BRAZILIAN JOURNAL OF MICROBIOLOGY

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Medical Microbiology

Investigation of *Helicobacter pylori* antigen in stool samples of patients with upper gastrointestinal complaints

Zeki Calik[a], *Murat Karamese*[a,*], *Osman Acar*[a], *Selina Aksak Karamese*[b], *Yalcin Dicle*[c], *Fatih Albayrak*[d], *Serpil Can*[e], *Bulent Guvendi*[f], *Alpgiray Turgut*[g], *Mustafa Cicek*[h], *Halil Yazgi*[i]

[a] *Department of Microbiology, Faculty of Medicine, Kafkas University, Kars, Turkey*
[b] *Department of Histology and Embryology, Faculty of Medicine, Kafkas University, Kars, Turkey*
[c] *Department of Nursing, School of Health, Mus Alparslan University, Mus, Turkey*
[d] *Department of Internal Medicine, Faculty of Medicine, Ataturk University, Erzurum, Turkey*
[e] *Department of Physiology, Faculty of Medicine, Kafkas University, Kars, Turkey*
[f] *Department of Genereal Surgery, Faculty of Medicine, Kafkas University, Kars, Turkey*
[g] *Department of Biochemistry, Faculty of Veterinary, Ataturk University, Erzurum, Turkey*
[h] *Department of Anatomy, Faculty of Medicine, Gazi Osman Pasa University, Tokat, Turkey*
[i] *Department of Microbiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey*

ARTICLE INFO

Article history:
Received 8 January 2015
Accepted 23 August 2015

Associate Editor: Ana Lúcia da Costa Darini

Keywords:
Helicobacter pylori
Upper gastrointestinal complaints
Rapid urease test
H. pylori card test

ABSTRACT

Helicobacter pylori infection is usually acquired in early childhood and it can persist throughout life without antibiotic treatment. This study aimed to compare the accuracy of the noninvasive *H. pylori* Stool Antigen Test-applied on the stool samples with the invasive gold standart Rapid Urease Test-applied on the gastric biopy samples of patients with upper gastrointestinal complaints. After endoscopy, biopsy and stool specimens were taken in 122 patients. The *infection* was detected with rapid urease test which is accepted as gold standart test. Rapid, one-step *H. pylori* card test was applied to all patients stool specimens. In this study 106 of the 122 patients (86.8%) were positive for *H. pylori* infection, while 16 of the 122 patients (13.2%) were negative. *H. pylori* card test was negative in 13 of the 16 patients and was positive in 98 of the 106. The sensitivity, specifity, positive and negative predictive values were 92.45%, 81.25%, 97.02%, and 61.90%, respectively. *H. pylori* card test is rapid, easy, noninvasive and inexpensive methods for detection *H. pylori* infection. This test showed high sensitivity and specificity. Additionally, it may be a good alternative to invasive tests for the detection of *H. pylori* infections especially in children.

* *Corresponding author.*
E-mail: murat_karamese@hotmail.com (M. Karamese).
http://dx.doi.org/10.1016/j.bjm.2015.11.022

Introduction

Helicobacter pylori (*H. pylori*) is classified as a gram-negative, spiral-shaped bacterium and a microaerophilic, fastidious, human pathogen. *H. pylori* infection is usually acquired in early childhood and it can persist throughout life without antibiotic treatment. It affects about 20% of the population in developed countries and more than 90% in the developing world.[1–4] Oral–oral and fecal–oral are the most common methods of transmission.[5]

H. pylori specifically colonizes on the gastic mucus layer, and it has developed a variety of mechanisms to survive in the harsh acidic environment of the gastric mucosa.[6] *H. pylori* contains many virulence factors that cause the infection and contributes to gastric inflammation.[7] It is a major cause of gastric and duodenal ulcer and gastritis, and the organism has been etiologically associated with Mucosal-Associated Lymphoid Tissue (MALT) lymphoma and gastric carcinoma.[8,9]

Invasive and non-invasive tests are used in the diagnosis of *H. pylori* infection. The invasive methods include culture, histology, and urease tests. Biopsy specimens obtained with upper gastrointestinal endoscopy are necessary for these tests.[10–12] The noninvasive methods include stool antigen test (SAT), urea breath test and serology.[13]

All the tests have advantages and disadvantages. The rapid urease test (RUT) is a gold standard method for the detection of *H. pylori*, and it is faster and cheaper than other invasive tests.[14,15] Proton pump inhibitors (PPIs), bismuth-containing compounds and antimicrobial agents may affect the performance of this test by inhibiting urease activity. In addition, other urease-producing microorganisms in the gastric mucosa can cause false positive results.[12,16] SATs are non-invasive and inexpensive methods to detect active *H. pylori* infection. This test has two versions: enzyme immunoassay and immunochromatography. Eradication of *H. pylori* infection is evaluated by SATs. Therefore this test is useful before and after *H. pylori* therapy.[2,16,17]

This study aimed to compare the accuracy of the noninvasive *H. pylori* Stool Antigen Test (SAT) applied on the stool samples with the invasive gold standart Rapid Urease Test (RUT) applied on the gastric biopy samples of patients with upper gastrointestinal complaints.

Materials and methods

Patient selection and collection of samples

This study was approved by the Local Ethics Committee of Ataturk University, Institute of Health Science with the number of 1466. The subjects were selected from patients with upper gastrointestinal complaints admitted to the Ataturk University, Medical Faculty and Department of Gastroenterology. Of those referred to the endoscopy unit for gastrointestinal endoscopy to evaluate dyspeptic complaints, 122 (49 male, 73 female) were included in this study. Patients taking bismuth preparations, PPIs, H2 receptor antagonists or antibiotics for the last month or taking anti-acids for the last two days were excluded from the study. The first stool samples of all patients were collected immediately after the endoscopy.

Detection of H. pylori in biopsy samples

The RUT (Salubris Helicheck, Boston, USA) was used for the detection of *H. pylori* on biopsy samples in this study. *H. pylori* produce an abundance of urease. The urease enzyme hydrolyses urea to release CO_2 and NH_3. The release of ammonia increases the pH of the medium. The urease activity causes a change in the pH indicator color for positive *H. pylori* results. All the biopsy specimens were taken from the patients during endoscopy and the RUTs were performed by the clinicians according to the manufacturer's protocol.[2,18]

Investigation of H. pylori antigens in stool samples

The rapid, one-step *H. pylori* card test (H+R *H. pylori* CARD, Madrid, Spain) was used to investigate the presence of *H. pylori* antigens in the stool samples. This test is a qualitative immunochromatographic assay for the determination of *H. pylori* in stool samples. The membrane is precoated with monoclonal antibodies, on the test band region, against *H. pylori* antigens. During testing, the sample is allowed to react with the colored conjugate (anti-*H. pylori* monoclonal antibodies-red polystyrene microspheres) predried on the test strip. The mixture then moves up the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. For a positive result, the specific antibodies present on the membrane will capture the colored conjugate. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, where a red band always appears. The presence of this red band serves as; an internal control for the reagents and verification that sufficient volume was added and proper flow was obtained.

The stool samples were evaluated by the card test according to the manufacturer's protocol. A single red band appearing across the central window in the site marked with the control line was considered negative. A red band appearing in the site marked with the result line and in the site marked with the control line was considered positive. A total absence of the control band, regardless of the appearance of the result site was considered invalid.

Statistical analysis

The statistical analysis was performed using SPSS for Windows Version 17.0 (Statistical Package for Social Sciences version 17.0). Positive predictive value, negative predictive value, sensitivity and specificity were evaluated with the following formulas.

Positive predictive value;

$$\text{True positives}/(\text{True positive} + \text{False positive}) \times 100$$

Negative predictive value;

$$\text{True negatives}/(\text{False negatives} + \text{True negatives}) \times 100$$