PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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World J Gastroenterol 2005;11(3):386-388
World Journal of Gastroenterology ISSN 1007-9327

• BRIEF REPORTS •

Accuracy of *Helicobacter pylori* stool antigen for the detection of *Helicobacter pylori* infection in dyspeptic patients

Ari Fahrial Syam, Abdul Aziz Rani, Murdani Abdullah, C. Manan, D. Makmun, M. Simadibrata, D. Djojoningrat, Daldiyono, Tadashi Sato

Ari Fahrial Syam, Abdul Aziz Rani, Murdani Abdullah, C. Manan, D. Makmun, M. Simadibrata, D.Djojoningrat, Daldiyono, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Indonesia
Tadashi Sato, First Department of Medicine Yamanashi Medical University, Yamanashi, Japan
Supported by Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Indonesia
Co-first-authors: Ari Fahrial Syam and Abdul Aziz Rani
Correspondence to: Ari Fahrial Syam, Jl Diponegoro No. 72 Jakarta Pusat 10430, Indonesia. ari_syam@hotmail.com
Telephone: +62-21-3153957 **Fax:** +62-21-3142454
Received: 2003-12-10 **Accepted:** 2004-01-30

Key words: *Helicobacter pylori* infection; Dyspepsia; HpSA; Enzyme Immunoassay

Syam AF, Rani AA, Abdullah M, Manan C, Makmun D, Simadibrata M, Djojoningrat D, Daldiyono, Sato T. Accuracy of *Helicobacter pylori* stool antigen for the detection of *Helicobacter pylori* infection in dyspeptic patients. *World J Gastroenterol* 2005; 11(3): 386-388
http://www.wjgnet.com/1007-9327/11/386.asp

Abstract

AIM: The premier platinum *Helicobacter pylori* (*H pylori*) stool antigen (HpSA) test is an enzyme immunoassay (EIA) that detects an *H pylori* antigen present in human stools. However, at present there is no uniformity about the cut off level required to consider the test as positive or negative. So we need the cut off level for our local population. The aim of this study was to evaluate the HpSA for the detection of *H pylori* infection in dyspeptic patients and to determine the sensitivity, specificity of the HpSA test in the diagnosis of *H pylori* infection, as compared to other standardized diagnostic techniques.

METHODS: Sixty-three dyspeptic patients were selected from patients who came to the Division of Gastrointestinal Clinic in Cipto Mangunkusumo Hospital, Jakarta, Indonesia. *H pylori* infection was confirmed in all patients by histology and rapid urease test (CLO test). Positive results for *H pylori* were based on positive results from both rapid urea test and microscopic detection of *H pylori.* Stool specimens were analyzed for *H pylori* antigen using HpSA immunoassay.

RESULTS: A total 63 patients consisted of 31 (49.2%) males and 32 (50.8%) females ranging in ages between 16 and 73 years with a mean age of 42.4±15 years. The mean age of men was 43.2±15.7 years and women was 41.6±14.4 years. Endoscopic findings in this study included gastric cancer 1.6%, peptic ulcer 4.8%, duodenal ulcer 7.9%, esophagitis 6.3%, gastritis 77.7%, and gastroduodenitis 4.8%. According to the predefined study criteria, 6 (9.5%) of 63 patients were positive for *H pylori*. In the diagnosis of infection, the area under the receiver operating characteristic (ROC) curve for the HpSA test was 0.722 (95% CI, 0.518-0.927). Using a cut-off value of 0.274 instead of 0.16 (as recommended by the manufacturer) the sensitivity and the specificity were 66.7% and 78.9% respectively.

CONCLUSION: The HpSA stool test, using a cut-off value of 0.274, may be useful for the primary diagnosis of *H pylori* infection, its specificity is similar to other standard tests but its sensitivity was lower.

INTRODUCTION

Helicobacter pylori (*H pylori*) were first isolated in the early 1 980s. Since then great attention has been paid to revealing its role in the pathogenesis of gastroduodenal diseases and to developing techniques for its accurate detection[1]. Various methods are available for detecting *H pylori*, but all have limitations. At present there are several techniques available for the detection of *H pylori*. The sensitivity and specificity of the used diagnostic methods differ depending on the handling of the specimens and the evaluation method. Advantages and disadvantages of each test have to be weighed against the reliability and patient acceptability.

Recently, there is no gold standard for the diagnosis of *H pylori* and the choice of diagnostic testing depends on the clinical situation, experience of the clinician, local resource and an estimate of cost effectiveness. When endoscopy is clinically indicated, for example, patients with an upper gastrointestinal bleeding, possible gastric cancer or suspected ulcer, the diagnosis of *H pylori* infection can be made by either urease rapid test (CLO, MIU or pyloric test), detection of *H pylori* on biopsy specimens or cultures. But when endoscopy is not clinically indicated, as in an asymptomatic patient with a history of duodenal ulcer with dyspeptic complaints of more than 2 wk, diagnosis can be made by serologic test or urea breath test (UBT). However, UBT is expensive and requires specialized equipments, while serologic test may have a lower specificity.

Since there is evidence that infected individuals excrete *H pylori* in feces, an immunoassay (EIA, HpSA) has been developed that can detect *H pylori* antigen in human feces[2]. Besides, *H pylori* in feces could be detected by PCR or even culture[3,4]. Therefore it is non-invasive; HpSA immunoassay would be particularly important for patients for whom endoscopy is not clinically indicated. The premier platinum HpSA test is an enzyme immunoassay (EIA) that detects an *H pylori* antigen present in human stools. However, at present there is no uniformity about the cut-off level required to consider the test as positive or negative. So we need to know the specific cut off level for our local population.

The aim of this study was to evaluate the HpSA for the detection of *H pylori* infection in dyspeptic patients and to determine the sensitivity, specificity and negative and postive predictive values of the HpSA test in the diagnosis of *H pylori*

infection, as compared to other standardized diagnostic techniques.

MATERIALS AND METHODS

Patients

Sixty-three dyspeptic patients were selected from patients who came to the Division of Gastrointestinal Clinic in Cipto Mangunkusumo Hospital, Jakarta, Indonesia from March 2001 to September 2001. Inclusion criteria were out patients clinically diagnosed as dyspeptic, who were aged 20-80 years, washed out for seven days of any medication that influenced the *H pylori* detection. Patients were informed of the procedures to be used in the study and they signed on informed consent document.

Patients had an upper endoscopy and four biopsy samples were taken from the antrum and corpus of each patient for hematoxylin and eosin staining, *H pylori* detection (Giemsa stain in both antrum and corpus) and rapid urease test (CLO test).

CLO test was performed according to the manufacturer's instructions and the results were interpreted after 24 h.

Biopsy specimens for histopathological examinations were fixed in buffered 4% formalin overnight and embedded in paraffin. Two 4 μm thick sections were stained with hematoxylin-eosin and one section was stained by the modified Giemsa procedure. The slides were microscopically examined by using a high power and at least five high-power fields were examined. If *H pylori* was observed the bacterial density was scored semiquatitatively on an ordinal scale (ranging from 0 to 3) by a single pathologist. A single pathologist performed the histology evaluation of gastric mucosa. Histology grades for *H pylori* density, polymorphonuclear neutrophil activity, chronic inflammation, glandular atrophy and intestinal metaplasia were determined according to the histologic classification of the updated Sydney system.

Positive results for *H pylori* were based on positive results from both rapid urease test and microscopic detection of *H pylori*.

Patients were asked to collect a specimen from their first stool after endoscopy. Stool samples were stored at -20 ℃ until use and independently tested by a private laboratory. The investigators were blinded to the results of the other *H pylori* tests. The stool specimens were analyzed for *H pylori* antigen using HpSA immunoassay as described by its manufacturer. A commercial kit, Primier platinum HpSA (Meridian Diagnostic, Cincinnati, Ohio USA) was used. Apposition of fecal sample was mixed with 200 μL of the sample diluents. One drop of enzyme conjugates was added to the microwells, which were incubated for 1 h at room temperature and washed five times. The reaction was terminated with one drop of stop solution and the results were read by spectrophotometry. An absorbance (450/630) $\geqslant$ 0.160 was considered positive as recommended by the manufacturer.

Statistical analysis

The sensitivity, specificity and positive and negative predictive values were measured. The different cut-off values between 0.100 and 0.300 were assessed. For each of these cut-off values the sensitivity and specificity were calculated. Statistical analysis was performed using SPSS.

RESULTS

Patient characteristic

A total of patient 63 patients, who consisted of 31 (49.2%) males and 32 (50.8%) females with a mean age of 42.4±15 years and ranging 16-73 years. The mean age of men was 43.2±15.7 years and women (41.6±14.4 years). Endoscopic findings in this study included were gastric cancer 1.6%, peptic ulcer 4.8%, duodenal ulcer 7.9%, esophagitis 6.3%, gastritis 77.7% and gastroduodenitis 4.8 % (Table 1).

Table 1 Endoscopic diagnosis of dyspeptic patients

Endoscopic findings	*n*	Percent (%)
Antral gastritis	49	77.7
Peptic ulcer	3	4.8
Duodenal ulcer	5	7.9
Gastric cancer	1	1.6
Gastroduodenitis	3	4.8
Esophagitis	4	6.3
Pangastritis	1	1.6

Diagnosis of H pylori infection

According to the predefined study criteria, 6 (9.5%) of 63 patients were positive for *H pylori*. Three patients (4.8%) were positive by the CLO test (Rapid urease test), 7 patients (11.1%) were positive by histology and 42 (66.7%) were positive by HpSA test. Histological examination, CLO test and HpSA test were all positive in 3 (4.8%) and negative in 20 patients (31.7%). (Table 2).

Table 2 Observed number of test results of *H pylori* tests (HpSA, Histology, and CLO test)

HpSA[1]	Histology	CLO test	*n*
Negative	Negative	Negative	30
Positive	Positive	Positive	3
Positive	Negative	Positive	3
Negative	Positive	Positive	3
Negative	Negative	Positive	3
Positive	Negative	Negative	42
Positive	Positive	Negative	6
Negative	Positive	Negative	7

[1]BBHpSA: *H pylori* stool antigen using a cut-off value of 0.160.

In the diagnosis of *H pylori* infection, the area under the ROC curve for the HpSA test was 0.722 (95% CI, 0.518-0.927). Using a cut-off value of 0.274 instead of 0.16 (as recommended by the manufacturer) the sensitivity and specificity were 66.7% and 78.9 % respectively. Using a cut-off value from the manufacturer, the sensitivity and specificity were 100% and 36.8% (Table 3). HpSA tests were positive for antral gastritis (63.4 %), gastric ulcer (66.7%), duodenal ulcer 80 % and esophagitis 75 % (Figure 1).

Table 3 Sensitivity and specificity of HpSA[1] with different cut-off values in dyspeptic patients with biopsy-proven *H pylori* status

Cut-off values	Sensitivity (%)	Specificity (%)
0.156	100.0	36.8
0.158	83.3	36.8
0.274	66.7	78.9
0.282	50.0	78.9
0.319	50.0	84.2

[1]HpSA : *H pylori* stool antigen.

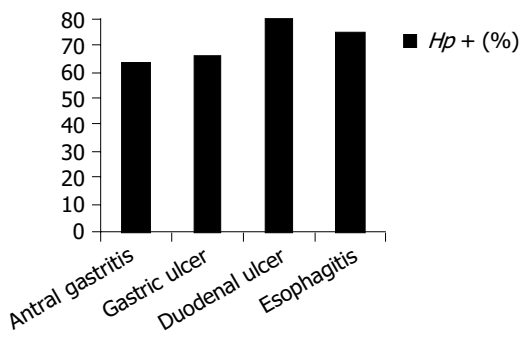

Figure 1 Endoscopic diagnosis of *H pylori* positive patients using *H pylori* stool antigen HpSA.

DISCUSSION

Recently many reliable methods for detecting *H pylori* infection are available. However, since invasive methods require endoscopy, they are not suitable for primary care physicians. In the absence of endoscopy facilities, primary care physicians require non-invasive methods to diagnose *H pylori* infection. HpSA stool test is an easy and quick procedure that does not require expensive equipments and can be used as an alternative to detect *H pylori* infection. This method also offer advantages as a simpler sampling method. The stool test seems to meet the requirements of general practitioners who treat most patients infected with *H pylori*, because it is easy to perform and requires no blood samples.

In the present study the sensitivity using a cut-off value of 0.274 was obtained (66.7%) that was lower than previously reported. However, the specificity (78.9%) was almost similar to other reports. In some reports, the efficacy of the HpSA test for the diagnosis of *H pylori* infection was investigated. A sensitivity of 89-94% and specificity of 90-94% for the diagnosis of *H pylori* infection in symptomatic untreated patients were observed[5-7]. Compared to C-UBT, the stool test was less sensitive and seemed to have an equal or even better specificity in toddlers. The HpSA revealed a sensitivity of 88.9% (95% CI 77.3-96.3) and specificity of 94.0% (95% CI 88.1-97.7) compared to[13] C-UBT, which had a sensitivity of 100% (95%CI 94.0-100) and a specificity of 98.8% (95% CI 94.7-100)[8].

It was reported that a cut-off value of 0.300 could provide the best diagnostic value. With this cut-off value, the sensitivity, specificity, and accuracy of HpSA were 93.9%, 95.7% and 94.8% respectively[9]. Using a cut-off value of 0.130, another study obtained a lower sensitivity (89.5%) and specificity (83.3%)[10]. By drawing an ROC curve for our patients, we found that a cut-off value of 0.274 provided the best accuracy. It is generally known that diagnostic kits sometimes provide different diagnostic accuracies in different patients. For this reason, we found the discrepancy between our results and other results.

In this study we found that HpSA was positive in 66.7% of gastric ulcer cases and 80% of duodenal ulcer cases. These observations suggest that HpSA is a highly reliable diagnostic method for peptic ulcer.

In conclusion, HpSA stool test, using a cut-off value of 0.274, may be useful for the primary diagnosis of *H pylori* infection; its specificity is similar to other standard tests but its sensitivity is lower. HpSA is a useful method for diagnosing *H pylori* infection in peptic ulcer.

ACKNOWLEDGEMENTS

The authors thank Prodia Laboratory Jakarta for kindly doing the *H pylori* stool antigen tests.

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Edited by Wang XL and Xu CT **Proofread by** Xu FM