Original Article

Determination of *Helicobacter pylori* Antigen in Stool Samples and Comparison with Rapid Urease Test in Patients Suspected of Helicobacter Infection

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A B S T R A C T

Background and Aims: *Helicobacter pylori (H. Pylori)* is the microorganism that infects nearly half of word's population. There are several invasive and non-invasive methods for diagnosis of infection. The main objective of this study was to evaluate antigen of *H. Pylori* in feces with *H. pylori* stool antigen (HpSA) test and comparison with rapid urease test (RUT) in the patients suspected to be infected.

Material and Methods: one hundred thirty-seven subjects (56 males, 81 females) were recruited from those patients undergoing a gastrointestinal endoscopic examination in the endoscopy units of Shahid Sadoughi university of medical sciences. One biopsy specimen was obtained from the stomach and each biopsy specimen per subject was tested for the presence of urease using the commercially available CLO test. Stool specimens were taken concurrently with the endoscopic examination and tested by Enzyme-linked immunosorbent assay (ELISA) method for presence of HpSA. In this study, RUT was considered as a gold standard test.

Results: The mean age was 40.4 ± 1.12 years. 13.3% of samples were shown HpSA-positive and negative RUT and 12.9% were shown HpSA-negative and positive RUT. Sensitivity and specificity of HpSA test was 86.6% and 87.1%, respectively. Positive and negative predictive values and accuracy were 89%, 84.3%, 86.2%, respectively.

Conclusions: Our findings showed that the stool enzyme immunoassay for *H. pylori* is a useful method for the primary diagnosis of *H. pylori* in the patients suspected to be infected.

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Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped microaerophilic bacterium, found mainly in the lining of the human stomach, duodenum and esophagus. *H. pylori* is not natural gastric flora, but may also cause chronic inflammation in infected individuals. The bacteria can be spread through person to person and mainly via the fecal-oral route. Entering the bacteria and damage to the gastric mucosa can lead to diseases such as peptic ulcers and stomach cancer [1, 2]. *H. pylori* can be colonized in the antrum (the distal thick and strong stomach muscles) and may cause a long-term infection. In fact, the only bacteria that can live and grow in the harsh environment of the stomach with the presence of gastric juice is *H. pylori*. The prevalence of infection with this bacterium in the middle age adults is over 80% in developing countries and in many industrialized countries is 20-50% [2]. The immune system is activated to deal with *H. pylori* challenge. Mucus thickness of stomach prevents the entry of immune cells. Due to the involvement of immune cells with *H. pylori*, immunoglobulin (Ig)G and IgA antibodies increase in blood plasma. Although *H. pylori* is not a direct cause of ulcers, but the inflammation of the stomach lining layer which is caused by it, may causes gastritis, ulcers, mucosal lymphoid tissue lymphoma, and gastric cancer [3, 4].

There are several invasive and non-invasive methods for diagnosis of *H. pylori* infection. Invasive techniques include histology, culture and rapid urease test (RUT) requiring endoscopy to obtain samples of gastric mucosa.

Noninvasive methods for the detection of *H. pylori* infection are urea breath test, serologic tests, stool antigen test by the enzymatic method, and by immunochromatography, which based on the analysis of blood or feces samples [3]. Most of the patients over 45 years of age and those with "danger signs" symptoms such as weight loss or bleeding require endoscopy and gastric malignancy so that invasive tests are needed. Non-invasive tests are useful for early detection or to monitor the success or failure of treatment. These methods are also used in the patients who could not endure the pain caused by endoscopy such as pediatric population and epidemiological studies. However, non-invasive diagnostic methods are considered as a global test. Previous studies have reported wide variations in the sensitivity and specificity of non-invasive diagnostic methods that most likely due to changes in the type of antibody, the different prevalence of *H. pylori* infection and different definitions of the standard method. Sensitivity and specificity of *H. pylori* stool antigen (HpSA) test by the enzymatic method before treatment have been reported 85-100%, and 66.6-97.8%, respectively. On the other hand, according to the reports, the sensitivity and specificity of immunochromatography method is 52.5-95.0% and 55.5-96.0%, respectively. RUT is the most common test for detection of *H.pylori*. The test has been reported to be sensitive (85%-95%) and specific (98%-100%). Its main advantage is high specificity, but its limitations are low sensitivity. The quality of the biopsy specimen is the

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probable cause of reducing the sensitivity and specificity of RUT, for example, a biopsy sample contamination with blood, stomach acid, and bile reflux reduces the sensitivity and specificity of the RUT. It should be noted that drugs such as antacids and proton pump inhibitors reduce the sensitivity of the test [5-8].

In the year 2000, it is shown that two non-invasive tests, urea breath test and HpSA test can be effective, convenient, and affordable and represent methods for detecting infection with *H. pylori* in the patients younger than 45 years without signs. RUT is useful, but it is expensive and may be required travel time to the hospital and the procedure is complicated for children. Benefits of HpSA test can be early detection and primary care and the method is simple and relatively inexpensive. According to some studies, sensitivity of the HpSA test by the enzymatic method was 92.1% and specificity was 91.9%, respectively [9-11].

The main objective of this study was to determine antigens of *H. pylori* in feces using an enzyme immunosorbent assay (ELISA) compared to the RUT in patients suspected to be infected.

Materials and Methods

One hundred and thirty-seven subjects (56 males, 81 females) were recruited from those patients undergoing a gastrointestinal endoscopic examination in the endoscopy unit at the laboratories of Shahid Sadoughi university of medical sciences during one year. The Ethical Committee of Shahid Sadoughi university of medical sciences, Yazd, Iran approved the study and the written informed consent was obtained

from each sample. All subjects were categorized according to age, and sex. A detailed medication history, including the use of nonsteroidal antiinflammatory drugs, antacids, Omeprazole, as well as antibiotics, was obtained. In order to be eligible for this study, the patients with non-specific dyspeptic complaints should not have been treated with antibiotics in the last four weeks, also not have been treated with nonsteroidal antiinflammatory drugs, antacids and should not have had a gastric surgery.

Endoscopy (Olympus GIFlT20) was performed under intravenous sedation (diazepam or midazolam), and the endoscopic findings were recorded. One biopsy specimen was taken from the stomach and each biopsy specimen per subject was tested for the presence of urease using the commercially available kit (CLO test, Tri-Med Specialties, Lenexa, KS). Subjects were classified as having a positive test if there was a change in color from yellow to orange, red or fuscia within 24 hour after obtaining the sample. Stool specimens were taken concurrently with the endoscopic examination and tested with a commercially available ELISA method (HpSA, GENERIC ASSAYS, GmbH, Germany).

Statistical analysis

In this study, RUT was considered as gold standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and 95% confidence intervals (CI) were calculated by standard methods.

Result

All samples were 137 with the mean age 40.4±1.11 years. Basic demographic characteristic of the samples are shown in table 1. HpSA and

RUT were positive in 86.7% of cases and in 87.1% of cases both tests were negative. 13.3% of samples results are shown HpSA-positive and negative RUT and 12.9% are shown HpSA- negative and positive RUT. The sensitivity of HpSA was 86.6% and specificity 87.1%. The results are presented in table 2.

Table1. Basic demographic characteristic of the participating subjects

RUT	Age (Mean ±SD)	Sex		Total
		Male	Female	
Positive	42.1±1.09	25 (44.6%)	48 (59.3%)	73 (53.3%)
Negative	38.9±1.12	31 (55.4%)	33 (40.7%)	64 (46.7%)

Table 2. Comparison of rapid urease test and stool antigen test in *H. pylori* infection diagnosis

RUT	HpSA		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Accuracy (%)
	Positive	Negative					
Positive	65	8	86.6%	87.1%	89%	84.3%	86.2%
Negative	10	54	(76.3-93)	(75.5-93.8)	(79-94.8)	(72.6-91.8)	

RUT= Rapid urease test; HpSA= *H.pylori* stool antigen; CI= Confidence intervals;
PPV= Positive predictive value; NPV= Negative predictive value

Discussion

In south and east Europe, south America, and Asia, the prevalence of *H. pylori* is often higher than 50% and in north European and north American the populations about one-third of adults are still infected so *H. pylori* is known as a common infection in the world [12, 13]. There are various diagnostic tests, including direct (invasive) and indirect (noninvasive) to detect the infection. Invasive methods to detect *H. pylori* infection such as histology, fluorescent in situ hybridization on histological preparations, culture, RUT, polymerase chain reaction (it can be performed on samples obtained by both invasive and noninvasive methods) and noninvasive methods such as serology, stool antigen test. A simple, low-cost, and accurate method is needed to diagnose the infection [14-16]. If sensitive, simple assay for diagnose *H. pylori* infection was possible using stool samples, it would be more convenient in clinical practice.

The present study has demonstrated high accuracy of *H. pylori* stool antigen enzyme immunoassay test compared to rapid urease test. Sensitivity of HpSA test was 86.6% and specificity was 87.1%. Furthermore, the test does not require technical expertise, special sample handling, or any additional equipment and thus allows considerable savings of diagnosis-related costs. It is a reliable method to diagnose an active infection and to confirm an effective treatment of infection, but may be affected by disorders of the digestive tract, proton pump inhibitors treatment, or the

presence of a bleeding ulcer [16]. A recent study compared the ability of three stool antigen test (SAT), Premier Platinum HpSA, FemtoLab Cnx, and HpAg stool antigen kits to detect *H. pylori* when compared with biopsy based diagnosis, the sensitivity, specificity of these three SAT methods compared with biopsy based on diagnosis were 63.6%, 88.0%, and 56.0% and 92.6%, 97.6%, and 97.6%, respectively [17]. Another study evaluated SAT method (Premier Platinum HpSA, Meridian Diagnostics, Cincinnati, OH) and 13C-urea breath test (13C-UBT) invasive technique for their ability to diagnose *H.pylori* infection in children. Their results revealed a sensitivity of 88.9% and a specificity of 94.0% for the HpSA compared to the 13C-UBT, 100% and 98.9%, respectively [18].

In this study, no equivocal result for the HpSA test was obtained from the patients and sensitivity, specificity was 86.6% and 87.1%, respectively, which is comparable to a part of a previous study result that compared the accuracy of the HpSA test with the various invasive tests using culture, histology and the RUT before eradication therapy of *H. pylori* infection and the sensitivity and specificity of the HpSA test was 98.3% and 95.0%, respectively [19]. Our results were in contrast with the results of another study in Iran that showed the sensitivity of HpSA test before

treatment in comparison with RUT in 61 patients was 65.8% and specificity was 91.3% [20]. In another study in Tabriz, Iran, they compared three diagnostic methods (histology, serological test and HpSA), HpSA sensitivity and specificity was 54.8% and 79.4%, respectively [21]. For explanation of these differences as mentioned in their article, storage and transportation, decrease in gastric bacterial load of the patients due to overconsumption of antibiotics in Iran, quality control and human error in laboratory performance are most likely to be considered, but in our study all these problems were reduced to a minimum.

Conclusions

In conclusion, the stool enzyme immunoassay for *H. pylori* is a useful method for the primary diagnosis of *H. pylori* in the patients. It delivered sufficiently accurate results and showed itself to be a suitable and cheaper alternative to invasive tests, thus the test seems suitable for both epidemiological studies and clinical purposes.

Conflict of Interest
The authors declare no competing interest.

Acknowledgments

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