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Non-invasive stool antigen test for screening of *Helicobacter pylori* infection and assessing efficacy of treatment in patients with peptic ulcer

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Abstract

Helicobacter pylori infection is one of the most common infections in humans, with an estimated 50% of the world population being infected. The infection is strongly associated with chronic gastritis, peptic ulcer, adenocarcinoma and non-Hodgkin's lymphoma of stomach. The prevalence of infection is high in developing countries, demanding a reliable diagnostic and treatment method. The present study was designed to investigate the monoclonal antibody-based *H. Pylori* stool antigen test to screen *H. pylori* infection and assess efficacy of treatment in patients with peptic ulcer. A total of 89 patients who underwent upper gastrointestinal endoscopy from July 2007 to June 2008 at Bangabandhu Sheikh Mujib Medical University Hospital, Dhaka, Bangladesh were included in the study. Endoscopic findings showed that out of 89 patients, 54 (60.7%) had duodenal ulcers, 24 (27%) had antral erosion and 5 (5.6%) had gastric ulcers. With RUT (rapid urease test) and histopathology of biopsy samples of 89 patients, 78 (87.6%) patients were found to be *H. pylori* positive. Stool antigen test was positive in 72 (92.3%) out of 78 *H. pylori* positive patients. The monoclonal stool antigen test (SAT) revealed 92.3% sensitivity and specificity of 100% before treatment. Among 52 follow-up patients (after treatment), 5 (9.6%) patients were detected positive by histology, RUT and stool antigen test, and 35 (67.3%) patients were negative by 3 tests. So the monoclonal SAT revealed 100% sensitivity and 100% specificity after treatment. The monoclonal stool antigen test is highly sensitive and a specific tool for diagnosis of *H. pylori* infection before therapy and can assess the success of eradication after therapy. It also offers the advantage of specificity and reliability over the invasive test. It is easy and quick to use, non-invasive and does not require any special technology.

Keywords: *Helicobacter pylori,* peptic ulcer, monoclonal SAT, non-invasive, Bangladesh.

Introduction

Helicobacter pylori (*H. pylori*), a gram-negative bacterium, was first isolated from humans in 1982.[1] *H. pylori* infection is one of the most common infections in humans, with estimated 50% of the world population being infected.[2] The infection is strongly associated with chronic gastritis, peptic ulcer, adenocarcinoma and non-Hodgkin's lymphoma of stomach.[3] An infected individual has an estimated lifetime risk of 10-20% for the development of peptic ulcer disease, which is at least 3-4 folds higher than in non-infected subjects, and <1% will develop gastric cancer.[2] *H. pylori* infection can be diagnosed in 90-100% of duodenal ulcer patients and in 60-100% of gastric ulcer patients.[4] A recent study found that the prevalence of *H. pylori* infection was higher in Bangladeshi than in Japanese subjects (60.2 and 45.1%, respectively) with abdominal complaints who underwent endoscopy examinations and had no history of *H. pylori* eradication.[5] *H. Pylori* infection is primarily acquired during childhood and typically clusters within families. A community-based cross-sectional study in asymptomatic young Bangladeshi children

Practice points

- *H. pylori* infection is one of the most common infections in humans, with an estimated 50% of the world population being infected.
- The prevalence of infection is high in developing countries, demanding a reliable diagnosis and treatment method.
- The monoclonal stool antigen test is easy and quick to use, non-invasive and does not require any special technology.
- In the present study, the monoclonal SAT revealed 92.3% sensitivity and specificity of 100% before treatment, and 100% sensitivity and 100% specificity after treatment.
- Based on this performance, the monoclonal SAT can be used for screening *H. pylori* infection and assessing efficacy of treatment in the healthcare settings of Bangladesh.

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(2-5 years) was done in rural and peri-urban areas by Urea Breath Tests (UBT) and found a 72% *H. Pylori* infection rate.[6] In Pakistan, the infection rate with *H. Pylori* was about 83% in adult patients undergoing upper GI endoscopy for various reasons.[7] The prevalence of *H. Pylori* infection varies with age, socioeconomic condition, education level.[8] The period of acquisition of infection is in childhood and the likelihood increases with age.[9] Between the ages of 40-60 years a prevalence rate of 30-40% was found in USA and Australia.[10] In the developing world, 80% of the populations are infected in adulthood.[11] Transmission occurs from person to person through fecal-oral, oral-oral and gastro-oral routes. The connection of *H. Pylori* with several clinical conditions has increased the demands for treatment of infection and thereby has increased the interest for reliable diagnostic method.

During the past decade several methods, both direct and indirect have been developed for diagnosing. Non-invasive tests are useful for primary diagnosis and are also useful in patients who cannot tolerate endoscopy.[12] Direct methods (invasive) involve endoscopy and examination of gastric biopsies from antrum and corpus, for culture, rapid urease test, histopathology. Noninvasive methods, include urea breath test, serology and stool antigen test. The urea breath test has a high degree of accuracy whether [^{14}C]- based or [^{13}C]-based, and is in may studies considered to be the reference standard.[13-15] However concerns over radiation may limit the use of [^{14}C]-urea and the employment of the [^{13}C]-urea breath test requires expensive equipment for analysis.[16]

In recent years a new diagnostic tool, the stool antigen test, has been available and the main advantages are the non-invasive nature of this procedure and patients can obtain a stool sample at home for laboratory analysis. It is very rapid and easy test and can be used in a situation where the culture facilities are not available. Result can be given to a patient within 2 hours after stool collection so that prompt therapy can be introduced. The first commercially available stool test was based on polyclonal antibodies and has been thoroughly evaluated with reports of a sensitivity in the range of 86-100%[17] and a specificity of 70-100%.[18] A second generation of kits, based on monoclonal antibodies, has already been used for several years. Though the results on monoclonal stool antigen test (SAT) were not satisfactory in study conducted by Quesada *et al*.;[19] however, Kolho *et al*.,[20] Hooton *et al*.,[21] and Frenck *et al*.,[22] demonstrated good results with monoclonal SAT. The present study was designed to investigate the monoclonal antibody-based *H. Pylori* stool antigen test to screen *H. pylori* infection and assess efficacy of treatment in patients with peptic ulcer at Bangabandhu Sheikh Mujib Medical University Hospital, Dhaka, Bangladesh.

Materials and methods

A cross-sectional study was conducted among adult patients with peptic ulcers in Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital, Dhaka, Bangladesh from July 2007 to June 2008. A structured questionnaire was used to collect the socio-demographic information of the patients. Patients were diagnosed by clinical history and subsequent endoscopic findings and a total of 89 peptic ulcer patients were recruited for the study. Three biopsy samples were taken from the patients for RUT (rapid urease test) and histopathology to assess the *H. pylori* status of the patients. Among 89 patients, 78 (87.6%) patients were found to be *H. pylori* positive, who then received *H. pylori* eradication therapy. All the *H.* positive subjects received the same eradication therapy. Out of 78 patients, 52 patients attended the clinic for further follow-up. The follow-up patients were then assessed for *H. pylori* again using similar procedure (biopsy samples for RUT and histopathology). Stools collected from 78 *H. pylori* positive and 52 follow-up patients and were stored -20^{0}C until tested by the monoclonal SAT. The research protocol was also approved by the ethics committee of the BSMMU. Written informed consent forms were signed by each participant.

Sample collection and transportation

Endoscopic examination of the upper GIT of the patients was done by the endoscopist. Three biopsy tissues were obtained from the gastric antrum and one from upper corpus from each patient. Two biopsy specimens of the antrum and one of the corpus was fixed in 10% buffered formalin for histopathology. The one remaining biopsy specimen was kept immediately for RUT. Stool samples were collected in an airtight transport container from all patients for stool antigen test.

Monoclonal SAT for H. pylori

Monoclonal SAT (Premier, Platinum HpSA PLUS, Meridian Bioscience, Italy), a commercially available enzyme immunoassay kit, was used to detect *H. pylori* antigens present in human stool. It utilizes a plurality of monoclonal and *H. pylori* capture antibodies adsorbed to microwells.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences, version 16.0 (SPSS, Inc, Chicago, IL). Descriptive data are given as frequencies and percentages. Differences in distributions were analyzed by chi square test. The level of significance was set at $P<0.05$.

Results

Table 1 outlines the details of the socio-demographic variables and *H. pylori* status of the study population. Endoscopic findings showed that out of 89 study population, 54 (60.7%) had duodenal ulcer, 24 (27%) had antral erosion and 5 (5.6%) had gastric ulcer (Table 2). With RUT and histopathology of biopsy samples of 89 patients, 78 (87.6%) patients were found *H. pylori* positive. Table 3 shows stool antigen test results and the *H. pylori* status of the patients before treatment. The stool antigen test was positive in 72 (92.3%) out of 78 *H. pylori* positive patients.

Table 1: ANC registration and facilitator

Variables	No of respondents	*H. pylori* status		
		Positive (%)	Negative (%)	Indeterminate (%)
Age				
<20 years	1	1 (100%)	0 (0%)	0 (0%)
21-40 years	59	51 (86.4%)	7 (11.9%)	1 (1.7%)
41-60 years	29	26 (89.7%)	1 (3.4%)	2 (6.9%)
Gender				
Male	66	55 (83.3%)	8 (12.1%)	3 (4.5%)
Female	23	23 (100%)	0 (0%)	0 (0%)
Marital status				
Married	76	67 (88.2%)	6 (7.9%)	3 (3.9%)
Unmarried	13	11 (84.6%)	2 (15.4%)	0 (0%)
Socio-economic status				
Lower (<5000/)	39	34 (87.2%)	3 (7.7%)	2 (5.1%)
Middle (5001/-15000/)	37	33 (89.2%)	3 (8.1%)	1 (2.7%)
Upper (>15000/)	13	11 (84.6%)	2 (15.4%)	0 (0%)
Educational status*				
Illiterate	16	15 (93.8%)	1 (6.3%)	0 (0%)
Up to V	32	28 (87.5%)	4 (12.5%)	0 (0%)
Up to SSC	8	8 (100%)	0 (0%)	0 (0%)
Up to HSC	14	10 (17.1%)	1 (7.1%)	3 (21.4%)
Graduate	12	10 (83.3%)	2 (16.7%)	0 (0%)
Post graduate	7	7 (100%)	0 (0%)	0 (0%)
Occupation				
Government	10	9 (90%)	1 (10%)	0 (0%)
Non government	3	2 (66.7%)	1 (33.3%)	0 (0%)
Business	12	11 (91.7%)	1 (8.3%)	0 (0%)
Student	13	11 (84.6%)	0 (0%)	2 (15.4%)
Housewife	18	18 (100%)	0 (0%)	0 (0%)
Day laborer	11	8 (72.7%)	3 (27.3%)	0 (0%)
Farmer	5	4 (80%)	1 (20%)	0 (0%)
Driver	5	4 (80%)	0 (0%)	1 (20%)
Unemployed	4	4 (100%)	0 (0%)	0 (0%)
Security guard	4	4 (100%)	0 (0%)	0 (0%)
Factory worker	4	3 (75%)	1 (25%)	0 (0%)
Smoking habit				
No	42	35 (83.3%)	5 (11.9%)	2 (4.8%)
Yes	47	43 (91.5%)	3 (6.45%)	1 (2.1%)
Number of sticks				
NA	41	35 (85.4%)	5 (12.2%)	1 (2.4%)
<5/day	4	4 (100%)	0 (0%)	0 (0%)
5-10/day	26	24 (92.3%)	1 (3.8%)	1 (3.8%)
11-15/day	11	9 (81.8%)	2 (18.2%)	0 (0%)
15-20/day	7	6 (85.7%)	0 (0%)	1 (14.3%)
Duration of smoking				
NA	41	35 (85.4%)	5 (12.2%)	1 (2.4%)
<5yRs	8	8 (100%)	0 (0%)	0 (0%)
6-10 yrs	21	19 (90.5%)	1 (4.8%)	1 (4.8%)
11-15 yrs	13	11 (84.6%)	1 (7.7%)	1 (7.7%)
16-20 yrs	5	4 (80%)	1 (20%)	0 (0%)
>20 yrs	1	1 (100%)	0 (0%)	0 (0%)
Betel leaf				
No	48	41 (85.4%)	5 (10.4%)	2 (4.2%)
Yes	41	37 (90.2%)	3 (7.3%)	1 (2.4%)
Jarda				
No	53	46 (86.8%)	5 (9.4%)	2 (3.8%)
Yes	36	32 (88.9%)	3 (8.3%)	1 (2.8%)

* Significant

Table 2: Endoscopic finding and *H. pylori* status of the study population (n=89)

Endoscopic finding (n=89)	*H. pylori* + status (n = 78)	*H. pylori* – status (n = 8)	Indeterminate (n=3)
Duodenal Ulcer (n=54)	47 (91.5%)	7 (8.5%)	0 (0%)
DU + DB (n=1)	1 (100%)	0 (0%)	0 (0%)
Gastric Ulcer (n=5)	5 (100%)	0 (0%)	0 (0%)
Antral Erosion (n=24)	23 (95.8%)	0 (0%)	1(4.2%)
GU + DU (n=4)	2 (50%)	1 (25%)	1(25%)
Prepyloric erosion (n=1)	0 (0%)	0 (0%)	1(100%)

So the monoclonal SAT revealed 92.3% sensitivity and specificity of 100% before treatment. Indeterminate results were excluded from the monoclonal stool antigen assay before treatment. Among 52 follow-up patients (after treatment), 5 (9.6%) patients were detected positive by histology, RUT and stool antigen test, and 35 (67.3%) patients were negative by 3 tests (Table 4). Remaining 12 (23.1%) indeterminate cases were excluded from further statistical analysis. So the monoclonal SAT revealed 100% sensitivity and 100% specificity after treatment.

Discussion

H. pylori infection is one of the most common infections in human beings worldwide, strongly associated with peptic ulcer disease and gastric cancer.[23,24] Detection of *H. pylori* infection has therefore become a key step in the management of patients referred to gastroenterologists. There are several methods available to detect *H. pylori* infection including invasive methods based on gastric biopsies and non-invasive methods like serology, urea breath tests, and stool antigen tests.[25,26] Stool antigen tests have recently been welcomed with great expectations as they are convenient to the patients and can be easily performed even in small laboratories.[27,28] However, the accuracy of stool antigen tests in different clinical situations and outside of controlled studies is a matter of concern.[29-31]

Table 3: Stool Antigen test and *H. pylori* status of the study population before treatment

H. pylori status	No of respondents	Stool Ag +ve	Stool Ag -ve
Positive	78	72 (92.3%)	6 (7.7%)
Negative	8	0 (0%)	8 (100%)

^{2}= 45.36, p<0.001

In the present study, 87.6% of patients with peptic ulcers were found to be *H. pylori* positive before treatment which corresponds to the findings (86.2%) of a study conducted by Karahan *et al*.[32] in Turkey. This is in contrast to lower detection rate of 44.2% *H. pylori* positive cases by Asfeldt *et al*.[16] in Norway and 55% by Trevisani *et al*.[33] in Italy. The higher detection rate in this study is probably due to that the study was done in developing country where 80-90% of population has *H. pylori*.

Table 4: Stool Antigen test and *H. pylori* status of the study

H. pylori status	No of respondents	Stool Ag +ve	Stool Ag -ve
Positive	5 (9.6%)	5 (100%)	0 (0%)
Negative	35 (67.3%)	0 (0%)	35 (100%)
Indeterminate	12 (23.1%)	0 (0%)	12 (100%)

^{2}= 52.00, p<0.001

population after 4 weeks treatment

The present study also demonstrated that *H. pylori* was found positive in 91.5% of duodenal ulcer and 95.8% antral erosion patients. Similarly Karahan *et al*.[32] found 100% and 90.5% *H. pylori* status positive among duodenal ulcer and antral erosion cases. Dominguez-Bello *et al*.[34] also found 96.7% *H. pylori* status positive among duodenal ulcer cases and 19% in erosive gastritis in Spain. This difference in erosive cases might be due to variation in age, economic status and ethnic background of the patients. The present study also demonstrated that monoclonal SAT was positive in 92.3% *H. pylori* positive status which is similar to the studies conducted in Turkey[32] (92%), Norway[16] (98.1%) and in Malaysia[35] (97.8%). We found 7.7% showed false negative results and no false positive results. Asfeldt *et al*.[16] identified 5.8% false negative and 1.8% false positive result. The reasons for false negative might be due to decreased bacterial density accompanied by low *H. pylori* stool antigen optical density leading to an erroneous diagnosis, and high genetic variability of bacterium leading to high variability of antigenic epitopes.

Sensitivity of monoclonal stool antigen for *H. pylori* in this study before treatment was 92.31% and specificity 100%. Asfeldt *et al*.[16] and Bhewa *et al*.[35] reported higher monoclonal SAT sensitivity and specificity. Gisbert *et al*.[36] carried out a systematic review and meta-analysis on the accuracy of these tests for diagnosis and for treatment

follow up and found pooled sensitivity and specificity were 94% and 97% respectively. High sensitivity and high specificity of monoclonal SAT due to use of monoclonal antibodies provides more concordant results between different kits. In case of the polyclonal *H. pylori* stool antigen test (rabbit origin), the antibodies in different kits come from different animals, giving rise to a different profile of antibodies. This leads inconsistency in results and overall a less reproducible test.

In this study, 9.6% were found to be *H. pylori* positive 4 weeks after treatment, 67.3% were found to be *H. pylori* negative, and remaining 23.1% had indeterminate result. Tasch *et al*.[37] also found 13.6% *H. pylori* positive and 86.4% *H. pylori* negative according to predefined criteria. Asfeldt *et al*.[16] similarly found 100% patients were negative by stool test and reference standard at follow up. In the present study, sensitivity and specificity of the monoclonal SAT was 100% and 100% respectively 4 wks after treatment. Similarly Asfeldt *et al*.[16] and Weingat *et al*.[38] found similar sensitivity and specificity after 6 weeks eradication therapy.

This study has a few limitations. First, the data was a cross-sectional; therefore, we can only postulate an association but not casualty between *H. pylori* and peptic ulceration. Secondly, the data were obtained in a specialized hospital from the population of the capital city and may not be generalized to other population of the country, especially rural areas.

Conclusion

The monoclonal SAT is a highly sensitive and specific tool for diagnosis of *H. pylori* infection, and can assess the success of eradication after therapy. It also offers the advantage of specificity and reliability over the invasive test. It is an easy to use, rapid test and does not require any special technology. In the present study, 87.6% of patients were found to be *H. pylori* positive before treatment. The monoclonal SAT was positive in 92.3% *H. pylori* positive patients, and 100% negative among *H. pylori* negative status patients. The monoclonal SAT revealed 92.3% sensitivity and specificity of 100% before treatment, and 100% sensitivity and 100% specificity after treatment. Based on this performance, monoclonal SAT can be used for screening *H. pylori* infection and assessing efficacy of treatment in the various healthcare settings of Bangladesh.

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