is a non-significant (P ≥0.05) association between *H. pylori* IgM antibodies and gender, but the females (32%) were more affected than males (27.2%). Also recorded significant (P ≤0.05) association between chronic *H. pylori* infection and gender (male 45% and female 55%), and highly significant association of blood groups phenotypes and Rh factor with *H. pylori* infection recorded by rapid IgG test (P ≤0.01), as the seropositivity of *H. pylori* according to rapid IgG test was (35, 15, 0 and 50) % for (A, B, AB and O) patients respectively, and (85and 15) % for Rh+ and Rh- patients, respectively.

Key words: *Helicobacter pylori*, ELISA, IgM, IgG

الملخص

لقد أجريت هذه الدراسة للكشف عن الاجسام المضادة IgG و IgM باستخدام تقنيات تشخيص مصلية مختلفة اختبار الاستشراب المناعي (IgG) سريع لـ *H. pylori* للكشف عن الأجسام المضادة IgG وفحص الامتزاز المناعي المرتبط بالانزيم للكشف عن الاجسام المضادةIgM. تعرض مائة و خمسة وعشرين عينة دم لمرضى تنظير المعدة و القولون في وحدة المناظير في مستشفى بغداد التعليمي من2013/1/10 إلى 2014/1/8، تم جمع عينات من الدم الوريدي من المرضى. سجل الاختبارين IgM و Rapid IgG نسبة الحساسية نفسها(89%), في حين سجل اختبار IgG السريع نسبة خصوصية (85.64%) اعلى من اختبار IgM (80.8%). أظهرت الدراسة نسبة أعلى من الأجسام المضادة IgM 31,2% للبكتريا عند الفئة العمرية (21-40) سنة، وارتفاعا طفيفا بنسبة 2,4% للفئة العمرية من (10-20) سنة كاصابة بكتيرية حادة، إحصائيا لم يكن هناك ارتباط بين IgM والجنس (P ≥ 0,05)، ولكن الإناث (32 %) كانوا أكثر اصابة من الذكور (27,2 %). سجلت الدراسة أيضا ارتباط معنوياً من الناحية الاحصائية بين الاصابة المزمنة والجنس (الذكور45% والإناث 55%)، (P ≤0,05) و ارتباط معنوياًللغاية من الناحية الاحصائية بين فصائل الدم وعاملRh مع الاصابة بالبكتيريا الحلزونية التي سجلها Rapid IgG Test (P ≤ 0,01)، كما ان إيجابية المصل للبكتيريا الحلزونية وفقا لاختبار Rapid IgG Test كان (35، 15 ، 0 و 50) % لـ (A ، B ، AB و O) من المرضى على التوالي و (85و15) % للمرضى +Rhو Rh- ، على التوالي.

الكلمات المفتاحية: البكتريا البوابية الحلزونية, اختبار الإليزا, الأجسام المضاد ة IgM,IgG

Introduction

Helicobacter pylori (*H. pylori*) is the main causative agent of gastrointestinal diseases including chronic gastritis, peptic ulcer associated disorders, gastric and duodenal carcinomas leading to morbidity and mortality in humans [1,2]. *H. pylori* has been ranked as a class I carcinogen by the

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International Research Agency on Cancer [3]. Once individual acquired the infection, *H. pylori* elicits a strong local and systemic humoral and cellular immune response, but it is not able to eliminate this bacteria [4]. The immune response to *H. pylori* include many factors produced by gastric mucosa that limit the proliferation of bacteria like gastric acidity, lactoferrin, beta – defensin [5] and Toll-like receptors-2 that presents on the surface of gastric epithelial cells and can recognize pathogen [6]. IgG antibodies is only a marker for a "chronic" infection with this bacterium and therefore no indicator of the time of acquisition of the infection, specific IgM antibodies are a more specific marker for a recently acquired infection with *H. pylori* [7].

Serological diagnosis simplest and least expensive, non –invasive method for IgG and or IgA antibodies, latex agglutination methods are quick tests, useful for screening purposes. ELISA based tests accurately quantities the amount of antibody (titer) present and are promising tool for assessing the efficacy of *H. pylori* eradication treatment, also for rapid office– based serologic test, using immunochromotography (ICM), and the immunoblott for the diagnosis of *H. pylori* [8]. Measurement of specific antibodies in serum has been used as non-invasive method by which *H. pylori* infection is detected [9].

This study is aimed to detection the frequency of *H. pylori* IgM and IgG antibodies to determine prevalence of acute and chronic infections in dyspeptic patients, using different serological methods and determine the relationship between the presence of these antibodies and some factors such as (gender, age, blood group phenotypes and Rh factor).

Materials and Methods

A total of 125 dyspeptic patients representing different age groups from both sexes. They underwent diagnostic upper gastrointestinal endoscopy at Endoscopy Department of Baghdad Medical City in Baghdad, Iraq at a period between 10/1/2013 to 8/1/2014.Informed written consent was obtained in advance from each patient includes sex, age, smoking, alcohol consumption and others.

Five milliliters of venous blood were taken in dry - sterile tube from each patient for serological tests. After clotting, the sera were obtained by centrifugation for 10min at 3000rpm, then divided into aliquots and stored at -20°C until use [10] for IgM test. Also whole blood samples were collected in tubes containing EDTA for plasma separation to use it in Rapid IgG Test.

Serological Tests

1. IgM Test

Bioelisa Helicobacter IgM is an ELISA test for quantitative and qualitative of IgM antibodies specific to *H. pylori* in human serum. It's based on an enzyme immuno assay technique with partially purified *H. pylori* bacteria antigen absorbed on a microplate and a detection antibody labeled with horse radish peroxidase (HRP). The procedure is conducted as manufacturer; Enzyme Linked Immunosorbent Assay (ELISA) Kit (Biohit /Finland).

2. Rapid IgG Test

The rapid IgG test according to Immunochromatography test (ICT) Kit (Abon biofarm /UK) instructions.

Statistical Analysis

The Statistical Analysis System- SAS [11] was used to find the effect of different factors on study parameters. Chi-square test was used to compare between percentages in this study. The efficacy of the tests was determined by calculating the sensitivity and specificity of each test.

Results and Discussion

1. IgM Detection

In this study, *H. pylori* specific IgM concentration was determined by ELISA method.

As shown in Table (1), out of 125 patients 74 (59.2%) were seropositive for IgM antibody, the higher prevalence of acute infection 18.4% was seen in age group (31-40) years followed by age group (21-30) years (12.8%), the IgM showed a slight rise from the age groups (10-20) and 71$\geq$ years (2.4%), also results revealed that the females 32% were more affected than males 27.2 % according to IgM ELISA test as acute infection. Also the sensitivity and specificity of this test were (89.21 and 80.8) %, respectively.

Table (1): Distribution of Dyspeptic Patients According to *H. pylori* IgM Seropositivity according To age and sex

Age groups (years)	No. patients IgM (+ve) Male	Female	Total of Positive (%)	Total of Negative (%)	Total Patients (%)
10-20	0	3	3(2.4)	7(5.6)	10 (8.0)
21-30	5	11	16(12.8)	4(3.2)	20 (16.0)
31-40	13	10	23(18.4)	5(4.0)	28 (22.4)
41-50	2	8	10(8.0)	12(9.6)	22 (17.6)
51-60	5	5	10(8.0)	8(6.4)	18 (14.4)
61-70	6	3	9(7.2)	9(7.2)	18 (14.4)
71>	3	0	3(2.4)	6(4.8)	9 (7.2)
P-value (χ^2)	---	---	0.0283 (4.683*)	0.1794 (1.604 NS)	0.0355 (4.371*)
Total	34 (27.2%)	40 (32%)	74 (59.2)	51 (40.8)	125 (100)
P-value(χ^2)	0.672 (1.027 NS)		0.0027 (7.024 **)		---
Sensitivity	89.21%		PPV		60.81%
Specificity	80.8%		NPV		69.21%

* ($P \leq 0.05$), ** ($P \leq 0.01$).

These results are similar to which reported by [12] who found that 62.7% were seropositive for IgM. Our results were different from that of [13-14-16] who found that patients were positive for IgM antibody (20,22.2,20.4and 21.1)%, respectively. This difference may be due to difference in nutritional behavior, socioeconomic status, water supply, educational level and environmental condition or others.

In this test the higher percentage of infection observed among age group (31-40) years followed by age group (21-30) years, the IgM showed a slight rise from the ≤ 20years age group.

Similar results recorded by [14] who found that the peak of infection among (31-40) years and showed a slight rise of IgM from the (10-20) years age group, also [17] found that the peak age of infection among (20-29) years and [15] found the lowest level of IgM was found among those ≤19 years. This high percent may be due to the infection associated with low socioeconomic status, poor hygiene and overcrowding [18].

Different results recorded by [16] who found that the higher percentage of infection observed among age group (15-30) years, but [15] recorded the highest level of IgM antibodies among the age group 50≥ years 19. Bakri, M.M. [19] recorded different results; the sensitivity and specificity of IgM test (ELISA) (94 and 93) %, respectively.

On the other hand our study showed that the females were more affected than males, but statistically there was no significant correlation between *H. pylori* antibodies and gender (P ≥0.05).

Our result was similar to [16,20] who found there was no significant difference between males and females for *H. pylori* antibodies.

In contrast, [21] found that the prevalence was significantly higher in boys. However, IgM antibody detection may reflect whether or not an acute infection exists [22].

2. Rapid IgG Test

Table (2) shown that, 80% of tested patients were positive (male 45% and female 55%), the seropositivity of *H. pylori* according to this test (35, 15, 0 and 50) % for (A, B, AB and O) patients, respectively, and (85 and 15) % for Rh+ and Rh- patients, respectively. Sensitivity and specificity of this test were (89.36 and 85.64) %, respectively. Results showed a significant association between *H. pylori* infection and gender, as P-value ≤ 0.05 and highly significant association of blood groups phenotypes and Rh factor with *H. pylori* infection recorded by ICT, as P-value ≤ 0.01.

Table (2): Rapid *H. pylori* IgG Test Seropositivity According to Gender, ABO Blood Groups and Rh Factor in Dyspeptic Patients.

Characteristic	No. patients IgG (%)		Total
	+ve	-ve	
Sex			
Male	45(45)	10(40)	55
Female	55 (55)	15(60)	70
Total	100(80)	25(20)	125
P-value	0.0439	0.0137	---
χ^2- value	4.601 *	7.034 **	---
Blood groups			
A	35 (35)	10 (40)	45
B	15 (15)	5(20)	20
AB	0 (0)	0 (0)	0
O	50 (50)	10 (40)	60
Total	100	25	125
P-value	0.00028	0.0035	---
χ^2- value	10.519 **	9.612 **	---
Rh+	85 (85)	20 (80)	105
Rh-	15 (15)	5 (20)	20
Total	100	25	125
P-value	0.0017	0.0019	---
χ^2- value	13.538 **	12.473 **	---
Sensitivity	89.36%		
Specificity	85.64%		
PPV	82%		
NPV	80%		

* (P≤0.05), ** (P≤0.01). PPV (Positive Predictive Value) NPV (Negative Predictive Value)

Similar results recorded by [23] found that 74.4% of tested patients were positive. Similar results also recorded by [24] recorded 64.8% seropositive patients 40.8% were male and 59.2% female, and he found that the seropositivity of *H. pylori* according to ICT were (32, 19.5, 6.7 and 41.8) % for (A, B, AB and O) patients, respectively, and (92.5 and 7.5) % for Rh+ and Rh- patients, respectively; but there is no significant differences in the frequency of ABO blood groups in seropositive patients. Treepongkaruna *et al.*, [25] and Rahman *et al.,* [26] recorded another result 96% and 93.2% of tested patients were positive, respectively. Sharma *et al.*, [27] recorded that the sensitivity and specificity of rapid IgG test are (96 and 95) %, respectively.

The *H. pylori* rapid antibody test addresses challenges related to ELISA as it is simple to test, has short test time (less than 30 min), does not require laboratory equipment or electricity supply, and use whole blood, plasma or serum as diagnostic sample [7].

Laboratory – based serologic testing using ELISA technology to detect IgM antibodies and ICT to detect IgG antibodies against *H. pylori* is inexpensive, noninvasive, and well –suited to primary care practice. Thus a positive IgG antibody test for *H. pylori* indicates a marker for infection rather than an indicator for active infection. While a positive IgM antibody test for *H. pylori* indicates a marker for an active infection.

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