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Immunological Study for *Helicobacter pylori* Bacteria in Human

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Abstract

Helicobacter pylorus is one of the most important human pathogens, infecting more than 50% of the human population. Commonly the first noninvasively testes used for *H. pylori* infection's detection were immunological tests like blood antibody test and stool antigen test. We investigated the more efficient susceptibility immunological test for detection of *H.pylori* infection in adult Egyptian patients by comparing the results of *H. Pylori* IgA (HpIgA), IgG in serum blood (HpIgG) and *H.pylori* Ag in stool (HpSAg) for 30 adult patient (males and females) against control group using ELISA technique.The results showed that each test could be used successfully for diagnosis of *H. pylori* with 100% specificity and PPV% with no gender relation. Finally HpSAg showed reliable results with high sensitivity (>95%) followed by HpIgG (87.5%), while HpIgA showed the lowest sensitivity (37.5%). Our findings confirms the use of the mentioned immunological tests for detectingthe *H. Pylori* infection and suggest the use *H. Pylori* Ag in stool as the most economic, sensitive and reliable method alone or followed by IgG antibody test as confirmatory test to be the first choices for early diagnosis of *H. Pylori* especially in developing countries.

Keywords: *Helicobacter pylori* • Immunological test • IgA,IgG • Patients

Introduction

Helicobacter pylori are a helix shaped, microaerophilic, Gram-negative, flagellated bacteria. *H. pylori* and mankind have an ancient relationship for at least 50,000 years. In 1983 *H pylori* was and first isolated from the human stomach [1]. A since then this bacterium are became one of the most important human pathogens, infecting more than 50% of the human population with high prevalence in developing countries *Helicobacter pylori* normally infect stomach, typically during childhood infect stomach, typically during childhood and persists for life. However, over 80% of individuals infected with the bacterium are asymptomatic. The infection can lead to peptic ulcer, gastritis, and the gastric cancer. Thus, being recognized as the principal agent leading to gastric cancer [2]. World Health Organization (WHO) has classified *H. pylori* as a class I carcinogen. Attributed to the poor socioeconomic status and overcrowded conditions, the lifetime risk of the infection is 90% in third world countries and much less in the a developed of world Infection in developed countries is less common in the young children and reaches up to 60% in older ages. A study in the Egypt revealed that about 91.7% has been found to be infected in this Egyptian population [3]. The rate of infection was different in different age groups with an increasing trend in older ages and suggested that the rate was increasing in rural areas of this country which make it a public-health issue. Testing for *H. pylori* is recommended if there is peptic ulcer disease, low grade gastric MALT lymphoma, after endoscopic resection of early gastric cancer, if there are first degree relatives for the gastric cancer, and in certain cases of dyspepsia. Many diagnostic tests could be used to detect *H. Pylori* include endoscopic and non-endoscopic methods. The techniques used may be direct (culture, microscopic demonstration of the organism) or indirect (using urease, stool antigen, or an antibody response as a marker of disease). Commonly the first noninvasively testes used for *H. pylori* infection's detection were immunological tests as they are commercially available, easy to perform and inexpensive. Studies showed that infection was associated with a specific gastric Immunoglobulin G (IgG) and Immunoglobulin A (IgA) response to the bacterium [4]. We investigated the more efficient dependable Immunological test for detection of *H.pylori* infection by comparing the results of *H. Pylori* IgA (HpIgA), IgG (HpIgG) in serum

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blood and *H.pylori* Ag in stool (HpSAg) in adult Egyptian patients [5].

Materials and Methods

This study was conducted including 50 individuals, A group of 30 patients diagnosed by *H.pylori* infection, The patients were 35 to 55 years old with a median age of 39 years old (39.5 males and 38.5 females). The control group consisted of 20 apparently healthy volunteers from 30 years-50 years old with median age of 42 years old (44 males and 37 female) with no history of previously been treated of gastric or duodenal ulcer. Blood drawn for serological testing was performed where 5 ml venous blood was taken from both groups collected in dry tube, after clotting, the sera were separated by centrifugation for (10 minutes at 3000 rpm) divided into aliquots and stored at (-20ºC) until used. Samples of stools from all individuals in each group were collected in dry clean tubes and stored at (-20ºC) until used. ELISA testing was performed using *H.Pylori* Antigen E32-320, *H.pylori* IgG E30-145 and *H.pylori* IgA E30-274 manufacturer instructions (Immunospec,USA) [6].

Statistical analysis

The efficacy of the tests was determined by calculating the Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of each test. Statistical analyses were performed using SPSS software (version 16) [7]. Chi-square test and independent t-test for significance used to compare proportions and mean between qualitative parameters. The result considered significant if the probability (P-value) was <0.05 [8].

Results

Out of 50 patients, only 30 serums' specimens were tested for HpIgG and HpIgA while all 50 stools' specimens were tested for HpSAg. The rest of patient refused to undergo the serological tests (Table 1).

Table 1. The statistical significance difference for Stool Ag, HPIgA, HPIgG in patients.

	Control	Patient	P value
HPAg (stool)	5.26 ± 3.92	**67.76 ± 23.30	<0.0 1
HPIgA-Ab	0.35 ± 0.18	** 1.85 ± 0.34	<0.0 1
HPIgG-Ab	0.49 ± 0.24	** 2.19 ± 0.61	< 0.01

For control group all tests performed showed negative results expressed by (5.26 ± 3.92) ranged from 0.11 to 0.82 for HpIgA, (0.35 ± 0.18) ranged from (0.142, 0.89) for HpIgG and (0.49 ± 0.24) ranged from (0.2, 15) for HpSAg.

There were significance difference increase in stool Ag in patient group (67.76 ± 23.30) When compared to control group (5.26 ± 3.92) There were significance difference increase in Serum the (IgAAb) in patient group (1.85 ± 0.34) When compared to the of control group (0.35 ± 0.18) There were significance difference increase in of Serum (IgGAb) in patient group (2.19 ± 0.61)When compared to control group (0.49 ± 0.24). where only 37.5% exhibited positive HpIgA are results with 1.728 (1.2, 2.1) the percentage increased in HpIgG results where 87.5%showed positive results with 1.991 (1.2, 3.8) and reached its maximum 96.7% in HpSAg results with 103.162 (20.3, 250) ng/ml (Figures 1-3).

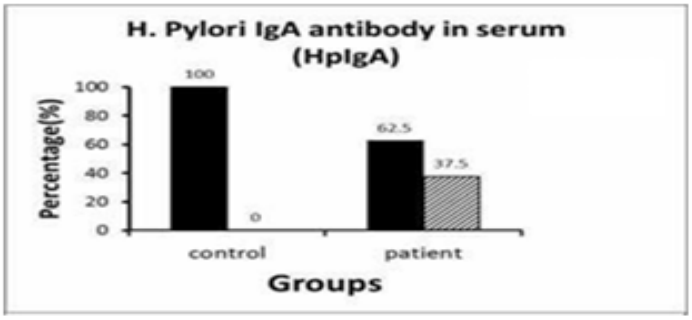

Figure 1. Diagnostic tests to detect *Helicobacter pylori* IgA Antibody in Serum (HpIgA) infection. **Note:** Negative, positive.

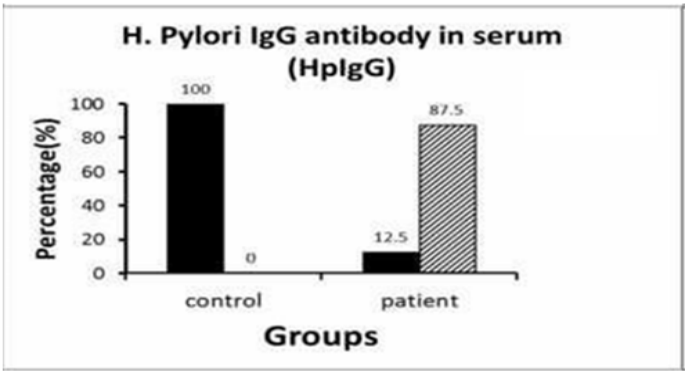

Figure 2. Diagnostic tests to detect *Helicobacter pylori* IgG Antibody in Serum (HpIgG) infection. **Note:** Negative, positive.

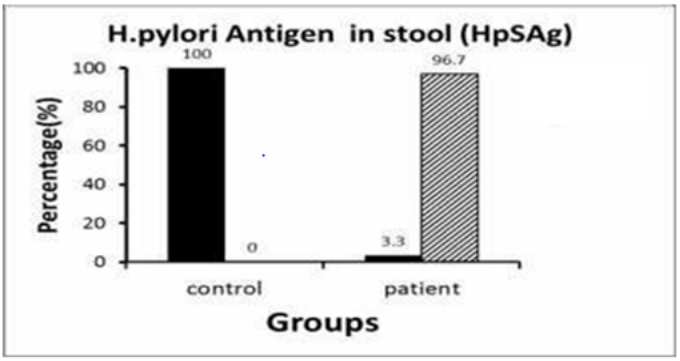

Figure 3. Diagnostic tests to detect *Helicobacter pylori* IgG Antibody in Serum (HpSAg) infection. **Note:** Negative, positive.

A significant difference between the results of both serological tests (HpIgG and HpIgA) was noted as the sensitivity of HpIgG was significantly higher than HpIgA ($p<0.001$). The same result was observed between HpSAg and HpIgA as the results of HpSAg were significant more sensitive than HpIgA ($p<0.001$), while the difference between HpIgG and HpSAg were equivalent ($p>0.1$) [9].

Discussion

H. Pylori infection develop no symptoms in about 85% of the infected patient, however acute infection may appear as an acute gastritis with abdominal pain (stomach ache) and nausea which are quite broad and confusing symptoms especially for elder people [10]. Knowing that the rate of infection increases with age and the infection tends to be common where sanitation is poor or living quarters are cramped as observed in the developing countries of the world. The infection remains localized to the gastric area, and probably persists unless specific treatment is given which may lead to peptic ulcer disease and gastric cancer to around 15% of the patients [11]. *H. pylori* remain highly prevalent in immigrants coming from countries with high prevalence of *H.Pylori*. However, the lower prevalence of infection in the younger generations suggests a further decline of *H.Pylori* prevalence in the coming decades. Low socioeconomic conditions in childhood are confirmed to be the most important risk factors for *H.Pylori* infection [12]. Although the way the infection is transmitted is still unclear, interpersonal transmission appears to be the main route. Finally, *H. pylori* recurrence after successful eradication can still occur, but seems to be an infrequent event [13]. Early diagnosis increases the chances to eradicate it and allow the ulcer to heal rapidly. Many tests could be used to detect *H. Pylori* divided into invasive and non-invasive techniques based upon the need for endoscopy. Commonly the first noninvasively testes used especially in developed countries are the most simple and least expensive method by immunological tests like blood antibody test and stool antigen test formed by provincial health laboratories [14]. Thus, in this study we evaluated the most common requested diagnostic tests to detect *H. Pylori* infection in Egypt. *H. pylori* infection can be detected by a variety of methods [15]. In the routine clinical diagnostics the urease test, histological examination, urea breath test, serology, bacterial culture and stool antigen test are valuable methods of detecting *H. pylori* infection. Histopathology has historically been considered as being the first diagnostic method for *H. pylori* detection and is still widely used as the main diagnostic tool in suspicious patients with upper gastrointestinal symptoms or in highly prevalent areas. This study investigates the more susceptibility for *H.pylori* infection by immunological study by comparing the results of *H. Pylori* IgA, IgG in serum blood and *H.pylori* Ag in stool for 30 patients, age (30-60 years) against control group using conventional ELISA test. In our study serological tests (HPIgA and HPIgG) gave average sensitivity results with mean above 50% (62.5%), average NPV 78.80% however they are mostly exhibited 100% specificity and PPV with clear distinctive results as

There was no indeterminate readings [16]. Our results are in consistent with different studies reported the performance of serological tests especially HPIgG where the sensitivity and specificity have found uniformly high sensitivity but variable specificity ranging from 30%-100%. The results of HPIgG were significant higher than that observed in HPIgA which are different from other studies observed HPIgA to be equal to HPIgG in performance with no additional benefit [17].

However in other study the sensitivity of HPIgA was inversely proportional with age as poor sensitivity increased with adults than children. Interestingly only 25% of the patient group showed positive results in both HPIgG and HPIgA, while 12.5% showed only positive HPIgA and 62.5% gave positive HPIgG only. Also there was no significant association between the results of HPIgG and HPIgA in all subjects. The same observation was reported in different studies showing presence of IgA antibody in absence of IgG and vice versa. These findings strongly suggested that HPIgA may not be the other choice to be used for detection of *H. Pylori* infection in elderly people. Also the presence of positive HPIgA indicates a positive confirmation for early stage of infection or recurrence even in absence of positive HPIgG which was found previously in other studies. Furthermore observed the predominantly IgG immune response to infection with *H. pylori* which explained why the mean of values for HPIgG produced in patient group was significantly higher than HPIgA (0.948 and 1.798) respectively [18].

The *Helicobacter pylori* Stool Antigen test (HPSAg) is an enzyme immunoassay which detects *H.pylori* antigens present in human stool samples for diagnosis, monitoring effectiveness of antibiotic therapy during the 14 days of treatment and for confirmation of eradication. HPSAg is FDA approved and recommended by both the American Gastroenterological Association (AGA) and the American College of Gastroenterologists (ACG).

HPSAg offers simpler sampling method; only one stool specimen is required and it does not require neither a technician nor nurse nor expensive equipment which make if suitable for children and Patients With Belonephobia. Comparing to serology tests, HPSAg is rapid, easy-to-use, non-invasive and had no confounding factors also [19].

HPSAg differentiate between active and latent infection; whereas, other serological tests only detects exposure to *H.Pylori*. Also large studies confirmed the high specificity and sensitivity of the test. In our study the results were consistent to the previously observation. HPSAg give the highest sensitivity and Negative Predictive Value (NPV) (>95%) which is significant higher than HPIgA but equivalent to the HPIgG. Also there was no significant association between the results of HPSAg and both serological tests (HPIgG and HPIgA) in patient group only. As only 37.5% from patient group exhibited positive results in both HPSAg and HPIgA which represented all the positive results in HPIgA test. We could conclude that HPIgA is strong positive confirmatory test if appeared but could not be used alone as sole detection test. While 81.25% gave positive results in both HPSAg and HPIgG and 56.25% of patient group showed positive HPSAg Only one patient had negative results of both HPSAg and HPIgA but positive HPIgG which

may refer to past infection or ongoing curing. Also 12.5% of patients group showed positive HPSAg, HpIgA and negative HPIgG results. Again as explained before these results may refer to early stage of infection. The overall findings confirmed the observation that serological tests are marker for infection rather than an indicator for active infection [20].

Opposite results were observed when the results of all the subjects including (both control and patient groups) were compared. There was significant relation between the results of HPSAg and either HPIgA or HPIgG ($p<0.01$) which was due to the common negative results in all control group. These suggested that all the tests were good indicator for absence of *H. Pylori* infection if combined together. That the meaning of if patient gave negative results in two of the three tests one of them (HPSAg), this means that the chance to have *H. Pylori* infection is negligible even if he exhibited similar symptoms [21].

Given the widespread prevalence of *H.pylori* infection, its socioeconomic impact, and the rising antibiotic resistance rates worldwide. This investigation was performed to draw attention of the physicians to the value and importance of immunological testing as non-invasive, relatively easy to be performed and convenient to patients for the recognition of *H.pylori* infection. Collectively our results suggested their utility as a reliable predictor of infection in high-prevalence developing country especially HPSAg test which exhibited best results to be the first choice to detect early infection followed by HpIgG as confirmatory test in adult patients, while HPIgA could not be used alone for diagnosis of *H.pylori* infection.

It is well recognized that women in many parts of the world experience lower socioeconomic status (a significant risk factor in Helicobacter infection and pathogenesis), often with reduced access to health care, than their male counterparts. It is intriguing, therefore, that males are more prone to infection, peptic ulcer disease and gastric cancer than females, suggesting this gender influence is potentially mediated by physiological factors. The most obvious physiological difference between males and females is the sex hormones. The predominant male hormone, testosterone, and the predominant female hormones, oestrogen and progesterone, can all influence immunity. It has long been recognized that inflammatory responses in men and women are disparate.

Broadly speaking, males are suspectible than the females in order to tend to develop more severe sequelae from the infectious disease, whereas females are more prone to autoimmune diseases. It is generally thought that females generate more robust cellular and humoral immune responses and thus are more resistant to certain infections than males. As it is seen in results that the percentage of infection in female is 46.6% ranging between 24.8 to 94.4 while in male 53.3% ranging between 28.8 to 100 for HPSAg, for the HPIgA antibody female results are 0.3 to 2.3 while in male are 0.3 to 2.4, whereas for HPIgG antibody female results are 0.6 to 2.8 while in male are 0.2 to 3.8 [22].

Conclusion

Given the widespread prevalence of *H.pylor*i infection, its socioeconomic impact, and the rising antibiotic resistance rates worldwide. This investigation was performed to draw attention of the physicians to the value and importance of immunological testing as non-invasive, relatively cheap easy to be performed and convenient to patients for the recognition of *H.pylori* infection. Collectively our results suggested their utility as a reliable predictor of infection in high-prevalence developing country especially HpSAg test which exhibited best results to be the first choice to detect early infection followed by HpIgG as confirmatory test in adult patients, while HpIgA could not be used alone for diagnosis of *H.pylori* infection.

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