Diagnostic Accuracy of a Monoclonal Antibody-based Stool Antigen Test in Helicobacter Pylori Infection in Iranian Patients

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Abstract: Helicobacter pylori has been associated with gastritis, peptic ulcer disease and gastric cancer. It is a curved and spiral Gram-negative microaerophilic bacillum. H. pylori infection occurs in all countries and its prevalence varies among different population groups. The purpose of this study was to evaluate the diagnostic accuracy of a monoclonal antibody-based stool antigen test in Helicobacter pylori infection in Iranian patients. A total of 148 patients entered the study and all of the four tests were performed for each patient. When the results of at least two tests of histopathology, rapid urease test and stool antigen were positive, H. pylori infection was confirmed. The accuracy, sensitivity, specificity, area under receiver operating characteristic (ROC) curve, positive predictive value and negative predictive value of these four tests were determined. Results showed that: The patients included 76 males and 72 females with a mean age of 42.4±10.3 years. The accuracy, sensitivity, specificity, positive predictive value, negative predictive value and area under ROC curve of these four tests are, respectively for histopathology: 95.3, 94.7, 97.1, 99.1, 85% and 0.912; RUT: 85.1, 84.1, 91.4, 96.9, 64% and 0.827; stool antigen test: 89.2, 91.2, 94.5, 96.4% and 0.889; serology 84.5, 92.9, 57.4, 71.4% and 0.623. We can concluded that the monoclonal antibody-based H. pylori stool antigen test has high accuracy and is a non-invasive, reliable and convenient method in the primary diagnosis of H. pylori infection.

Key words: Helicobacter pylori · Histopathology · Rapid Urease Test · Stool Antigen Test

INTRODUCTION

H. pylori belongs to a genus of spiral-shaped Gram-negative bacteria named Helicobacter [1]. In tissues H. pylori lies close to the gastric epithelial cells. Their morphology can facilitate motility in the mucus layer of the stomach [2]. H. pylori is microaerophilic, which means that it prefers a reduced amount of oxygen for growth [3]. The bacterium infects more than half of the population of the world and is more common in developing countries [4]. Infection may be asymptomatic or result in varying degrees of dyspepsia and in some people it causes gastritis, peptic ulceration or gastric cancer [5]. Because of the potentially subsequences of infection, accurate diagnosis and appropriate therapy are important.

H. pylori can be diagnosed by invasive or noninvasive methods or both [6]. Endoscopic biopsy of gastric mucosa is the common invasive method; the ability of H. pylori to produce urease allows to rapidly detecting the organism in gastric biopsy material. Noninvasive tests are serologic tests that detect serum antibodies to H. pylori, urea breath tests (UBT) and fecal antigen tests [7]. According to guidelines of the Infectious Diseases Society of America (IDSA) and American Gastroenterology Association (AGA), serologic testing should not be used to diagnose active H pylori infection since the presence of antibodies may simply reflect past exposure to the organism rather than contemporary infection [8]. Predication of cure requires two negative tests (UBT and fecal antigen), at 4 weeks.
and at 6 to 8 weeks after treatment [9]. The purpose of this study was to evaluate the diagnostic accuracy of monoclonal antibody-based stool antigen test in Helicobacter pylori infection in Iranian patients.

**MATERIALS AND METHODS**

**Patients and Samples:** The study involved 314 consecutive patients with symptoms of recurrent burning pain (indigestion) in upper abdomen, nausea, vomiting and feeling of fullness after eating who were referred to the Imam Reza poly-clinic of Arak University of Medical Sciences from March 2013 to February 2014 to undergoing gastro-duodenoscopy with biopsy. Finally, one hundred forty eight dyspeptic patients, who did not have exclusion criteria, entered the study after providing written informed consent. Exclusion criteria were the use of H2-blocker, proton pump inhibitors or antibiotics during the four weeks prior to examination, active GI bleeding, breast-feeding, pregnancy, taking immunosuppressive drugs and history of gastrectomy. This study was approved by the Research Council of Faculty of Basic Sciences in Islamic Azad University of Qom.

**Definition of H. pylori Status:** H. pylori infection was defined when the results of at least two tests of histopathology, rapid urease test and stool antigen were positive simultaneously. A negative H. pylori infection was confirmed when all invasive tests were negative.

**Rapid Urease Test (RUT):** A rapid urease test was obtained by adding a biopsy specimen to a urea broth (Urea test broth is prepared by adding 9.5 g of Na2HPO4, 9.1 g of KH2PO4, 20 g of urea, 0.01 g of phenol red and 0.1g of yeast extract.) The result of the test was considered positive if there was a change of urea broth color.

**Histopathology:** Endoscopy was performed using Olympus videoscope QX140 or GF100 (Olympus, Tokyo, Japan). Several biopsy specimens from 2 sites (the gastric body and the antrum) were obtained for histologic examination and urease rapid test. For histopathology gastric biopsy specimens were stained by modified Giemsa and hematoxylin-eosin. All sections were randomly renumbered before examination by observer and were assessed separately without knowledge of previous results.

**Stool Antigen Tests Based on Immunoassay:** Fecal samples were stored at -20°C until analyzed. A monoclonal antibody-based enzyme-linked immunosorbent assay (FemtoLab H. pylori Cnx; Connex, Martinsried, Germany) was used to detect H. pylori-specific antigen in stool samples by a modified method. Color developed in the presence of bound enzyme and the results were interpreted spectrophotometricaly (450 and 630 nm, double wavelength). According to manufacturer's guidelines, an optical density (OD) of <0.150 was defined as negative for H. pylori and an OD of >0.150 was considered a positive test result.

**Serology:** Patient's blood samples were taken in the reference laboratory for anti H. pylori antibody (IgG, IgA) examination by ELISA method (Biotek microplate reader ELX800).

**Statistics:** All analyses were performed using the SPSS (ver.18) software. Sensitivity and specificity were combined into a single parameter, positive and negative predictive values, the likelihood ratio (LR) and the area under the ROC curve. Additionally, the accuracy was calculated as well as the corresponding 95% confidence intervals for all tests.

**RESULTS**

Totally, 148 patients were included and 166 patients were not enrolled, because of unwillingness to participate in study or the presence of exclusion criteria. The patients included 76 males and 72 females with a mean age of 42.4±10.3 years (range, 19-76 years).

Among the patients 64.2, 27.7 and 14.2% had a history of abdominal fullness and burning pain, gastero-esophasial reflux and peptic ulcer, respectively. Among 148 patients who were eligible for analysis, 113 had H. pylori infection according to the definition of H. pylori status. Prevalence of H. pylori infection was thus 76.4% in the study population. The accuracy, sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio and area under ROC curve of these tests have been shown in Table 1 and Figure 1.
Table 1: Diagnostic characteristics of four Helicobacter pylori diagnostic tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV$^1$</th>
<th>NPV$^2$</th>
<th>LR$^+$</th>
<th>LR$^-$</th>
<th>Accuracy</th>
<th>AUC$^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology</td>
<td>94.7</td>
<td>97.1</td>
<td>99.1</td>
<td>85</td>
<td>33.1</td>
<td>0.05</td>
<td>93.5</td>
<td>0.912</td>
</tr>
<tr>
<td>Rapid Urease Test</td>
<td>84.1</td>
<td>91.4</td>
<td>96.9</td>
<td>64</td>
<td>9.8</td>
<td>0.17</td>
<td>85.8</td>
<td>0.827</td>
</tr>
<tr>
<td>Stool Antigen Test</td>
<td>91.2</td>
<td>82.9</td>
<td>94.5</td>
<td>74.4</td>
<td>5.3</td>
<td>0.11</td>
<td>89.2</td>
<td>0.889</td>
</tr>
<tr>
<td>Serum Antibodies</td>
<td>92.9</td>
<td>57.4</td>
<td>87.5</td>
<td>71.4</td>
<td>2.2</td>
<td>0.12</td>
<td>84.5</td>
<td>0.623</td>
</tr>
</tbody>
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1- Positive Predictive Value 2- Negative Predictive Value 3- Positive Likelihood Ratio 4- Negative Likelihood Ratio 5- Area under Curve

Fig. 1: ROC curve of histology (Black), stool antigen test (Red), rapid urease test (Blue) and serology (Green) for H. pylori infection diagnosis

**DISCUSSION**

*H. pylori* colonizes the human stomach and survives for a long time [10]. Diagnosis of helicobacter pylori and detection of the simple and accurate method for diagnosis is very important. Because *H. pylori* spp. and the methods of laboratory tests are different in each area validity of each method must be determined in each region [11]. *H. pylori* infection has 20 – 50% prevalence in middle age adults in developed countries and >80% prevalence in middle age adults in developing countries [12].

Choi *et al.* [13] reported that the sensitivity of histology and rapid urease test, ranged from 89.1 to 97.6% and their specificity was > 98%, while serology had high sensitivity, but low specificity. They showed that sensitivity, specificity, positive and negative predictive values and accuracy of the stool antigen test were 93.1, 94.6, 95.1, 92.3 and 93.8%, respectively.

Khalifehgholi *et al.* [14] showed RUT has the highest specificity (100%) and the specificities of stool antigen test, histology and serology, were 86.7, 77.8 and 55.6%, respectively, the highest sensitivity (95.6%) belonged to histology and RUT. The sensitivities of serology and stool antigen test were 91.3 and 73.9%, respectively.

Kazemi *et al.* [15] reported that the sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under ROC curve of 4 tests were respectively as, 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.

Redéen *et al.* [16] reported the sensitivity was 99% for serology, 90% for RUT and histological examination. The specificities were 82, 98 and 97%, respectively. The accuracy was 86% for serology, 95% for RUT, 93% for culture and 95% for histology.

A meta-analysis demonstrated that ELISA-based stool antigen test using monoclonal antibodies is highly accurate for the initial and post-treatment diagnosis of *H. pylori* infection [17].

In the current study, sensitivity and specificity of stool antigen were 91.2 and 82.9% respectively, which has been shown to be as reliable as histological examination. The accuracy was about 90% and its easiness makes it very suitable for the use in clinical practice particularly in pediatrics and geriatrics. The efficacy of stool tests for detecting *H. pylori* infection depends greatly on the antigen selected for test. Indeed, it was shown that polyclonal antibody tests, which have different antigenic compounds, showed very high variability and low reliability and their results are far less reliable than those of monoclonal antibody stool tests [18]. Of course, not all monoclonal antibody tests detect the same antigen and genetic variations of *H. pylori* strains could lead to regional variations in diagnostic validity [19]. For this reason, their usefulness should be tested regionally, as well as the method of detection of the antigen is also important because immunoassays are more reliable than in-office immune-chromatographic tests [20].
CONCLUSIONS

Our study showed that stool antigen test can be a very suitable test for diagnosing \textit{H. pylori} infection and can be considered as a routine diagnostic test in patients. The use of monoclonal antibodies has improved the accuracy of this test but more studies are needed to evaluate accuracy of stool antigen test in follow up of treated patients. We concluded that the monoclonal antibody-based \textit{H. pylori} antigen stool test is a reliable and convenient method in the primary diagnosis of \textit{H. pylori} infection.

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REFERENCES


