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Original article

Helicobacter pylori Stool Antigen Test: Validation Study Using a Selected Group of Sri Lankan Patients

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Abstract. The use of *Helicobacter pylori* stool antigen test is limited in Sri Lanka. The validity of the amplified IDEIA HpSt AR monoclonal antibody test was examined in 51 patients who underwent upper gastrointestinal endoscopy and multiple gastric biopsies for dyspeptic symptoms. Histology with both H&E and modified Giemsa was considered as the reference standard. There were 33 males and the mean age (SD) of study subjects was 49.4(15.4) years. Endoscopically, forty-three (84.3%) patients had abnormal findings in the stomach predominated by prepyloric erosions (53.5%). Histologically, all patients had features of chronic gastritis and activity was detected in 94.1% of patients. Histologically (both H&E and modified Giemsa) 82.4% of patients had morphological evidence of *H. pylori* in the gastric mucosa. Stool antigen test was positive only in 7 out of 51 (13.7%) patients. The sensitivity and specificity of the stool antigen test in this study were 11.9% and 77.8%, respectively. The negative and positive predictive values were 15.9% and 71.4%, respectively. A low concentration of *H. pylori* stool antigen below the required level of detection could be a plausible explanation for false negative results. Furthermore, decreased shedding of the organism with increasing chronicity of infection may be another reason. Infection of the local population with a hitherto unknown strain of *H. pylori* is worth considering. In conclusion, until further testing is done, the validity of amplified IDEIA HpSt AR monoclonal antibody test as *H. pylori* detection technique in the local population cannot be supported by our observations.

Keywords: *H. pylori*, Stool antigen test, Histology, Sri Lanka

INTRODUCTION

Diagnosis of Helicobacter pylori infection is based on an array of diagnostic tests ranging from invasive diagnostic tests that require biopsy samples to non-invasive tests that are based on characteristics and immunological properties of the organism.

In Sri Lanka, the diagnosis of *H. pylori* infection is mostly dependent upon invasive diagnostic techniques where biopsy specimens obtained during upper gastrointestinal endoscopy are subjected to histological examinations and/or rapid urease tests (1-3). Since endoscopy and histopathology services are available free of charge at every tertiary care hospital in Sri Lanka, the demonstration of the organism in the gastric mucosa by histology is the preferred method. Currently, the clinical use of non-invasive tests such as *H. pylori* antibody and breath tests is limited and confined mainly to research settings (4, 5).

Even though the prevalence of *H. pylori* infection is expected to be high in concordance with data from other developing countries in the region, previous studies done in Sri Lanka reveal varying prevalence figures (2,6,7). However, no country-wide, population-based screening for *H. pylori* infection has been done partly because of the assumption that the prevalence of infection is high in the local population. However, there are few studies that show a low prevalence of infection among Sri Lankans (1, 6). Most of the studies have used histology and /or urease tests to confirm the diagnosis. Therefore, the need of other techniques particularly non-invasive detection techniques should be considered.

The stool antigen test to detect *H. pylori* infection is not being widely used in Asia and its applicability is not well known. The high cost of these tests may have partly contributed to their limited use. We intended to compare the performance of stool antigen tests with histology. In this study, we

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evaluated the Amplified IDEIA HpSt AR monoclonal antibody test in a group of adult patients presented with dyspeptic symptoms at an endoscopy unit in Southern Sri Lanka.

METHODS

We studied 58 consecutive adult patients who underwent gastroscopy for persisting upper gastrointestinal symptoms during the year 2010 at the Medical Endoscopy Unit, Teaching Hospital Karapitiya. Patients who had consumed antibiotics within the period of 4 weeks preceding the test and had consumed proton pump inhibitors and/or bismuth preparations within the period of 2 weeks preceding the test were excluded from the study. In addition, those patients who did not provide a subsequent stool sample within the given period of time were also excluded from the final analysis. Informed consent was obtained from all participants and the ethical approval for the study was granted by the Ethics Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka in a letter dated 06.05.2009

During upper gastrointestinal endoscopy, five gastric biopsy specimens (3-antral, 2-corporal) were obtained from each patient for the detection of *H. pylori* histologically. Two staining techniques were used; Hematoxylin & Eosin (H&E) as the routine stain and modified Giemsa as the special stain (8). A patient was considered as positive for *H. pylori* when morphological evidence of colonization by *H. pylori* was seen in at least one biopsy specimen either with H&E or with modified Giemsa. Each patient was provided with a sterile container without any transport media to collect stool samples and advised to bring a fresh stool sample within 48 hrs of completion of the endoscopy. Those who brought the stool sample later than 48 hours were excluded from the study. The stool samples were stored below -20^0 C till the time of analysis. The stool samples were subject-ted to Enzyme enzyme-linked immuno-sorbent Assay (ELISA) using an Amplified IDEIA HpSt AR monoclonal antibody test kit to detect the presence of *H. pylori* stool antigen. All stool samples were doubled and positive and negative controls provided with the test kit were used and the test was performed according to the manufacturer's guidelines (Oxoid Manual for the protocol of Amplified IDEIA HP St AR, Oxoid Limited, UK). The frozen stool samples were thawed and each stool sample was mixed with a wooden stick and a pea-sized stool sample was added to the sample diluent provided. If the stool sample was liquid in consistency, 100µl of stool sample was used. To each of the microwell, 50 µl of the stool supernatant was added, followed by 50 µl of conjugate and 100µl of conjugate after washing the microwells thoroughly. With appropriate incubation periods followed, 100µl of stop solution was added to stop the reaction. Photometric reading was taken using an EIA plate reader set at 450nm (single wavelength method). The cut-off was taken as 0.190 and those specimens with absorbance values more than 0.190 were considered as positives.

Histology with either H&E or modified Giemsa providing morphological evidence of *H. pylori* was taken as the reference standard (9). Sensitivity, specificity, positive predictive and negative predictive values of stool antigen test were calculated.

RESULTS

Out of 58 patients, 7 were excluded since they did not provide the stool sample on time. Data of 51 remaining patients were analyzed. Out of them, 33 were males. The mean age (SD) was 49.4(15.4) years.

Endoscopically, 43 (84.3%) patients had some abnormality detectable in the upper gastrointestinal tract while in 8 (15.7%) the endoscopic examination was normal. The major abnormality detected was prepyloric erosions which constituted 53.5% (23/43) of all abnormalities detected. Antral gastritis was common constituting 27.9% (12/43) of all abnormalities. There were four patients who had gastric ulcers located in the prepyloric region. Only one patient had evidence of duodenitis while none had esophageal abnormalities.

Histologically, all patients had chronic gastritis. Infiltration of gastric mucosa by polymorphonuclear neutrophils, which is an index of disease activity, was present in 94.1% (48/51) of patients.

With H&E staining, 74.5% (38/51) of patients had morphological evidence of *H. pylori* either in the antrum or corpus or in both. With modified Giemsa, 82.4% (42/51) of patients became positive for H. pylori. H&E staining failed to identify four patients who

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had *H. pylori* in their gastric mucosa detected by the modified Giemsa method. None of Giemsa negative patients were positive with H&E.

Stool antigen test was positive in 13.7% (7/51) of patients. There were two false positives and 37 false negatives. The sensitivity and the specificity of stool antigen test in this study were 11.9% and 77.8% respectively. The negative predictive value of the test was 15.9% while the positive predictive value was 71.4% (Table 1).

Table 1. Sensitivity, specificity, positive and negative predictive values of *H. pylori* stool antigen test

	Histology (morphological evidence of *H. pylori*)				
Stool antigen test	**Present**	**n**	**Absent**	**n**	**Total**
Positive	True Positive	a=5	False Positive	c=2	a + c = **7**
Negative	False Negative	b=37	True Negative	d=7	b + d = **44**
Total		a + b = **42**		c + d = **9**	
Sensitivity	11.90%	(95%CI: 3.98 - 25.63)			
Specificity	77.78%	(39.99 - 97.19)			
Disease prevalence	82.35%	(69.13 - 91.60)			
Positive Predictive Value	71.43%	(36.42 - 91.60)			
Negative Predictive Value	15.91%	(11.59 - 21.44)			

DISCUSSION

The stool antigen test is one of the recent diagnostic techniques made available for the diagnosis of *H. pylori* infection. There are a variety of stool antigen tests in use, especially in Western countries where the prevalence of *H. pylori* infection is low. These have been used to screen asymptomatic individuals, monitor therapeutic response and also to confirm the elimination of infection (9, 10).

The basis of the test is to detect the presence of *H. pylori* antigen in patients' stools. The antibodies used in these test kits are either polyclonal or monoclonal. Test kits in which monoclonal antibodies are used have shown very high sensitivity and specificity values. The kit which was used in this study was the Amplified IDEIA HpSt AR monoclonal antibody test kit that contains monoclonal antibodies specific for H. pylori. In previous studies, this test has produced sensitivity and specificity values ranging from 80 to 90% (11, 12). One Asian study also reported high sensitivity and specificity values for this test (13). The same test kit, however, showed very low sensitivity (11.9%) and moderate specificity (77.8%) in our study. However, there are several recent studies where the stool antigen tests have shown lower sensitivity and specificity figures as well (14-16).

During the collection and storage of stool samples, the guidelines given by the manufacturer were strictly adhered to. The reagents of the test kit were stored under recommended conditions till the time of analysis. The analysis was done at the first attempt and therefore the problem of repeated thawing and freezing of stool samples did not occur. Since the performance guidelines were followed strictly, the low sensitivity and specificity values we observed were unlikely to be due to technical issues with the assay.

Patients who used medications likely to interfere with the test performance were excluded from the study and the selected patients produced sufficient quantity of stool for the analysis. The preparation of the stool sample for the test was thorough. Adequate mixing of samples was done before obtaining the required amount of stool for the test.

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Despite adequate mixing during this process, a sampling error might have occurred due to unequal distribution of the *H. pylori* antigen in stool. In addition, insufficient concentration of the antigen in stool could also have resulted in a false negative result. The assay has been tested for each strain of *H. pylori* in a concentration of ≥1x10^8 organisms/ml in sample buffer. If the concentration of the organism in stool is below the level of detection, there is a chance of a false negative test.

In our patient group, the mean age (SD) was 49.4(15.4) years indicating that the patient group is relatively older. In one study, it was shown that the proportion of confirmed *H. pylori* in transient-positive stools (46%) was higher than that in persistent-positive stools (12.5%). This study suggests that the shedding of the organism diminishes with increasing chronicity of infection (17). This might explain the low detection rate of *H. pylori* in the stools of elderly individuals which is also evident in our study.

A false negative test could also be due to a different *H. pylori* strain that may inhabit local people hitherto undetected. There are many strains identified so far in different geographical locations and studies have shown that the genetic sequence of *H. pylori* can be used to distinguish between closely related human populations and they are even finer than classical human genetic markers (18, 19). Therefore, it is a possibility that local people could inhabit a different strain of the organism that would not answer the test.

A false positive test could be due to non-pylori Helicobacter species that are known to cause similar symptomatology and positive stool antigen tests (18). Yet, both *H. pylori* and non-pylori Helicobacter species would appear similar with microscopy. Cross-reaction with other organisms is highly unlikely since the assay has been tested negative with a wide range of possible microorganisms listed in the manual. The patchy distribution of organisms in the gastric mucosa that escapes the sampling during gastric biopsy could be another possibility (20).

All histologically positive cases (82.4%) demonstrated morphological evidence of the organism in at least a single biopsy specimen with either H&E, modified Giemsa or both staining techniques. Histology with H&E combined with a special stain e.g. modified Giemsa, Warthin starry etc. is the recommended test to visualize this organism microscopically and is considered the reference method for confirming the *H. pylori* infection (9). All patients in this study had chronic gastritis histologically. Considering the other features of the inflamed gastric mucosa, activity; and infiltration of gastric mucosa by polymorphonuclear neutrophils, which is considered as an indirect indication for the presence of *H. pylori*, was seen in over 90% of patients (21, 22). Interestingly, all histologically positive patients had infiltration of polymorphonuclear neutrophils, in the gastric mucosa. Only three patients who had no infiltration in the gastric mucosa had no *H. pylori* detectable histologically. Therefore, the histological detection of the organism seems reasonable and accurate. In our previous study where four detection techniques of *H. pylori* were compared, histology was the most sensitive and most specific detection technique among the four tests studied (3).
Our data indicate the poor concordance of stool antigen test and histology, two detection methods of *H. pylori* infection and the cause of this disparity is not clear to us. We suggest repeating this test using other patient groups and other test kits which are now available such as stool antigen rapid test kits to examine the reproducibility of our observations. In conclusion, with this data and also considering the cost of the kit we cannot recommend the stool antigen test as a detection test of *H. pylori* infection in Sri Lanka.

Limitations. Due to financial constraints, the collected stool samples were kept at -20^0C till the stool antigen kits were purchased with a grant received subsequently. However, the period of storage was within the recommendation of the manufacturer's guidelines.

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