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ORIGINAL ARTICLE

Evaluation of diagnostic value of Helicobacter pylori stool antigen test before and after eradication

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ABSTRACT

Aim: To evaluate the diagnostic value of Helicobacter pylori stool antigen test before and after eradication

Background: There are different methods for diagnosis and follow up of Helicobacter pylori infection, including invasive methods done with endoscopy and non invasive ones such as UBT and urine analysis antibody. One of non invasive new methods is analysis of Helicobacter pylori stool antigen, used for primary diagnosis and post eradication follow up.

Patients and methods: A total of 54 patients with epigastric discomfort referred to Taleghani hospital for upper gastrointestinal endoscopy were enrolled in this study. Helicobacter infection was confirmed with rapid urease test, culture and histological methods. Gold standards for positive infection were positive culture or positive RUT and histology. Patients with positive infection (26 patients) were treated with omeprazol, tetracycline, metronidazol, bismuth subsalsylate, for 2 weeks, and then 1 and 3 month later were reevaluated with gold standard tests and Helicobacter pylori stool antigen test.

Results: Sensitivity, specificity and accuracy of Helicobacter pylori stool antigen test were 78.6%, 92.3%, and 85.2% respectively before eradication, 100% and 56.5% and 61.5% 1 month after eradication and 85.7%, 89.5% and 88.5%, 3 months after treatment.

Conclusion: Helicobacter pylori stool antigen test is an excellent method for pretreatment diagnosis of Helicobacter pylori infection, and also in 3-month follow up of eradication, but the specificity and accuracy were low in early period follow up.

Keywords: *Helicobacter pylori stool antigen, Mucosal associated lymph tissue (MALT), Rapid urease test (RUT), Urea breath test (UBT), Polymerase chain reaction (PCR).*

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INTRODUCTION

One of the most important infections of Gastrointestinal (GI) system is induced by Helicobacter pylori. This infection causes a broad spectrum of disorders such as gastritis, duodenum ulcer, gastric ulcer and gastric cancer in the form of lymphoma or mucosal associated lymphoid tissue (1).

Discovering this bacterium provoked a great change in management and treatment of GI diseases, thus many health care centers are focused on diagnosis of this infection (1).

There is a common understanding that Helicobacter infection is acquired in childhood

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and teenage and is distributed from one country to another, therefore diagnosis of infection in children is important. Overall, there are two means of diagnosis of Helicobacter infection, which consist of invasive and non-invasive methods (2). Invasive methods are limited to the tests that require endoscopic sampling and consist of histological and rapid urease test which are not well tolerated by patients (3).

On the other hand there are non-invasive tests, such as respiratory urease test (RUT), serology, polymerase chain reaction (PCR) and Helicobacter pylori stool antigen test which do not require endoscopy. Each of these methods has advantages and disadvantages and they are not affected by inappropriate sample collection. Helicobacter pylori stool antigen test is introduced as a highly sensitivite and specific test (4, 5).

In this study we evaluated the results of this new diagnostic method for pre and post treatment period of Helicobacter pylori infection and its utilization in the form of simple, fast and accurate test in diagnosis (5).

PATIENTS and METHODS

A total of 54 patients were included in this study. They were admitted in endoscopic department of Taleghani hospital with dyspepsia and upper GI symptoms during a two-year period (2006-2007). All patients' data were collected in specific questioners containing demographic, clinical, endoscopic and laboratory results. The study was approved by the ethics committee of Research Institute for Gastroenterology and Liver Disease and all patients were asked to sign an informed consent before participating in the study.

Exclusion criteria were as follows: recent use of antibiotics in the last 2 months and use of anti-acid drugs such as proton pump inhibitors in at least 15 days before treatment.

Helicobacter pylori infection was diagnosed by taking two biopsy samples from gastric antrum, one for culture and the other for rapid urease test and one or two more samples for histological examination. All samples were taken by an expert gastroenterologist.

All samples for rapid urease test were kept up to 24 hours and studied in this period.

The samples were incubated in Taio medium and sent to microbiology laboratory of Health faculty (Shahid Beheshti university, M.C., Tehran, Iran) immediately for culture.

Culture was performed from biopsy sample in Campilobacter medium and a smear was prepared. Results were observed after 4 days.

Biopsy samples were sent to pathology department for making block sections and staining for Helicobacter pylori (Giemsa staining method). Stool sample of each patient was taken in endoscopy department and sent to immunology department soon after, to be prepared and frozen for the next study.

ELISA (Enzyme-Linked ImmunoSorbent Assay) test was utilized to identify Helicobacter pylori antigen in stool. The commercial set of HPAGT (Generesis, England) was performed according to the manufacturer's protocol. Optic density was measured with a 450 nanometer filter. In this test, values equall to and greater than 9 ng/milliliter were marked as positive and less than 9 ng/milliliter marked as negative.

In 26 patients who had received quadruple therapy (consisting of metronidazole, bismuth subcitrate, tetracycline and omeprazole), standard tests were performed after one month and three month, including rapid urease test, culture, histology and Helicobacter pylori stool antigen test (HPSAT).

For comparison of results , the gold standard test was positive rapid urease test and histology together or culture alone for confirmation of the true positive and negative cultures and RUT and histology together for true negative results .[6]

Statistical analyses were performed using Fisher's exact test and linear association of Mentel

Haeszel. Sensitivity, specificity and predictive value of positive and negative results were calculated. A $p<0.05$ was considered statistically significant.

RESULTS

Of 54 studied patients, 21 were 15 to 30 years, 20 were 31 to 50 years and 13 patients were over 50 years. There were equal males and females in each group (27 males and 27 females).

Endoscopic results were as follows: 18 (33.3%) normal, 11 (20.4%) gastritis, 12 (22.2%) gastrodeudenitis, 4 (7.4%) esophagitis, 4 (7.4%) duodenal ulcer, 3 (5.6%) gastric ulcer and 2 (3.7%) duodenitis.

Sensitivity, specificity, PPV (Positive Predictive Value), NPV (Negative Predictive Value) and accuracy of HPSAT before treatment in comparison to other tests were calculated (Data shown in Table 1).

Table 1. Comparison of Helicobacter pylori stool antigen test results with gold standard pre-treatment

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
RUT*	84.6	92.9	91.7	86.7	88.9
Culture	84	89.7	87.5	86.7	87
Biopsy	75	80	75	80	77.8
HPSAT†	78.6	92.3	91.7	79.9	85.2

* Rapid Urease Test
† Helicobacter pylori stool antigen test

All results of HPSAT were compared with standard methods, one and three months after treatment (shown in Tables 2 and 3).

Table 2. Comparison of Helicobacter pylori stool antigen test results with gold standard one month after treatment

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
RUT*	100	54.2	15.4	100	57.74
Culture	100	56.5	23.1	100	61.5
Biopsy	66.7	52.3	15.4	92.3	53.8
HPSAT§	100	56.5	23.1	100	61.5

* Rapid Urease Test
† Helicobacter pylori stool antigen test

Table 3. Comparison of Helicobacter pylori stool antigen test results with gold standard three months after treatment

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
RUT*	100	78.3	37.5	100	80.8
Culture	85.7	89.5	75	94.4	88.5
Biopsy	80	81	50	94.4	80.8
HPSAT†	85.7	89.5	75	94.4	88.5

* Rapid Urease Test
† Helicobacter pylori stool antigen test

DISCUSSION

The main aim of this study was to evaluate HPSAT accuracy, sensitivity and specificity in comparison to endoscopic and other invasive methods.

In our study, sensitivity, specificity and accuracy of HPSAT before and three months after eradication show that this test is acceptable in late follow up after eradication. But in early period after eradication (one month), specificity and accuracy of the test were very low. Makristhatis et al. illustrated that positive results for HPSAT test of dead bacteria in stool (7). Normally, Helicobacter pylori is seen in form of bacillus but as a result of antibiotic therapy or sometimes in unknown conditions, it appear as non infective cocci or coccoid forms that could cause false positive results (8, 9). After eradication, the

proteins of destructed bacterial antigens may cause positive results.

HPSAT may show cross reaction with other Helicobacters that are colonized in human GI system, such as Helicobacter hilmani or Helicobacter pilon (2, 7, 9). In a recent study, the specificity of HPSAT was 68.3% for Helicobacter in one month after eradication. It could be concluded that HPSAT does not have an acceptable accuracy and specificity in short period after eradication. In one prospective multi-center study at eleven European institutes, the sensitivity and specificity of HPSAT were reported 94.1% and 91.8% respectively in late post treatment (after 3 months) (1, 2).

In a study in children, after 2 months the sensitivity, specificity and accuracy were reported 93.9%, 95.7 and 94.8%, respectively (10). In other study, sensitivity, specificity, PPV and NPV were observed 96%, 75%, 80.6 and 75.8%, respectively (11). In last study with similar sample size to our study, sensitivity, specificity, accuracy was 78.6%, 93.2% and 85.2% before treatment and 85.7%, 89.5% and 88.5% three months after eradication (12).

Consequently, the HPSAT is a non invasive, very simple and fast method; therefore we suggest this test as a non invasive with high accuracy, sensitivity and specificity for pretreatment diagnosis and at least three months after eradication follow up. While early follow up with HPAST after eradication needs more investigations.

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