

Evaluation of Invasive and Non-Invasive Methods for the Diagnosis of *H. pylori* in Dyspepsia Patients

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Abstract

Background: *H. pylori* has been established as the major causative agent of chronic gastritis, and represents the main etiological cause of other gastroduodenal diseases such as peptic ulceration, gastric lymphoma, and gastric cancer. Therefore, screening and treatment of the bacterium is an important strategy for preventing gastric cancer.

Objective: To compare some invasive classical histological tests to non-invasive serological antibody and stool antigen tests for the diagnosis of *H. pylori* infections.

Patients and Methods: The study population comprised of 171 patients with symptoms of dyspepsia and other gastritis related symptoms. Three biopsy specimens were taken and collected from the stomach and sent for histopathologic study. H and E staining and modified Giemsa staining were performed on tissue sections of each case. Serology antibody and Stool antigen tests were also used as non-invasive tests in this study.

Results: Four tests had been done to detect the presence of *H. pylori* Bacteria. The mean age (+ SD) of the studied sample was 43.8 + 14.7 years, ranging from 20 to 74 years. The median was 44 years. The total agreement between the serum Ab test and the stool Ag test was 88.3%. Significant differences were detected between the two tests ($p < 0.001$). It is evident that the serum Ab test is highly sensitive (sensitivity = 98.4%), but the Predictive value positive (76.3%) was not so high. The Giemsa test was highly specific (specificity = 98.2%), and highly sensitive (sensitivity = 93.5%).

Conclusion: The main advantage of histopathology tests is not only restricted to detection purpose, it also can detect any abnormalities of gastric mucosa by bacteria such as peptic ulcer bleeding, atrophic gastritis, intestinal metaplasia, and gastric cancer. The modified Giemsa staining method could be used as a method of choice for the detection of *H. pylori* due to its sensitivity, simplicity and consistency.

Keywords: *H. pylori*, gastritis, peptic ulcer, histology, H & E, Giemsa, Stool Ag and serum Ab test

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Introduction

Helicobacter pylori (*H. pylori*) has been established as the major causative agent of chronic gastritis, and represents the main etiological cause of other gastroduodenal diseases such as peptic ulceration, gastric lymphoma, and gastric cancer [1]. The association of the pathogen with these infections including gastric cancer has been reported all over the world [2], and is one of the most common known risk factor for the development of gastric cancer. Therefore, screening and treatment of the bacterium is an important strategy for preventing gastric cancer [3], therefore, diagnosing *H. pylori* infections is of paramount importance for the effective treatment of these infections [4]. Several methods have been used for the diagnosis of *H. pylori* infections, which can be classified into two main methods invasive and non-invasive methods. Invasive methods require an endoscopic gastric biopsy specimen which include, culture, histological examination, and rapid urease test, CLO (Campylobacter like organism) test, smear examination, and molecular studies. Noninvasive methods include fecal antigen detection, serologic, and urea breaths tests [5]. Sensitivity of any of these techniques in detecting *H. pylori* relies on how density of the bacterial cells in the specimens taken by biopsy (recent use of disease related medications specifically antibiotics and proton-pump inhibitors can reduce the density of the cells), pathologist expertise, and type and quality of the stain used for detection purposes [6]. Stool antigen test is also one of the common methods for the

diagnosis of infections caused by *H. pylori*, in which monoclonal antibodies to *H. pylori* native catalase was developed and use of this test is one of the popular tests in the diagnosis of these infections in Japan [7]. The sensitivity of any techniques is calculated by finding the rate of patients whose test positive, and who are known to have the disease [8].

The aim of this study was to compare some invasive classical histological tests to non-invasive serological antibody and stool antigen tests. And to find the sensitivity and specificity of the diagnostic methods used in this study.

Patients and Methods

This study was conducted in the Bacteriology and Histopathology laboratory of Rizgary Hospital, Erbil, Iraq, within the period of May 2018 to November 2018. The study population comprised of 171 patients of any age and gender with symptoms of dyspepsia and other gastritis related symptoms. A questioner regarding the age and weather they were taking any dyspepsia related medications was included and filled by all patients. Any patient received antibiotics for *H. pylori* infection, or any other GIT related medications such as proton pump inhibitors (PPI) within the previous 3 months were excluded, due to the fact that these medications known to have effect on the density and even disappearance of the pathogen in the antrum. Three biopsy specimens from the antrum and the corpus were taken and sent for histopathologic study. In the histopathological unit, the

biopsy specimens of all 171 patients were fixed in 10% buffered formalin for at least 24hrs, and then embaded in paraffin wax. Hematoxylin and Eosin (H and E) staining and modified Giemsa staining were performed on tissue sections of each case. Three sections for each specimens were deparaffinized and hydrated in descending grades of alcohol, cut in sequential 4- μ m sections. The sections were then stained with H and E stain and modified Giemsa stain to demonstrate the presence of *H. pylori* and gastritis. The *H. pylori* were clearly detected as curved bacilli on the surface of the gastric epithelial cells. The slides were evaluated by histopathologist and assigned to each morphological variable. Regarding the serology tests, blood samples from of all patients selected for endoscopy in this study were taken by venipuncture and serum separated and used for *H. pylori* serology to detect IgG antibodies by Immunochromatography technique using VIDAS® *H. pylori* IgG kit (Biomeriux). The kit was used by following the kit instructions. Briefly, the blood samples were left at room temperature for about one hour; centrifuging at 1,000- 2,000xg for 10 mins to remove the

clot. The serum samples were immediately collected in a sterile eppendorf tube using a Pasteur pipette, and were maintained at 2-8°C while handling and stored at -20°C until using for the test. For stool antigen test, stool samples from the same patients were taken and stored at -80°C and tested for the prevalence of *H. pylori* antigen using commercial kits BIONEXIA® *H. pylori* Ag (Biokerieux) for detection of *H. pylori* antigen in human stool according to manufacturer's instructions. A patient was classified to be positive on the basis of stool antigen test, as this test was considered as the gold standard test for data analysis in this study. The study protocol was reviewed and approved by Scientific and Research Ethics Committee of the College of Health Sciences/ Hawler Medical University.

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS, version 22). Frequencies and percentages were calculated. McNemar test was used (in the 2X2 table) when the results of the studied tests like serum Ab test were compared with the stool Ag test; as in the following table:

		Stool Ag test		P (By McNemar's)
		Positive	Negative	
A test like serum Ab i2	Positive	TP	FP	TP+FP
	Negative	FN	TN	FN+TN
Total		TP+FN	FP+TN	Grand total

*TP=True positive; TN=True negative; FP=False positive; FN=False negative.

**Sensitivity = $TP / (TP+FN) * 100$; Specificity = $TN / (FP+TN) * 100$; Predictive value positive (PV+): $TP / (TP+FP) * 100$; Predictive value negative (PV -): $TN / (FN+TN) * 100$; Total agreement = $(TP + TN) / Grand total$.

***A p value of ≤ 0.05 was considered as statistically significant.

Results

Table (1): Age and gender distribution.

	No.	%
Age		
20-29	40	23.4
30-39	33	19.3
40-49	31	18.1
≥ 50	67	39.2
Gender		
Male	65	38.0
Female	106	62.0
Total	171	100.0

The total number of the studied sample was 171. Four tests had been done to detect the presence of H. pylori Bacteria. The mean age (+ SD) of the studied sample was 43.8 + 14.7 years, ranging from 20 to 74 years. The

median was 44 years. Around two thirds (62%) of the sample were females. The age and gender distribution are presented in Table (1).

Table (2): Validity of serum Ab test compared with stool Ag test.

Serum Ab test	Stool Ag test			Validity measures	Values	P value
	Positive	Negative	Total			
				Sensitivity	98.4%	<0.001
Positive	61	19	80	Specificity	82.6%	
Negative	1	90	91	PV+	76.3%	
Total	62	109	171	PV-	98.9%	
				T. Agreement	88.3%	

*By McNemar's test

The total agreement between the serum Ab test and the stool Ag test was 88.3% as presented in Table 2. Significant differences were detected between the two tests ($p <$

0.001). It is evident in the table that the serum Ab test is highly sensitive (sensitivity = 98.4%), but the PV+ (76.3%) was not so high Table (2).

Table (3): Validity of Giemsa test compared with stool Ag test.

Giemsa	Stool Ag test			Validity measures	Values	P value
	Positive	Negative	Total			
				Sensitivity	93.5%	0.687*
Positive	58	2	60	Specificity	98.2%	
Negative	4	107	111	PV+	96.7%	
Total	62	109	171	PV-	96.4%	
				T. Agreement	96.5%	

*By McNemar's test

It is evident in Table 3 that the total agreement between the Giemsa test and the stool Ag test was 96.5% with no significant difference detected between the two tests (p

= 0.687). The Giemsa test was highly specific (specificity = 98.2%), and highly sensitive (sensitivity = 93.5%) as presented in Table (3).

Table (4): Validity of H/E test compared with stool Ag test.

H/ E	Stool Ag test			Validity measures	Values	P value
	Positive	Negative	Total			
				Sensitivity	83.9%	0.012*
Positive	52	1	53	Specificity	99.1%	
Negative	10	108	118	PV+	98.1%	
Total	62	109	171	PV-	91.5%	
				T. Agreement	93.6%	

*By McNemar's test

Table(4) shows that the total agreement between H / E and the stool Ag test was 93.6%, but the difference between the two tests results was significant ($p = 0.012$). The test was highly specific (specificity = 99.1%), and its sensitivity was relatively high (sensitivity = 83.9%).

Discussion

The high rates of infections caused by *H. pylori* were mostly detected in high population densities and low socioeconomic communities, and the prevalence of the bacteria is different between countries [8]. The present study composed of 171 patients of either gender ranging from 18-70 years; these patients were suffering from dyspepsia and requiring an upper gastrointestinal endoscopy. The highest rate of infected patients were among ages of over ≥ 50 years with the percentage of 39.2%. On the other hand 23.4% of patients with age group 20-29 were also positive for *H. pylori* infection. These data could support the conviction that *H. pylori* infection was developed early in

life, leading to multifocal gastritis later in life. The results of this study is in agreement to the results obtained by Brown, 2000 who indicated that the rate of infected people increases with age as 50% of infected people were among those ages of over 60 years. Other studies observed that chronic gastritis detected in relatively younger age group with a mean age of 47 years [9]. Similar to these results Udoh 2012 [10] also reported chronic gastritis cases in younger age group with mean age of 48.6 years. Data obtained from this study showed that around two third of the sample were female, which is in a good accordance with results produced by Shahram [11], who reported that female rate were significantly higher than male rates, similarly in [12] study the prevalence of *H. pylori* infection in female were 52% was higher as compared by male patients. In contrast to these observations a higher incidence was seen in males with a male: female ratio of 2.1: 1 [13]. Chen [14] found a slight preponderance of *H. pylori* infection in

males (57.6%) as compared to females. However, some reports indicated that gender and age were not significant associated factors for *H. pylori* infection [15].

In the present study, three diagnostic methods were used and compared for detection of *H. pylori* infection using stool antigen test as a gold standard method for this purpose. The sensitivity and specificity of serum antibody test were 98.4% and 82.6% respectively. These results were similar to those of Rajan, [16] who found lower values for specificity 59.25% and 90% For sensitivity. In another study done by [17] there results were the diagnostic odds ratios for serology, and stool antigen test, the sensitivity was 95% estimated at a fixed specificity (84%) for serology, which is in a good accordance with our data. The data of this study shows that the total agreement between the serum antibody test and the stool antigen test was (88.3%) table (2). According to these data there was one case, which gave seronegative but tested positive for the stool antigen, considering that monoclonal antibodies used for stool antigen have very high specificity [18] it could be explained that that most of the cases with negative serum antibody test results obtained for positive cases of stool antigen test were most likely false positive. Our data, however, showed (18) cases tested positive for serum antibody test, but negative for stool antigen test. This may be due to low specificity of serum antibody test, which is usually explained for remaining anti *H. pylori*

antibodies in the patients serum after treated infections for a period of time.

Sensitivity and specificity of both invasive methods, H & E and Modified Giemsa were somewhat similar in results obtained in our study, which were 83% and 99% for H & E respectively, and 93% and 98% for Modified Giemsa method. It is well known that H & E staining method can be used directly for identification of *H. pylori*, in addition to the evaluate the degree of inflammation. However, sometimes it is difficult to see the organism, especially when the density of *H. pylori* is low. Since it has been reported that the sensitivity and specificity of H & E stain was 69-93% and 87-90% respectively [19].

However, similar to our results, by using special stains like modified Giemsa stain the specificity can be increased to 90-100% [19]. Data of present study showed the total agreement between the Giemsa test and stool antigen test was 96.5% with no significant difference detected between the two tests. However, the total agreement between the H & E test and stool antigen test was 93.6%, so the difference between the two tests results was significant. This was comparable to the results reported by [16] the prevalence of positive tests for *H. pylori* was higher in Giemsa stain 55% as compared to H&E test 47%.

In a study done by [1] who compared the sensitivity and specificity of different methods for detection of *H. pylori* in patients. It has been reported that among 30 positive cases confirmed by histopathology using the modified Giemsa stain. The positive results

for serum antibody and culture tests were 27 and 29 cases respectively. Which indicate some differences in their specificities, this result confirmed that Giemsa stain was the best method with a high sensitivity 98% as compared to other tests [1]. Additionally, The accuracy of some methods for *H. pylori* detection lowering in some cases of infection such as peptic ulcer bleeding. However, it has been indicated that most accurate method was histopathology in particular modified Giemsa stain [20].

There were [20] cases in the present study were negative for *H. pylori* on histopathology but had a positive result in serum antibody test. This may be occurred in cases of chronic atrophic gastritis, which is a common clinical appearance in the development of gastritis caused by the pathogen. As in these types of infections, the gastric cavity becomes suitable place for colonisation of the bacteria. Therefore, the organism hides from the mucosa, while the antibody titres remain in the range of diagnostic standards to give positive results.

Serum antibody tests for the diagnosis of *H. pylori*, is economic and widely used in patients before treatment [8]. However, the sensitivity is lower compared to other tests due to remaining the antibody in the serum after treatment for a period and low density of bacteria or bacterial product in gastric cavity rendering the results to be negative in endoscope related staining method.

Conclusions

To conclude, the main advantage of histopathology tests is not only restricted to

detection purpose, it also can detect any abnormalities of gastric mucosa by bacteria such as peptic ulcer bleeding, atrophic gastritis, intestinal metaplasia, and gastric cancer. The modified Giemsa staining method could be use a method of choice for the detection of *H. pylori* due to its sensitivity, simplicity and consistency.

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