

Original Article**Diagnostic values of Helicobacter pylori diagnostic tests: stool antigen test, urea breath test, rapid urease test, serology and histology***

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Abstract

BACKGROUND: The purpose of this study is to compare validity of 5 diagnostic tests of helicobacter pylori with each other: stool antigen test, urea breath test (UBT), rapid urease test (RUT), serology and histology.

METHODS: A total of 94 patients who had indication of endoscopy entered the study. All of the 5 tests were performed for each patient. When the results of at least 2 tests were positive (except serology), Helicobacter pylori infection was considered to be positive. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under receiver operating characteristic (ROC) curve of these 5 tests were determined.

RESULTS: The sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under ROC curve of these 5 tests are as below, respectively. Histology: 89%, 78%, 93%, 91%, 85% and 0.881; RUT: 93%, 75%, 95%, 94%, 86% and 0.831; serology: 50%, 54%, 46%, 61%, 52% and 0.563; stool antigen test: 96%, 83%, 98%, 96%, 91% and 0.897; UBT: 89%, 73%, 92%, 90%, 82% and 0.892.

CONCLUSIONS: Stool antigen test is the most accurate test for Helicobacter pylori diagnosis before eradication of these bacteria.

KEYWORDS: Helicobacter Pylori, Stool Antigen Test, Urea Breath Test.

J Res Med Sci 2011; 16(9): 1097-1104

Helicobacter pylori has been considered to be the etiologic cause of gastritis, peptic ulcer disease and associated with development of gastric cancer.¹⁻³ It is a spiral or curved gram-negative microaerophilic flagellate bacilli. Prevalence of infection is different worldwide, depending on the socioeconomic status and sanitation conditions.⁴ In the developed countries, its prevalence is under 40% but in developing countries it is more than 80%.⁵

The diagnosis of Helicobacter pylori can be classified as invasive and noninvasive methods. Invasive methods include urease test, which has sensitivity from 79.7% to 97.5% and

specificity from 97.2% to 100%,⁶⁻⁹ performed in the endoscopy, suits as a rapid indirect test to confirm the presence of Helicobacter pylori in biopsy samples; and histology of the modified Giemsa stained gastric biopsies with sensitivity from 94% to 97.5% and specificity from 97.2% to 99%.^{6,7,9} Noninvasive methods are often the first line diagnostic tests and include serology,¹³ C-urea breath test,¹⁴ and a new developed diagnostic test based on the detection of Helicobacter stool antigen. Serologic tests are available and relatively cost-effective tests which are often used for screening or documentation of infection in patients whose other tests yielded borderline results. However,

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these tests are not suitable to diagnose active infection or follow-up of eradication because of its low accuracy.

Urea breath tests are considered to be the gold standard of diagnosis, with sensitivity and also specificity from 90% to 100%.^{6,10-12} The stool antigen tests based on immunoassay (ELISA), have been tested in several laboratories of the world. The results have been satisfactory and in some researches it has been considered to be the preferable strategy for diagnosis of *Helicobacter pylori* in primary care.^{13, 14} In Iran, its validation for diagnosis of *Helicobacter pylori* was reported in children, with sensitivity and specificity of 100% and 83.4% respectively,¹⁵ but its diagnostic value has not yet been tested in adults. The major advantages of this test are its cost, when compared to C-urea breath test¹³ and also the possibility of being performed in any laboratory.

The aim of this study was to evaluate the diagnostic value of 5 widely used diagnostic tests of *Helicobacter pylori* [histology, rapid urease test, serology, stool antigen test and urea breath test (UBT)] and to compare them with each other.

Methods

This study was a diagnostic accuracy study and the protocol of this work was reviewed and approved by the Institutional Ethics Committee of the Faculty of Isfahan University of Medical Sciences. We enrolled 110 patients in our study but the final cases were 94 patients because of case failure. Outpatients that were sent to the Endoscopy Unit of Poursina-Hakim institution for dyspeptic symptoms were prospectively recruited for the study. Patients were contacted before the endoscopic examination and were asked to participate in the study. Before the endoscopic examination, the patient signed an informed consent form which included name, age, gender, chief complaint, drug history and past medical history.

A total of 110 dyspeptic patients, who had indication of upper GI endoscopy and did not have exclusion criteria, entered the study after providing written informed consent. 16 pa-

tients were excluded, because either UBT test results or fecal samples or histological examination findings were unavailable. The remaining 94 patients were available for analysis. Exclusion criteria were as follows: taking antibiotics in past 8 weeks, proton pump inhibitors in past 2 weeks or H₂-blocker agents in past 1 week, taking immunosuppressive agents, active GI bleeding, pregnancy, breast-feeding and history of gastrectomy.

When the results of at least 2 tests (except serology) were positive *Helicobacter pylori* infection was considered to be positive. Separation of serology was because of documented lower accuracy of this test in comparison to other tests. This test is not suitable for diagnosis of *Helicobacter pylori*.¹⁴ All of the tests were performed within 2 weeks and no medication was prescribed in this time distance. The description aforementioned tests are as follows:

Rapid Urease Test (RUT): 1-3 biopsies from antrum and body were taken for RUT from each patient before taking samples for histology. Samples were maintained in urea at temperature of 32-37 °C. The RUT results were read within 24 hours.

C-Urea Breath test (UBT):¹⁴ Patients were fasted for 12 hours, brushing the teeth before using meal, composed of 75 mg of C-labeled urea dissolved in 200 ml water. Breath samples were collected in aluminized plastic bag to determine the baseline value before ingestion of urea-water meal and at 30 minutes after ingestion of the meal. Breath samples were analyzed by Infrared spectroscopy (Bioscan Inc., Washington D.C.; Model: B-LC-1000).

Histology: During endoscopy, 4 gastric biopsies (from antrum and body) were taken from each patient. Biopsies were sent to the laboratory in formalin solution for histological examination (Hematoxylin and eosin stain and modified giemsa stain).

Serology: Patient's blood samples were taken in the reference laboratory for *Helicobacter pylori* antibody examination (Vitek Immunodiagnostic Assay System-VIDAS-method).

Stool Antigen test: Stool specimens were taken in reference laboratory and tested using

the stool antigen test kits (Rapid Immunoassay method, GA GENERIC ASSAYS GmbH, Germany).

Statistical analysis was performed using SPSS software version 18. Using the results of Helicobacter pylori infection as defined based on Helicobacter pylori status, sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under a Receiver Operating Characteristic (ROC) curve for each test were determined (with 95% confidence interval) and were compared with each other. P-value < 0.05 was considered significant.

Results

There were 39 males and 55 females, age range was from 20 to 72 years and mean age was 46.14 years. 37 patients out of the 94 (39.3%) participants were positive for Helicobacter pylori, by using predefined criteria.

A ROC curve was generated for each test by plotting the true-positive rate (sensitivity) against the false-positive rate (1-specificity). The results from ROC curve analyses are shown in Figures 1 to 5. According to the predefined criteria, the sensitivity, specificity, positive predictive value (PPV), negative predic-

tive value (NPV), accuracy and area under ROC curve for each test are shown in table 1.

Discussion

H.pylori colonizes the human stomach during childhood¹⁷ and survives in the human stomach for the lifetime of the carrier.¹⁸ In most of the individuals H.pylori infection may be asymptomatic, causing chronic gastritis. Around 20% to 30% of the infected individuals may develop peptic ulcer disease,¹⁹ and less than 2% gastric may develop cancer.⁵ Therefore, diagnosis of helicobacter pylori and detection of the best method for diagnosis is very important. Accuracy of each method must be determined in any society separately, because bacteria spp. and the fact that methods of laboratory tests are different in each society. In Iran, there has been no major study with the aim of determination of H.Pylori diagnostic tests accuracy till now. As Helicobacter pylori is a very common infection in developing countries such as Iran, studies on cost-effectiveness of diagnostic methods and follow up of infected patients after treatment, needs a basic study on accuracy of diagnostic methods. We decided to perform this study in which, sensitivity, specificity and accuracy of 5

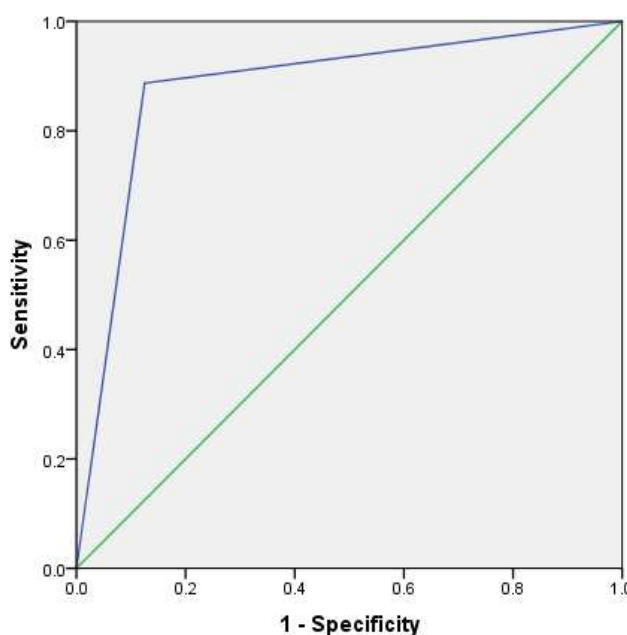


Figure 1. ROC curve of histology for H. pylori infection diagnosis

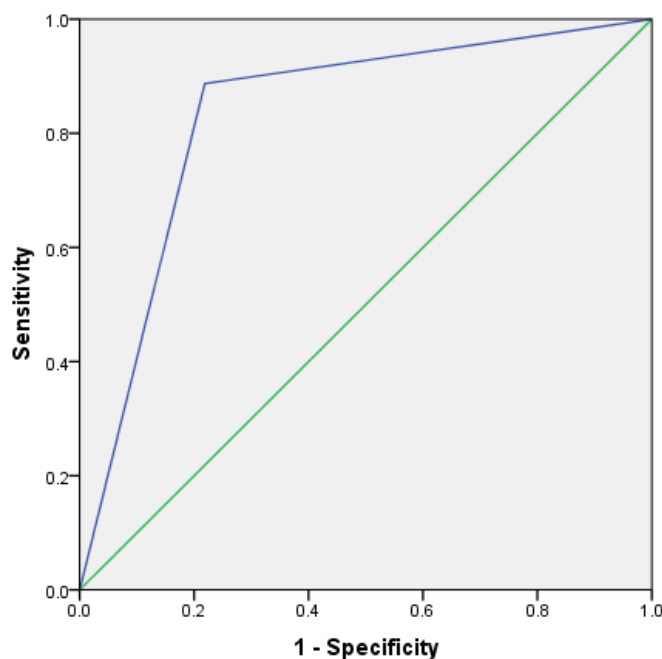


Figure 2. ROC curve of urea breath test (UBT) for *H. pylori* infection diagnosis

routine diagnostic tests of *H. Pylori* in Iran were determined and compared.

As expected, serology had the lowest accuracy which makes it not suitable for primary diagnosis of helicobacter pylori. Results of histology and RUT were relatively the same as other studies.⁶⁻⁸

Sensitivity and specificity of UBT was 89% and 73% respectively, which are unexpectedly low values for UBT, the most striking finding of this study. UBT has been shown to be as reliable as histological examination if adequately performed. The poor performance of the test in our study may be attributable to 3 main reasons.

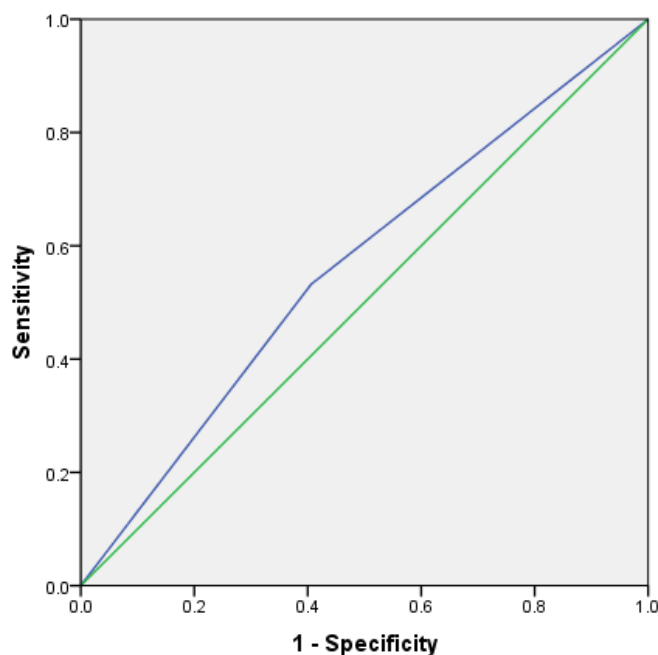


Figure 3. ROC curve of serology for *H. pylori* infection diagnosis

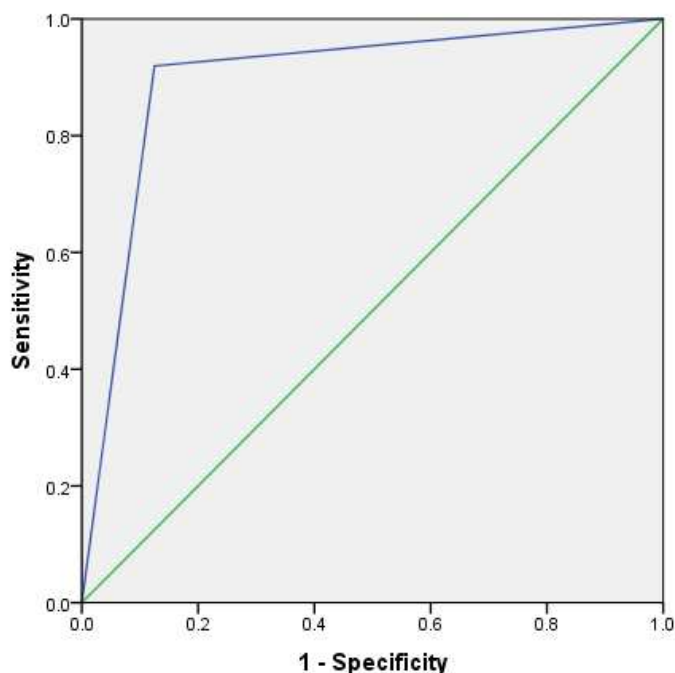


Figure 4. ROC curve of stool Antigen test for *H. pylori* infection diagnosis

First, the majority of *Helicobacter pylori* spp. in our society may be different from other societies where UBT accuracies were higher. This question may be answered by the major basic pathological studies on *Helicobacter pylori* in our society. Second, the drugs which induce false negative UBT may be used by patients in

the short time before performance of UBT. Third, American kits by Bioscan manufacture that were used for UBT in our study do not use concomitant citric acid in its meal. Most studies evaluating the need for citric acid in UBT showed higher delta values with citric acid when compared with other tests.²⁰⁻²³ Citric acid

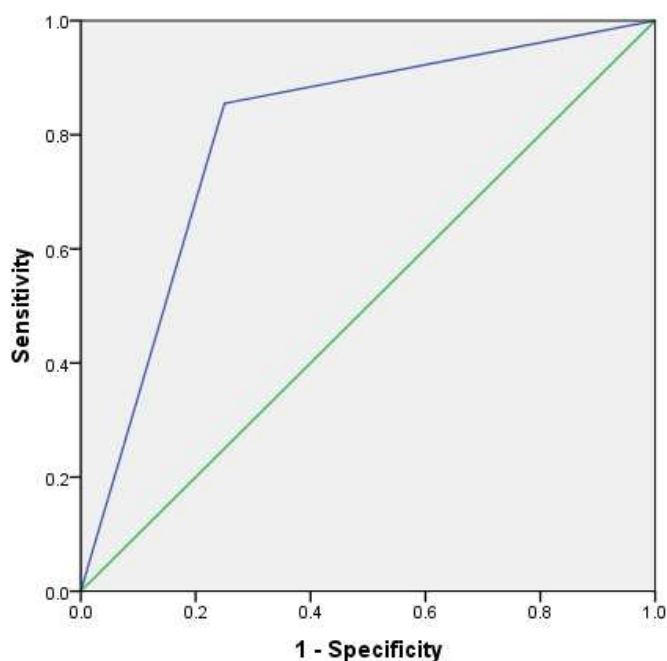


Figure 5. ROC curve of urease breathe test (UBT) for *H. pylori* infection diagnosis

Table 1. Diagnostic characteristics of 5 Helicobacter pylori diagnostic tests (P-value < 0.05)

| Result | Test | Histology | RUT [§] | Serology | Stool Antigen Test | UBT [¶] |
|--------------------------|------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Sensitivity | | 89% | 93% | 50% | 96% | 89% |
| Specificity | | 78% | 75% | 54% | 83% | 73% |
| PPV [†] | | 93% | 95% | 46% | 98% | 92% |
| NPV [‡] | | 91% | 94% | 61% | 96% | 90% |
| Accuracy | | 85% | 86% | 52% | 91% | 82% |
| area under the ROC curve | | 0.839 (0.748-0.930)* | 0.843 (0.752-0.935)* | 0.525 (0.405-0.645)* | 0.901 (0.826-0.977)* | 0.812 (0.715-0.909)* |

§ Rapid Urease Test

¶ Urea Breath Test

† Positive Predictive Value

‡ Negative Predictive Value

* 95% confidence interval

is expected to increase delta values in infected patients and not change delta values in uninfected ones. Adding citric acid may, therefore, well increase the discriminative capacity of the test, reducing the number of patients with borderline delta values.^{21, 23} However, the improvement is expected to be modest. Citric acid would probably upgrade the results of the test from good to excellent by improving the discriminative power of UBT in the 5-15% of patients with borderline delta values.^{21, 23} Therefore, our results strongly suggest that the UBT should be modified by including citric acid co-administration to improve its diagnostic accuracy. Additional studies are necessary to evaluate the need for citric acid to improvement of the diagnostic reliability of UBT.

This study also showed that stool antigen test can be a very good standard for diagnosing H. pylori infection. This test was the only test with accuracy > 90%, making it very suitable for the use in clinical practice. However, this result applies only to this particular kit. The efficacy of stool tests for detecting H. pylori infection depends greatly on the antigen selected for detection. Indeed, it was shown that polyclonal antibody tests, which have different antigenic composition, showed very large variability^{24, 25} and poor reliability.^{26, 27} Overall, the results are far less reliable than

those of monoclonal antibody stool tests.²⁶ In addition, not all monoclonal antibody tests detect the same antigen. Hence, genetic variations of H. pylori strains could lead to geographical variations in diagnostic efficacy. Their usefulness should, therefore, be tested regionally. Finally, the method of detection of the antigen is also important; immunoassays are more reliable than in-office immunochromatographic tests.

Advantages of this study were including all 5 available diagnostic tests and performing these tests prospectively within a short time interval (2 weeks) which decreased the rate of interactions that may have made the tests false positive or negative. A limitation of the study was that we could not fully analyze the reasons for the poor performance of the UBT. The likely efficacy of adding citric acid to improve test results should be tested in additional studies.

In conclusion, our study showed that stool antigen test can be a very suitable test for diagnosing H. pylori infection instead of UBT in non treated patients. It can be considered as a noninvasive first-line routine diagnostic test in our region. More studies are needed to evaluate accuracy of these tests in follow up of treated patients.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

All the authors have carried out the study, participated in the design of the study and acquisition of data performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

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