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Evaluation the Efficacy of ELISA IgG, IgM and IgA Tests for Diagnosis of *Helicobacter pylori*

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Abstract: ***It has been well recognized throughout the world that Helicobacter pylori is the main cause of gastric ulcer and stomach carcinoma. Laboratory diagnosis of H. pylori infection is made by invasive and non-invasive methods. Invasive methods require endoscopy which is uncomfortable an unacceptable by the most patients. Therefore, non-invasive methods particularly serological tests are easier and comfortable for patients. A total of 86 patients with ages ranging from 18-77 years old (43 males and 43 females) who were referred to the Duhok Hepatology & Gastroenterology center/Azadi Teaching Hospital for endoscopic examination from June to October, 2013 were enrolled in the study. From each patient 5 ml of blood was collected under a septic condition and sera were separated for serology. Data from each participant were recorded in a special questionnaire form after consent agreed upon on ethical and scientific committee of the Azadi hospital. The efficacy of three different ELISA tests (IgG, IgM, IgA) was assessed taking culture as a gold standard method.***

H. pylori was found in 70.93%, 30.23% and 5.81% by ELISA IgG, IgA and IgM tests respectively. The highest percentage (70.93%) of H. pylori positive cases were found by ELISA IgG and the lowest percentage (5.81%) was recorded in ELISA IgM. The study found a significant relationship between age groups and H. pylori positive cases by ELISA IgG in which positive cases increased with the increase of ages.

No statistical correlation was found between the sex, smoking status and residency of studied patients with H. pylori infection. A significant correlation was found between patients without endoscopic lesions and positive cases for H. pylori by ELISA IgG in which 75.67% of patients without endoscopic lesions were positive for anti-H. pylori antibodies compared to 41.66% in patients with endoscopic lesions.

The efficacy of IgG, IgM and IgA tests was calculated compared with golden standard tests and ELISA IgG characterized by the highest values of sensitivity (75.75%), negative predictive value (36.00%) and accuracy (86.00%) but with lowest specificity (45.00%) and similar positive predictive value (81.96%) with IgM and IgA. The lowest sensitivity was 5.97% and accuracy 25.56% with ELISA Ig M. From the results of the present study we can conclude that among ELISA tests, anti-H. pylori IgG is a reliable serological test for the diagnosis of H. pylori infection.

Keywords: ELISA, Antibody, Evaluation, *H. pylori*

1. INTRODUCTION

Since the discovery of *H. pylori* by Barry Marshall and Robin Warren from Perth, Western Australia in 1983, a pivotal change has occurred in the overview of both microbiologists and clinicians toward peptic ulcer etiology and treatment strategies. Before this discovery, microbiologists believed that no microorganisms could thrive in such harsh destructive gastric environment and physicians also believed that peptic ulcer is mainly due to the overproduction of gastric acids against which treatment was directed to suppress acid production. Nowadays, it is well recognized that *H. pylori* is the principal cause of gastric ulcer and the main hazard factor for the development of gastric carcinoma and mucous associated lymphoid tissue lymphoma. Clinicians started to change their treatment strategy from acid suppressing drugs to both anti-acid and antimicrobial drugs regime [1].

H. pylori is a Gram negative, curved bacilli with 4-6 unipolar sheathed flagella. It grows slowly. It is also fastidious in its growth requirements in that needs special media supplemented with antimicrobial agents and provided with micro aerobic conditions (5% O_2, 5-10% CO_2, 85% N_2) [2].

H. pylori was first named *Campylobacter* like organism, later named *Campylobacter pylori*. Based on the DNA sequence, *H. pylori* was placed under new genus of *Helicobacter* [3]. The distribution of *H. pylori* infection varies among developed and developing nation in which 70-90% of the people living in developing nations are colonized by *H. pylori* compared to 50% in those living in developed countries [4]. Colonization with *H. pylori* varies even among populations of the same country according to age, socioeconomic status, hygienic practice, geographical area and overcrowding conditions [5].

Laboratory identification of *H. pylori* infection is made by several different techniques including both invasive and non-invasive method. Invasive methods are based on gastric biopsies taken during endoscopy like culture, histopathology and rapid urease test (RUT). Invasive methods are costly and inconvenient for most patients

they might also give false negative results, particularly in patchy colonized and treated patients. Furthermore, these methods are not suitable as a screening test for large scale of population studies. Therefore, non-invasive methods particularly serological tests remain the alternative choice for screening the sero-prevalence of *H. pylori* in large populations during epidemiological studies [6]. Serological tests like ELISA may be superior to invasive methods in cases with gastric atrophy in which the number of microorganisms is so small to be undetectable by invasive methods. Serological tests also reveal antibodies generated by intracellular survival *H. pylori* after treatment [7].

2. LITERATURE REVIEW

Among serological tests, Enzyme-linked immunosorbent assay (ELISA) is the most commonly used for detecting specific antibodies in the serum, as it is easy to perform, in-expensive, widely available, and suitable also for large-scale screening [1]. *H. pylori* ELISA kit (ACON Lab, USA) was used by [17] for detecting anti-*H. pylori* IgG antibodies in the serum samples collected from patients with gastritis. They found that 81% of 58 patients were positive. *H. pylori* IgG and IgA ELISA kits (Germany) was used by [14] and found that 41(58.5%) and 48 (68.5%) from 70 studied patients were positive by IgG and IgA, respectively. The efficacy of ELISA IgG for the detection anti- *H. pylori* antibodies was assessed by [27] and found that 119 (39.1%) out of 304 tested serum samples were positive with a sensitivity of 99% and specificity of 82%.Another study by [24] found that 487 (69.0%) out of 706 tested serum samples were positive for anti-*H. pylori* IgG by ELISA technique (Orgenics® Immunocomb II), while [12] found 85 (73.91%) from 115 patients were positive for anti-*H. pylori* IgG antibody titers by ELISA IgG kit. They found that the test had high sensitivity (100%) but with low specificity (55.3%) in comparison to urease test, culture, histopathology and leukostix test. A study conducted by [13] in Iran using ELISA IgG, IgM and IgA kits (IBL International GMBH) for detecting seroprevalence of *H. pylori* in 339 patients (114 male and 225 female) who suffered from gastric symptoms from northwest of Iran. They found that the overall seropositivity rates were as follows: anti *H. pylori* IgG 73%; anti *H. pylori* IgM 43% and anti *H. pylori* IgA 25%. In a study carried out by [16] and used ELISA IgG, IgM and IgA (Monobind Inc., USA) for serological distribution of anti- *H. pylori* Ab in population of Sulaimani governorate/Iraq. A total of 335 adult and children volunteers from Chamchamal and Sulaimani cities in Kurdistan region/ Iraq were tested and found that 20.4% had positive IgM, 32.3% positive IgG and 58.2% positive IgA. ELISA IgG, IgM and IgA kits (NovaLisa, NovaTec, Germany) used by [15] to screen the seroprevalence of *H. pylori* among asymptomatic healthy Omani blood donated individuals. Among 133 healthy asymptomatic individuals, IgG *H. pylori* antibody was found in 83 (62.4%), IgM *H. pylori* antibody in 21 (15.1%) and IgA *H. pylori* antibody in 11 (8.7%). In another study by [11] using ELISA kits for IgG, IgA and IgM and found that 68 from 102 patients were positive .Out of these, IgG type was positive in 49 (72%), IgA in 56 (82.3%), and IgM in 25 (36.8%).

3. MATERIALS AND METHODS

Serological tests

Each serum sample was analyzed by ELISA IgG, IgM and IgA kits (Monobind Inc., USA) following the procedure supplied by kits.

Statistical analysis

Data was statistically analyzed using (statistical analysis system) SAS software (Version, 2010) by Log linear model.

The first Model: $Y_{ij} = \mu+A_i+E_{ij}$ used for analyzing the effect of age, smoking, gender and resident on Serological tests (IgG, IgM and IgA).

Y_{ij} = the studied test

μ =over all mean

A_i = the independent factor

E_{ij} = random error

The second Model: $Y_{ijk}=\mu+A_i+B_i+(A^*B)_{ij}+E_{ijk}$ used for analyzing the effect of Culture on Serological test(IgG, IgM and IgA).

Y_{ijk} = the studied serological test.

μ = over all mean

A_i = the effect of first standard

B_i = the effect of second standard

$(A^*B)_{ij}$ = the interaction of both standard

E_{ijk}= random error

(SAS, LTD, 2010)

4. RESULTS

Three different ELISA kits (IgG, IgM and IgA) were used for the detection of *H. pylori* infection in 86 patients using a culture method as a gold stander method. *H. pylori* was detected in 37.2%, 70.93%, 5.81% and 30.23% by ELISA IgG, ELISA IgM and ELISA IgA respectively compared to 37.2% by cultural method as shown in Table 1. The maximum percentage of *H. pylori* was recorded with ELISA IgG (70.93%) followed by ELISA IgA (30.23%), while the lowest percentage of *H. pylori* was recorded with ELISA IgM test (5.81%).

Table 1. Serological tests and cultural methods used for detection of *H. pylori*.

Tests	Positive for *H. pylori*		Negative for *H. Pylori*	
	number	%	number	%
IgG	61	70.93	25	29.06
IgM	5	5.81	81	94.18
IgA	26	30.23	60	69.76
Culture	32	37.2	54	62.79

A high percentage of *H. pylori* infection was found with increased ages with ELISA IgG in which a high percentage 88.23% was found in individuals with the age group 41-50 years old compared to 50% in the age group less than 20 years old. ELISA IgA recorded high percentages of positive cases 70% in young age less than 20 years old compared to low percentages with increasing

ages, while in ELISA IgM, the picture was irregular in which only 11.53% and 12.5% of cases were positive for *H. pylori* in age 20-30 and 31-40, respectively, as shown in Figure 1. An important correlation was found between ages and *H. pylori* positive cases with ELISA IgG in which positive cases increased with the increase of age but no significant correlation was detected between age groups ELISA IgM and IgA tests for *H. pylori* infection.

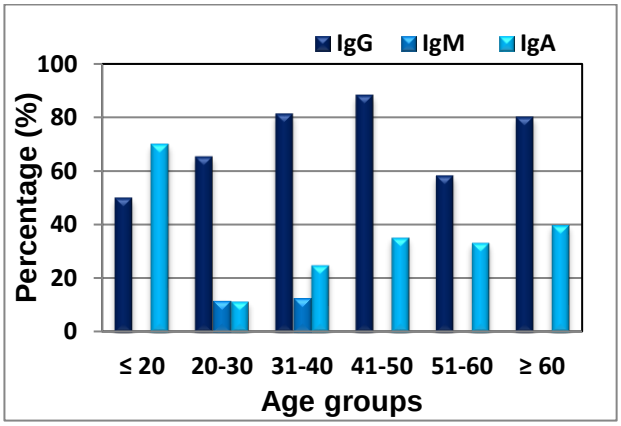

Figure1. Percentages of *H. pylori* infection in age groups using ELISA techniques.

H. pylori was detected in 34 (74.06%), 3 (6.97%) and 15 (34.88%) in female patients by ELISA IgG, IgM and IgA respectively. Among male patients, *H. pylori* was detected in 27 (62.79%), 2 (4.65%) and 11 (25.58%) by ELISA IgG, IgM and IgA, respectively, as shown in Figure 2. No significant correlation was found between sex and ELISA (IgG, IgM and IgA) methods for screening *H. pylori.*

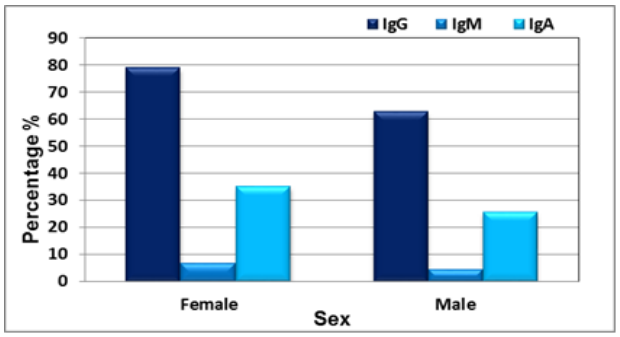

Figure 2. Percentages of *H. pylori* infection in sex groups by ELISA IgG, IgM and IgA tests.

H. pylori was detected in 13 (65%), 1(5%) and 2 (10%) smoker patients by ELISA IgG, IgM and IgA, respectively, while 14 (21.21%, 0 (0%) and 8 (12.12%) were positive for *H. pylori* in non-smoker patients by ELISA IgG, IgM and IgA respectively, as shown in figure 3. No significant correlation was found between smoking and ELISA techniques.

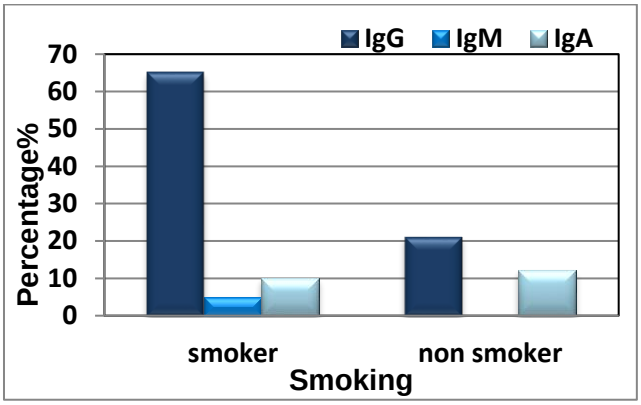

Figure 3. Percentages of *H. pylori* infection in smokers and non-smokers patients by ELISA IgG, IgM and IgA tests.

H. pylori was found in 33 (60%), 2(4%) and 14 (28%) in patients lived in the urban areas by ELISA IgG, IgM and IgA, respectively, while 28 (77.77%), 3(8.33%) and 12 (33.33%) were positive for anti- *H. pylori* antibodies in patients who lived in rural areas by ELISA IgG, IgM and IgA respectively, as shown in Figure 4. No significant correlation was found between residence and ELISA tests.

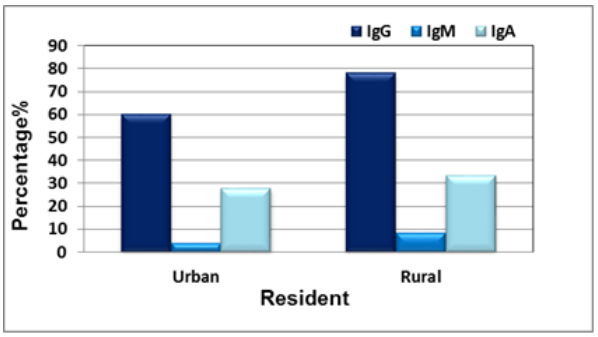

Figure 4. Percentages of *H. pylori* infection among resident groups using ELISA techniques.

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of ELISA IgG, IgM and IgA were calculated using culture as golden standard methods. Among 86 patients, 61 were positive and 25 were negative by ELISA IgG test in which the true positive, false positive, true negative and false negative results were 50, 11, 9 and 16 cases, respectively. Only 5 cases were positive and 81 cases were negative by ELISA IgM test in which the true positive, false positive, true negative and false negative results were 4, 1, 18 and 63 cases, respectively. By ELISA IgA test 26 cases were positive while 60 cases were negative in which true positive, false positive, true negative and false negative results were 22, 4, 16 and 44 cases, respectively, as shown in Table 2.

Table 2. True positive, false positive, true negative and false negative results of ELISA IgG, IgM and IgA tests.

Test	+ve	True +ve	False +ve	-ve	True – ve	False -ve
IgG	61	50	11	25	9	16
IgM	5	4	1	81	18	63
IgA	26	22	4	60	16	44

5. DISCUSSION

Among serological tests, Enzyme-linked immunosorbent assay (ELISA) is the most commonly used for detecting specific antibodies in the serum, as it is easy to perform, in-expensive, widely available, and suitable also for large-scale screening [1].

The results of ELISA IgG were in agreement with those recorded by other researchers [8, 9, 10, 11, 12, 13] who found 76%, 69%, 73.68%, 73.9%, and 73% respectively. While the results were in disagreement with the results of [14,15,16,17] who found 58.5%, 62.4%, 32.3% and 81%. The causes of these dissimilar results may be attributed to the kind of captured antigens used for detection of IgG, geographical distribution of the *H. pylori* infection and stage of infection. The ELISA IgA results were similar to those found by [13], who found that 25% of patients were positive for *H. pylori* infection, while dissimilar to that found by [14,11,15,16], who found 68.5%, 82.3%, 8.7% and 58.2% respectively. This dissimilarity in the results can be attributed to the same factors mentioned in ELISA IgG.

The results of ELISA IgM were dissimilar to those found by most studies, such as [1,11,13,15,16] who found 44% ,43%, 36.8%, 20.4%, 15.1%, respectively. This dissimilarity of the results may be due to the low number of samples covered by the study or the patients were in the chronic stages of the infection. Positive cases of *H. pylori* increased with the increase in age by ELISA IgG in which a high percentage (88.23%) of positive cases was in the age group 41-50 years old patients compared to 50% in the age group less than 20 years old (Figure 1). A significant relation was found between *H. pylori* positive cases and the increase of ages. The picture was different with the results of ELISA IgA in which a high percentage (70%) of positive cases was found in young patients under 20 years old compared to 40% in older patients more than 60 years old, but did not show significant correlation. The results were in disagreement with those of [13], who found that *H. pylori* seropositivity for IgA increased with the increase in age. The pattern of positive cases by ELISA IgM was not significant and irregular in which no positive cases were found in the age group less than 20 years old, 41-50, 51-60 and above 60 years old patients. The findings of the study were similar to those found by [18,16,19, 20, 13, 21] who found a significant effect of age on the levels of *H. pylori* positive cases in which high percentages of positive cases for anti-*H. pylori* IgG antibodies were increased with the increase in ages, these results in disagreement with those of [12] who found no correlation between *H. pylori* seropositivity and age. This may be due to the chances of acquisition of infection which tend to increase with age. The results of ELISA IgM were inconsistent and not significant this may be due to several factors, such as low number of patients tested because of the cost constraint, the nature of the antigen used in the kit or the patients were in the chronic stage of infection and IgM antibodies appeared in the early stage of infection then decline.

No significant correlation was detected between sex and (IgG, IgM and IgA ELISA`s Tests) for finding of positive cases of *H. pylori*. The results were similar to those found by [1] for IgM, [23, 22, 24] for IgG and [16] for IgG, IgM and IgA, while in disagreement with the results of [15] who found that males were more infected than females by ELISA IgG, IgM and IgA. No significant correlation was found between the smoking factor and seroprevalence of *H. pylori*. The results were dissimilar to those found by [18,21,10], who found a significant effect of smoking on the prevalence of *H. pylori*. This discrepancy in the results could be contributed to the number of analyzed samples, Methodology, socio-economic status and geographical location.

In this study, no statistical correlation was found between residence and *H. pylori* seropositivity by serology IgG, IgM and IgA tests. The results were comparable to the results of [22] and [19] who found no significant impact of residence on the seropositivity of *H. pylori* by ELISA techniques.

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of ELISA IgG were 75.75%, 45.00%, 81.96%, 36.00% and 68.60% respectively, for ELISA IgM were 5.97%, 94.73%, 80.00%, 22.22% and 25.58%, respectively and for ELISA IgA were 33.33%, 80.00%, 84.61%, 26.60% and 44.18% respectively, as shown in Table 2.

In the present study, ELISA IgG was characterized by high sensitivity, high negative predictive value and high accuracy in comparison to both IgM and IgA. Lowest sensitivity, lowest positive predictive value, lowest negative predictive value and lowest accuracy were found with IgM. The results were analogous to those reported by [14,25], but generally the results of this study were lower than those found by [26,27]. The lower results of the present study may be due to inadequate number of analyzed serum samples because of the cost constraints and the antigenicity of the antigens used in the kits.

4. CONCLUSION

1. ELISA IgG was superior to ELISA IgM and IgA for the detection of anti-*H. pylori* antibodies.
2. ELISA IgG was characterized by high sensitivity, negative predictive value and accuracy but with lower specificity.
3. ELISA IgM was characterized by the highest specificity but with the lowest sensitivity.
4. ELISA IgA was characterized by moderate sensitivity, specificity, positive predictive value, negative predictive value and accuracy.
5. No significant effect of smoking and residence was found on *H. pylori* infection.
6. A significant correlation was found between age and *H. pylori* infection by ELISA IgG in which *H. Pylori* infection increased with the increase in age.

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