

Challenges of Accurate Diagnosis of *Helicobacter pylori* in Iran



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Abstract

Background: *Helicobacter pylori*-associated gastritis is regarded as the most prevalent gastrointestinal infection, as well as the major cause of several other diseases. Despite being discovered over 30 years ago, no golden diagnostic method has yet been suggested for this microorganism.

Objective: The present study aimed to compare two common diagnostic methods of histology and serology.

Materials and Methods: This cross-sectional study began in July, 2016, and was conducted at Imam Jafar Sadegh Hospital (a university hospital) in Maybod, Yazd, Iran, on 70 patients. All the patients underwent endoscopy, and biopsy samples were taken for histology, as well as blood samples for ELISA test. Finally, sensitivity, specificity, NPV (negative predictive values), and PPV (positive predictive values) were calculated and analyzed.

Results: The sensitivity, specificity, accuracy, PPV, and NPV of the ELISA method with histology as the gold standard were 87.5%, 36.9%, 54.2%, 42%, and 85%, respectively. Still, the histology method appears to be the most reliable test for the diagnosis of *H. pylori* in Iran.

Conclusion: Regarding the high prevalence of *H. pylori* in Iran, the present study recommends the use of histology as a diagnostic method in combination with other diagnostic methods for detecting *H. pylori* infection.

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Background

Helicobacter pylori is recognized as a spiral, gram-negative, microaerophilic, and motile bacterium which is well colonized in the gastric mucosa.¹ *H. pylori* infection exists all over the world; however, its colonization percentage varies in different regions.² *H. pylori* is acquired at early childhood and can be transmitted through fecal-oral and oral-oral routes.³ However, only in a small percentage of patients, *H. pylori* infection leads to severe diseases such as gastric cancer.⁴ Although this bacterium was discovered over 30 years ago, no golden method has yet been suggested for the diagnosis of this microorganism, particularly in epidemiologic studies.^{5,6} Therefore, various methods have been employed in numerous studies.^{5,7,8} Several invasive (e.g., histology, culture, rapid urease test, and molecular methods) and non-invasive (e.g., urea breath test, stool antigen test, and serology) diagnostic tests are currently available for detecting *H. pylori* infection.⁶ Nonetheless, selection of a test or a diagnostic method depends on the sensitivity and specificity of the method, equipment, access to diagnostic tests, quality of laboratory, clinical

conditions of the patient, and positive/negative results of tests at various clinical conditions.⁹

Owing to the lack of a standard guideline for *H. pylori* diagnosis and its prevalence of 75% to 91% in the Iranian population⁷, the present study aimed to compare two common diagnostic methods, namely histology (the most reliable current diagnostic method) and serology (the most common and non-expensive method) and evaluate the effect of demographic factors on obtained results. Endoscopy is normally used to diagnose *H. pylori*-associated diseases such as peptic ulcers, atrophic gastritis, and various gastric cancers. However, several factors affect the accuracy of histology results, such as sampling location, size and number of biopsies, staining method, and the use of antibiotics.¹⁰ Serologic tests detect *H. pylori*-IgG antibody and are useful in epidemiological studies, though they may be associated with false results. In addition, serologic tests are not affected by bleeding, gastric atrophy, and the use of proton pump inhibitors and antibiotics.¹¹

Materials and Methods

This cross-sectional study began in July, 2016, and was conducted at Imam Jafar Sadegh Hospital (a university hospital) in Maybod, Yazd, Iran, on 70 patients including 39 males and 31 females who were suspicious for ulcer. All participants signed the informed consent form prior to the enrollment. The data pertaining demographics and medications were recorded using a questionnaire.

Sampling

In order to measure Anti *H. pylori* IgG, 5 mL venous blood was taken from each patient and sent to the hospital laboratory. Since peptic ulcer is usually confined to the mucosa and submucosa in the stomach and duodenum, endoscopic samples were taken by Endoscopy machine (Fujifilm, Japan MED-169-KRT).

Histology Method

The biopsy samples were sent to the Pathology laboratory of the hospital in 10% formalin, and were placed inside the Tissue Processor device and after the completion of formalin fixation, they were embedded in a block of paraffin. Then they were cut by a microtome machine and the slides prepared for complete drying were placed in the incubator at 37 °C. After the sequential steps of staining with Giemsa and rinsing with 3% alcohol acid solution, slides were prepared for observation under microscope.

ELISA Method

To measure Anti *H. pylori* IgG, serum samples were centrifuged at 1372 g for 3 minutes (Behdad Co., Iran), and ELISA was performed according to the manufacturers' instructions (Monobind Company Kit, Cat No.1425-300, USA).

Statistical Analyses

The obtained results were analyzed using SPSS software version 20.0 through chi-square test, and specificity, sensitivity, negative predictive values (NPV), and positive predictive values (PPV) were also calculated.

Results

Demographic Information

The mean age of the participants was 46 years. The highest prevalence was within the age group of 20-40 years (n=26) and the lowest prevalence was within the age group below 20 years (n=4).

Histology

Positive histologic results with Giemsa staining are shown in Figure 1.

Situation of Age Groups According to ELISA and Histology Results

No significant relationship was observed between age and



Figure 1. Tissue Smear Stained With Giemsa.

H. pylori infection via ELISA method ($P>0.05$). However, results of statistical analyses showed a significant relationship between these two variables via histology method ($P<0.05$). Comparison of the results revealed that the most likely occurrence of *H. pylori* infection was in the age group of 20-40 years (Table 1).

Comparison of Discrete Variables in the Population Studied

Comparison of the following considerations was noteworthy in histology method:

(1) *Use of Antibiotics*: Only 4 out of the 24 patients taking antibiotics were diagnosed to be infected (16.66%) compared to 21 out of 46 patients who had not used antibiotics (45.65%).

(2) *Smoking*: Fourteen out of 52 non-smokers were diagnosed to be infected with *H. pylori* (27%) compared to 10 out of 18 smokers (55.5%).

(3) *Hospitalization*: Only 4 out of the 24 patients hospitalized were diagnosed to be infected (16.66%) compared to 20 out of 45 patients who had not been hospitalized (44.44%). These results showed that the risk of infection among smokers and outpatients, as well as those who did not use antibiotics was higher.

Comparison of ELISA and Histology Results

The results of ELISA and histology, sensitivity, specificity, NPV, and PPV are presented in Table 2. In this study, the results of sensitivity and specificity for ELISA were calculated as 87.5% and 36.9 %, compared to histology results.

Table 1. Comparison of Age Groups Regarding the Histology Results

	Age	<20	20-40	40-60	60-80	>80	P Value
Positive histology	No.	1	14	8	1	0	0.035
	%	25	53.84	33.33	10	0	
Negative histology	No.	3	12	16	9	6	0.035
	%	75	46.16	66.67	90	100	

Table 2. Comparison of ELISA Method With Gold Standard

Variable	Gold standard		Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
	Negative	Positive					
ELISA	17	21	87.5	36.9	54.2	42	85

Abbreviations: PPV, positive predictive values; NPV, negative predictive values

Discussion

Currently various methods are used for the diagnosis of *H. pylori*, including urine test, urea breath test, culture, PCR, stool antigen test, rapid urease test, histopathology, and immunohistochemistry.⁶ Owing to a number of reasons, including the difficulty and time-consuming nature of bacterial growth in culture media, the probability of occurrence of false positive results in PCR, anaphylactic reactions, and the risk of allergy in urea breath test, the present study compared two common diagnostic methods, namely ELISA and histology. These methods are recognized as common diagnostic methods in Iran because of their ease of implementation and low costs. Histology is an invasive method while ELISA is a non-invasive method. Moreover, ELISA is an easier technique, which causes less pain and is less expensive. However, its specificity has been reported as 36.9% in experiments, which is worth reflecting. The low specificity of ELISA may be attributed to the transience of *H. pylori* infection, which is cleaned in a short time. ELISA can show positive results even after the treatment of the infection. In the histology method, however, if various factors including sampling location (i.e., Antrum and Corpus), size and number of biopsies, staining method, inhibitors of proton pump, and the use of antibiotics are considered, the accuracy of this method will significantly improve.¹⁰

In this study, histology was considered a gold standard method for the diagnosis of *H. pylori* infection. Evidence from previous studies supports this assumption.¹²⁻¹⁴ In addition, sensitivity and specificity of ELISA test have been calculated and reported in various studies. For example, in a study conducted by Iqbal et al in Pakistan, the sensitivity, specificity, NPV, PPV, and accuracy of serology method were reported to be 95%, 80%, 96%, 76%, and 86%, respectively, in comparison with histology results.¹⁵ Moreover, in a study by Pourakbari et al on 89 patients in Iran, the sensitivity, specificity, NPV, PPV, and accuracy of anti *H. pylori* IgG were 50%, 83.3%, 79.3%, 56.6%, and 65%, respectively, in comparison with biopsy method, while the results were 100%, 90%, 92.5%, 100%, and 95% for histology method.¹⁶ In another study conducted by Wang et al, the sensitivity and specificity of serology method were reported as 97.6% and 96.2% compared to histology.¹¹ The values studied in these works are higher than those in the present study. As can be concluded, histology has higher sensitivity and specificity in comparison with serology. Differences between sensitivity and specificity

percentages for serology methods in the present study and similar researches depend on the antigen used in commercial kit and the prevalence rate of specific *H. pylori* strains employed as the source of antigen. The serological tests also cannot distinguish between active infection and past exposure to *H. pylori*. The above items can explain the controversial results.

The reason for higher prevalence of *H. pylori* infection in the age group 20-40 is the lifestyle and inappropriate nutritional habits of these individuals. In respect of the age, Awuku et al and Frugis et al stated that the risk of the infection was higher in lower ages and the reason was the possibility of acquiring infection through fecal-oral and oral-oral routes.^{17,18} This finding disagrees with the study conducted by Asahi Hishida et al. In their study, the risk of obtaining *H. pylori* infection increased with the increase of age.¹⁹ Another factor examined in the present study was smoking. In this regard, Ghosh et al and Hishida et al stated that the prevalence of *H. pylori* infection was higher among smokers. This is due to the fact that smoking causes various ulcers in the respiratory and digestive tracts, which can lead to infection and gastric cancer.^{19,20} Likewise, a significant relationship was found in our study between *H. pylori* infection and smoking ($P=0.027$). In terms of antibiotics, it seems that the use of antibiotics results in cross reactions and, subsequently, in false positive results in ELISA test. Therefore, histology is more reliable than ELISA in these subjects.

Helicobacter pylori infection is a global concern, the prevalence of which has been estimated to be 30% in developed countries compared to over 80% in developing countries.²¹ South Korea, China, Japan, the United States, Europe, and Asia-Oceania have specific guidelines for the diagnosis of the infection.⁶ Furthermore, various studies have suggested different methods. Gholi et al compared histology, rapid urease test, polymerase chain reaction, stool antigen test, and serology with endoscopic-based techniques, and concluded that the highest specificity was for histology and rapid urease test (95.6%) while the lowest specificity was for stool antigen test (73.9%). In this study, the highest (100%) and the lowest (55.6%) specificity were reported for rapid urease test and serology test, respectively.⁸ The results of a study by Safari et al who used different gold standards on 127 patients showed that non-invasive methods (e.g., ELISA) have the lowest accuracy while invasive methods (e.g., histology) have the highest.²² In the study of Kazemi et al, in which urea breath test was considered as the gold standard, it was concluded

that stool antigen test was the most accurate method for the diagnosis of *H. pylori*.²³ Moreover, in another similar study, Pourakbari et al compared histopathology, rapid urease test, stool antigen test, serology, and PCR, and concluded that histopathology had a specificity of 100% and serology had the lowest sensitivity. They also found that the accuracy of rapid urease test, histopathology, and PCR was the same. They recommended that because of the harmful effect of invasive methods (i.e., histopathology and rapid urease test), non-invasive stool antigen test is used for detecting *H. pylori* infection.¹⁶ The above-mentioned studies confirm that the reliability of histology is greater than that of serology.

Conclusion

Although ELISA may be preferred to invasive methods such as histology due to being non-invasive and its ease of implementation, its low specificity leads to the occurrence of many false positive results. Hence, it cannot be solely employed in the definite diagnosis of *H. pylori* infection. Moreover, as long as more non-invasive methods are not provided for *H. pylori* diagnosis, it is impossible to ignore invasive and more reliable methods, such as histology. However, it is clear that more than one single method must be employed to diagnose this infection. Therefore, when endoscopy is not needed, *H. pylori* stool antigen test (HpSA) and C-urea breath test would be potentially very helpful in diagnosing *H. pylori* infection.²⁴ Finally, histology method is suggested as a diagnostic method along with other diagnostic methods for detecting *H. pylori* infection.

Authors' Contributions

All authors have equally contributed to the study and therefore are responsible for its content.

Ethical Approval

All procedures and sampling of patients were performed with the permission of laboratory management of the hospital.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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