

RAPID COMMUNICATION

Comparison of invasive methods and two different stool antigen tests for diagnosis of *H pylori* infection in patients with gastric bleeding

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Abstract

AIM: To compare two different *H pylori* stool antigen tests as noninvasive diagnostic methods.

METHODS: The study population consisted of 22 upper gastrointestinal system bleeding patients. Urea breath test (UBT), rapid urease test (RUT) and histopathological examination were applied to all patients. Stool specimens from these patients were examined by rapid STRİP!HpSA and one step simple *H pylori* antigen cassette test for the detection of *H pylori* antigens.

RESULTS: For these 22 patients, 15 (68.2%) were diagnosed as positive and seven (31.8%) were diagnosed negative for *H pylori* infection by the gold standard methods. Whereas 10 (45.5%) were positive and 12 (54.5%) were diagnosed negative by the rapid STRİP!HpSA test. The sensitivity, specificity, positive and negative predictive values were 60%, 86%, 90% and 50%, respectively. When compared to the gold standard methods, these differences were not significant. However, six patients (27.3%) were positive, and 16 (72.7%) were negative by the simple *H pylori* stool antigen cassette test. The sensitivity, specificity, positive and negative predictive values were 33%, 86%, 83% and 38%, respectively. Compared to the gold standard methods, the simple *H pylori* stool antigen cassette test results were significantly different ($P = 0.012$).

CONCLUSION: Rapid STRİP!HpSA test could be used as a routine diagnostic tool in the microbiology laboratory for assessing clinical significance and eradication control of *H pylori* in upper gastrointestinal system bleeding patients.

INTRODUCTION

H pylori is a microaerophilic gram-negative spiral shaped bacterium^[1-3]. It is recognized as the major cause of gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma, however little is known about its role in functional dyspepsia^[4,5]. *H pylori* colonizes the gastric mucosa and attaches to the gastric epithelial cells^[6]. The prevalence of *H pylori* infection is 70%-90% in developing countries and 25%-50% in developed countries^[2,7]. Person-to-person spread is the most probable mode of transmission. Faecal-oral and oral-oral transmissions have been reported^[8]. Chronic *H pylori* infections of the stomach are increasingly recognized as a major risk factor for the development of gastroduodenal disease. *H pylori* can be detected by noninvasive and invasive methods, the latter requiring endoscopy. Noninvasive testing for *H pylori* can be done by measuring exhaled ¹³C urea breath test (UBT), by serology, by stool antigen tests, by a simple stool PCR and by analyzing body materials such as saliva and urine^[2,9]. Although histopathology and culture of the organism, which are not easily and routinely performed, is considered the gold standard for the diagnosis of *H pylori* infection, we need rapid, accurate and reliable noninvasive methods^[2].

In the case of upper gastrointestinal bleeding which is a major cause of morbidity, mortality and medical care costs, peptic ulcer is the most frequent source of bleeding in these patients. Treatment of *H pylori* infection is more effective than antisecretory non-eradicating therapy in preventing recurrent upper gastrointestinal bleeding from peptic ulcer. Consequently, all patients with peptic ulcer

bleeding should be tested for *H pylori*, and eradication therapy should be prescribed to infected patients^[10].

In the presence of upper gastrointestinal bleeding, the diagnosis of *H pylori* infection may be compromised. The UBT is responsible for a high number of false negative results when it is used to diagnose *H pylori* in patients with upper gastrointestinal bleeding. Coagulation disorders or anticoagulation may prevent a biopsy being taken. The UBT may not be feasible in patients on artificial respiration, or in the presence of impaired consciousness or acute abdominal disease. Therefore, it is suggested that noninvasive methods, such as serology or UBT, be used to identify *H pylori* infection in these patients. In some cases, the indication for *H pylori* eradication therapy is based only on a serological test. Serology alone, however, is a rather inaccurate diagnosis method^[10-12]. An ideal noninvasive test for *H pylori* infection should be safe and acceptable to patients, inexpensive and easy to perform, and with a high degree of sensitivity and specificity^[13].

The diagnostic role of HpSA test and simple *H pylori* antigen cassette test in patients with upper gastrointestinal bleeding remains unclear. Only a few reports have discussed the results of the HpSA test and simple *H pylori* antigen cassette test in these patients^[10-12,14]. The aim of this study was to evaluate diagnostic accuracy of stool antigen tests of rapid STRIP!HpSA and simple *H pylori* antigen cassette test other than HpSA in patients with upper gastrointestinal bleeding. We also compared two different *H pylori* stool antigen tests as noninvasive diagnostic methods with UBT, RUT and histopathology as gold standard methods.

MATERIALS AND METHODS

Patients

The study population consisted of 22 upper gastrointestinal system bleeding patients (13 males, 9 females; mean age, 58 \pm 18 years; age range, 20 to 86 years) between September 2004 and January 2005. UBT (Infai, Germany), rapid urease test (RUT) and histopathological examination were applied to all patients.

The diagnosis of *H pylori* was defined as positive for UBT alone or for histopathology and (or) RUT results defined as gold standards. A patient was classified as *H pylori* negative when UBT and (or) histopathological examination and urease test were both negative.

Endoscopy and biopsy sampling

Two antrum and two corpus biopsy specimens were taken from each patient undergoing upper endoscopy from the same location in the stomach: one from the antrum and the corpus. One of these was used for the rapid urease test and the other two were immediately fixed and transported in 10% phosphate-buffered formalin solution for histopathologic examination.

Histopathologic examination of biopsy specimens

Paraffin-embedded gastric biopsy specimens were routinely processed. Haematoxylin-eosin, Alcian blue and Giemsa stains were used for morphologic examination

of *Helicobacter*-like organisms (HLO) and updated Sydney system was used^[15]. Histopathology was performed by a specialized pathologist.

Stool specimens

Stool specimens from these patients were collected and were kept at -20°C until used. They were examined by rapid STRIP!HpSA (Meridian Bioscience Europe) and one step simple *H pylori* antigen cassette test (Linear Chemicals, S.L. Spain) for the detection of *H pylori* antigens. Both tests were performed in accordance with the manufacturer's specifications.

Stool antigen tests

Rapid STRIP!HpSA (Meridian Bioscience Europe) is a rapid immunoassay using a monoclonal anti-*H pylori* antibody on a strip for the detection of *H pylori* infections in stool specimens. The strip is introduced in a tube containing diluted patient samples and the appearance of a pink-red line in the reading area indicates a positive result after 5 min of incubation at room temperature. A positive test result is evaluated according to the blue band (control line), a distinguishable pink-red band (test line) also appears across the white central zone of the reaction strip. Any pink-red line, even very weak, must be considered as a positive result. By contrast, the sample is considered negative when only one blue coloured band (control line) appears across the white central area of the reaction strip.

Simple *H pylori* antigen cassette test (Linear Chemicals, S.L. Spain) is a rapid immunochromatographic test using a single monoclonal antibody. A diluted fecal sample is placed in an immunochromatographic support and the result is read after 5 min. A positive test will display a red line in the reading area next to a blue control line. Even a minimal trace of a red line was considered positive. By contrast, the sample is considered negative when only the control blue line develops. If no line appears in the reading area the test is considered null^[14,16].

RESULTS

The diagnosis of *H pylori* was defined as positive for UBT alone or for histopathology and (or) RUT results defined by gold standard methods. A patient was classified as *H pylori* negative when histopathological examination and urease test were both negative.

For those 22 patients, 15 (68.2%) were diagnosed as positive and seven (31.8%) negative for *H pylori* infection by the gold standard methods. Meanwhile, 10 (45.5%) were positive and 12 (54.5%) were negative by the rapid STRIP!HpSA test. The sensitivity, specificity, positive and negative predictive values were 60%, 86%, 90% and 50%, respectively. When compared to the gold standard methods, these differences were not statistically significant. However, six patients (27.3%) were positive, and 16 (72.7%) were negative by the simple *H pylori* stool antigen cassette test. The sensitivity, specificity, positive and negative predictive values were 33%, 86%, 83% and 38%, respectively. Compared to the results by the gold standard methods, the simple *H pylori* stool antigen cassette test

Table 1 Results of invasive and noninvasive methods for diagnosis of *H pylori* infection in patients with gastric bleeding

	Positive n (%)	Negative n (%)	False positive n (%)	False negative n (%)
Urea breath test	12 (54.54)	10 (45.45)	2 (9.09)	2 (9.09)
Rapid urease test and/or histopathology	15 (68.18)	7 (31.82)	-	-
Rapid STRIP!HpSA stool antigen test	10 (45.45)	12 (54.54)	1 (4.54)	6 (27.27)
Simple <i>H pylori</i> stool antigen cassette test	6 (27.27)	16 (72.73)	1 (4.54)	10 (45.45)

results were statistically different ($P = 0.012$) (Table 1).

DISCUSSION

Many studies have described the use of ELISA-based HpSA stool antigen kits with either polyclonal or monoclonal antibodies, for diagnosis of *H pylori* infections. In this study we evaluated the diagnostic accuracy of two non-ELISA-based kits, rapid STRIP!HpSA and simple *H pylori* antigen cassette, in patients with upper gastrointestinal bleeding.

Meridian Bioscience, Inc. introduced the concept of detecting *H pylori* antigens in stool specimens, with a microtiter based immunoassay, in 1997. Primer Platinum HpSA, after extensive evaluation, was accepted as an accurate tool for non-invasive *H pylori* infection diagnosis^[17-19]. Recent official European Guidelines recommend the use of either stool antigen or urea breath test for diagnosis and confirmation of eradication four weeks after the end of the treatment^[20].

To our knowledge, three recent studies have evaluated the HpSA test in the presence of gastrointestinal bleeding: Gisbert *et al* ($n = 34$ hospitalized patients; sensitivity of polyclonal ELISA, monoclonal ELISA and monoclonal immunochromatographic test was 74%, 94%, 60%, respectively), Lin *et al* ($n = 93$ patients with bleeding peptic ulcers and 59 patients with nonbleeding peptic ulcers; sensitivity 82% and specificity 68%) and Peitz *et al* ($n = 114$ patients; the sensitivity 84% and specificity 90%)^[10-12].

In another study, Erzin *et al* compared two different stool antigen tests for the primary diagnosis of *H pylori* infection in Turkish patients with dyspepsia. A total of 151 patients who were referred to the endoscopy unit were included. They used FemtoLab *H pylori* enzyme immunoassay and Premier Platinum. The sensitivity and specificity of the monoclonal FemtoLab *H pylori* were 93% and 90% respectively, and of the polyclonal Premier Platinum HpSA were 84% and 67%, respectively. They concluded that Femtolab *H pylori* was an excellent tool for primary diagnosis of *H pylori* in Turkish patients with dyspepsia^[21].

Still in another study, Kato *et al* compared rapid lateral flow stool antigen immunoassay (LFI) and stool antigen enzyme immunoassay (EIA) for the diagnosis of *H pylori* infection in children. One hundred and eighty-two children and adolescents were studied. The sensitivity, specificity and accuracy of the LFI method were 90.6%, 95.8% and

94% respectively and for the EIA method, sensitivity, specificity and accuracy were 96.8%, 99.2% and 98.3%, respectively^[22].

Islam *et al* assessed the performance of the HpSA for the diagnosis of *H pylori* infection and confirming post-therapy eradication compared to generally well accepted clinical reference standard. HpSA was used for the 127 patients. The sensitivity and specificity of HpSA were 67% and 100%, respectively. As a result, HpSA was found to be a reasonably useful diagnostic test for *H pylori*. The HpSA may prove to be useful for the primary care physicians as part of the test-and-treat strategy for dyspepsia, but this may need further study^[23].

Ito *et al* investigated the clinical usefulness of HpSA test for the evaluation of the success of eradication therapy by comparing it with the UBT. A total of 105 patients with *H pylori* infection were enrolled in that study. The diagnostic accuracy of the UBT and the HpSA test was 94.3% and 97.1%, respectively. As a result, HpSA is a very useful and non-invasive diagnostic tool for the evaluation of eradication therapy of *H pylori*. A combination of the HpSA test and the UBT is very practical in the clinical evaluation of eradication therapy of *H pylori*^[24].

Manes *et al* compared the accuracy of HpSA, FemtoLab and UBT in the assessment of eradication of *H pylori* infection 4-8 wk after the completion of antibiotic treatment. Three hundred and forty-six patients were studied. The sensitivity and specificity of HpSA were 73.4% and 97.8%, respectively. The sensitivity and specificity of FemtoLab were 88.3% and 97.8%, respectively. They concluded that both the new stool antigen tests, although less accurate, may represent valuable alternatives to UBT since they were cheap and easy to perform and did not need the use of expensive isotope ratio mass spectrometers. Thus, due to its high level of sensitivity, the new monoclonal stool test could be preferred for the post-eradication setting of *H pylori* infection^[25].

Five recent studies done by Shaikh *et al* ($n = 86$ children; sensitivity 76% and specificity 61%), Raguza *et al* (133 children; sensitivity 94.6% and specificity 96.6%), de Carvalho Costa Cardinali *et al* (161 children; sensitivity 96.9% and specificity 100%), van Doorn *et al* (106 children; sensitivity 100% and specificity 92%) and Elitsur *et al* (121 children; sensitivity 67% and specificity 99%) evaluated the HpSA test in asymptomatic children with *H pylori* infection compared to other methods. It was demonstrated that the commercial polyclonal HpSA test can not replace histopathologic findings as the best standard for the diagnosis of *H pylori* infection in children^[26-30].

Syam *et al* evaluated the HpSA for the detection of *H pylori* infection in 63 dyspeptic patients. The sensitivity and specificity of HpSA were 66.7% and 78.9%, respectively. They concluded that HpSA stool test may be useful for the primary diagnosis of *H pylori* infection in peptic ulcer^[31].

Arikan *et al* conducted a prospective study to examine the reliability of the HpSA test. The HpSA test had a 91% sensitivity and 83% specificity. HpSA test proved to be as reliable as pathological examination for confirming the existence of *H pylori* in humans. Thus, the HpSA test was a useful method for detecting *H pylori* in patients for whom

endoscopy was not indicated^[32].

Lopez *et al* compared the efficacy of several non-invasive methods and assessed comparative reliability of the stool tests. Eighty-six patients were applied FemtoLab, HpSA and Simple *H pylori* tests. The sensitivity and specificity of HpSA were 58% and 96%, respectively and the sensitivity and specificity of simple *H pylori* were 61% and 78%, respectively. According to the results, they suggested that UBT was the most reliable diagnostic examination for determining *H pylori* status in patients with chronic renal failure on dialysis because stool tests showed heterogeneous results^[14].

Inelmen *et al* evaluated the accuracy of HpSA in the diagnosis of *H pylori* infection in 85 elderly patients affected by medication. Among 56 patients who were not taking PPIs, the sensitivity and specificity of the HpSA test were 76% and 93%, respectively. Among 29 patients who had received pharmacological therapy with PPIs, the sensitivity and specificity of HpSA test were, respectively, 82% and 83%. They concluded that HpSA was a useful test in elderly people. The test was easy, simple to perform and non-invasive^[13].

Chisholm *et al* conducted a comparative evaluation of the performances of Premier Platinum HpSA ELISA, Amplified IDEIA HpStAR ELISA and ImmunoCard STAT!HpSA kits. ImmunoCard STAT!HpSA was demonstrated easier to perform than ELISA and was more sensitive than the HpSA kit. Compared with the IDEIA HpStAR kit, the ImmunoCard test was less sensitive (87.8% versus 95.9%, respectively) and specific (89.4% versus 100.0%, respectively). The Amplified IDEIA HpStAR kit was the most sensitive and specific of the three tests and it was available for pre-treatment, noninvasive detection of *H pylori* in stool samples in an English adult dyspeptic population^[33].

Previously we studied forty adult Turkish dyspeptic patients. Two antrum and corpus biopsies were taken from each patient and RUT and histopathology were applied to all patients as gold standard methods for the diagnosis of *H pylori* infection. Stool specimens were examined by polyclonal Premier Platinum HpSA and simple *H pylori* cassette test before and after the eradication therapy. When we compared with gold standard methods, the diagnostic accuracy of Premier Platinum HpSA and simple *H pylori* cassette tests were 75% and 87.5%, respectively.

In that study the HpSA test was polyclonal test but we wanted to compare it with the available monoclonal Simple *H pylori* cassette test^[34]. The new Rapid STRIP!HpSA test was also used in this group of patients (unpublished data). As no results have been published to date on the rapid STRIP!HpSA test and the simple *H pylori* stool antigen cassette test, we undertook the present study. The simple *H pylori* stool antigen cassette test, although easy to use, gave a sensitivity that was too low (33%) for the reliable diagnosis of *H pylori* infection. However, the rapid STRIP!HpSA test, which was also convenient to use, was more sensitive (60%). We conclude that because it is a monoclonal test, rapid STRIP!HpSA, can be used as a routine diagnostic tool in the microbiology laboratory for assessing clinical significance and eradication control of *H pylori* in upper gastrointestinal bleeding patients.

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