Original Article

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Comparison between stool antigen test and rapid urease test for Helicobacter pylori diagnosis in symptomatic cases of peptic ulcer disease at a tertiary care centre

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Abstract

Background: Peptic ulcer disease is defined as a breach in gastric epithelium of the inner lining of the gastrointestinal tract due to imbalance between destructive gastric acid, pepsin, and protective gastric mucosa. *Helicobacter pylori (H. pylori)* is a gram-negative, flagellated bacilli. *H. pylori* infection eradication is possible with antimicrobial therapy, so its prompt and appropriate diagnosis is very important.

Objectives: To compare between the results of stool antigen test (SAT) and rapid urease test (RUT), with assessment of sensitivity and specificity.

Methods: A hospital-based descriptive cross-sectional study in department of Internal Medicine at Birat Medical College and Teaching Hospital was conducted from 2023 January 2 to 2023 July 2 after institutional ethical clearance. Symptomatic gastritis patients visited in outpatient department, admitted and who gave consent were enrolled through consecutive sampling methods in this study. Biopsy sample obtained by upper gastrointestinal endoscopy of these patients were examined by RUT. ABON™ One Step *H. pylori* SAT Device was used for the detection of *H. pylori* antigens in the Stool Specimens obtained from these patients. Microsoft Excel sheet and SPSS v.23 were used for data analysis.

Results: More than two-thirds (105, 67.7%) were positive in upper gastrointestinal endoscopy and nearly two-thirds (100, 64.5%) had positive stool antigen tests.

Conclusion: This study showed the *H. pylori* stool antigen test is effective non-invasive way to find out *H. pylori* infection demonstrating no significant difference in specificity and sensitivity in comparison to RUT and can be considered as a routine diagnostic tool for surveying clinical significance as well as eradication of *H. pylori*.

Key words: Diagnosis; *Helicobacter pylori*; Peptic ulcer disease; Rapid urease test; Stool antigen test; Symptomatic gastritis.

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INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative helical or curved flagellated microaerophilic bacillus. It is triadic positive bacteria (catalase positive, oxidase positive, and urease positive). It has been usually considered as one of the commonest cause of gastritis, peptic ulcer disease (PUD), gastric adenocarcinoma, and gastric mucosa associated lymphoid tissue lymphoma.[1,2,3]

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Non-invasive diagnostic tests for *H. pylori* are C-urea breath test, serology and stool antigen test (SAT), whereas invasive test includes endoscopy and gastric mucosa biopsy which is examined using rapid urease test (RUT), based on the production of urease enzyme, or histopathological examination for detecting infection by *H. pylori*.[3-9] The RUT, which involves inserting tissue samples from the gastric mucosa into a commercially available analysis kit whose results are based on change in colour, is the most widely used invasive approach for detecting *H. pylori*.[6,7]

H. pylori infection effective treatment and eradication is possible by treatment with antimicrobial therapy, so it's prompt and appropriate diagnosis is very important.[3] Henceforth, in this research the outcome of SAT and RUT was studied with the objective to compare between the results of SAT and RUT, with assessment of sensitivity and specificity.

METHODOLOGY

A hospital-based analytical cross-sectional study was conducted in the department of Internal Medicine in Birat Medical College and Teaching Hospital. All symptomatic gastritis patients visiting the department of medicine in Birat Medical College and Teaching Hospital (BMCTH) without prior treatment were the samples of the study. All symptomatic gastritis patients visited in outpatient department (OPD), admitted and who gave informed consent were enrolled in this study. Consecutive sampling methods were conducted in the study. All the participants within the six months of the period from 2023 January 2 to 2023 July 2 were included in the study. The sample size was calculated using formula, $n = Z^2pq/e^2 = 151$; where prevalence, $p = 0.11$ (11%); $q = 1-p$; e = margin of error = 0.05 (5%) at confidence level of 95%. The total samples collected within the six months study period was 155. Microsoft Excel sheet was used for data recording, and Statistical Package for Social Sciences version 23 was used for data analysis. Cases were selected based on inpatient and OPDs of Internal Medicine, BMCTH on the basis of inclusion and exclusion criteria. All in-patients and OPD patients above age 18 years who gave informed consent and presented with in the study duration were included.

The terminally ill with serious medical condition and/or not in the state of giving consent were excluded. The ethical approval was obtained from the institutional review committee of BMCTH on 2023 January 2 (Ref. IRC-PA-263/2023). Informed consent was obtained from all the participants of the study.

The diagnosis of *H. pylori* infection was defined as positive for SAT positive results alone or RUT positive results in gastric mucosa biopsy specimens obtained from upper gastrointestinal (UGI) endoscopy, considered as gold standard methods. A patient was considered as *H. pylori* negative when RUT and SAT were both negative. The RUT was done with H-P Test RUT kit manufactured by Lenus Medicare and Research One Person Company (OPC) private limited (Kolkata, India) (ISO 13485:2016) which works as urea in sodium phosphate buffer, pH 6.5, whenever urease from *H. pylori* interacts turns into phenol red due to increase in pH and formation of sodium azide, ammonium and carbon dioxide.

The stool samples collected from these patients were kept at room temperature until used. Was used for the detection of *H. pylori* antigens in the stool specimens. The test was performed according to specifications provided by the manufacturer.

ABON™ One Step *H. pylori* Antigen Test Device (faeces) is a rapid immunochromatographic assay. It uses a strip mounted by anti-*H. pylori* monoclonal antibody, for the *H. pylori* infections detection in stool specimens of patients. Patient samples were taken by an applicator provided in the kit and mixed with the buffer provided in the test kit, and then a diluted sample was introduced to the kit and the kit was placed at room temperature and the results were observed within five minutes, the observation of the result was done within 20 minutes as there would be chances of false positives if result observation and interpretation took longer than 20 minutes. The complexes from the stool sample placed on the kit will bind to the antibody mounted on the membrane on reaching the test line and form a red colour line which indicates the positive result described by presence of *H. pylori* antigen on the provided stool sample. As the complexes moved forward on the membrane inside the kit another red line appeared on the control which indicated that the test kit was functioning properly and the test was valid .The red line on the control only indicated negative results. The red line only on the test but not on the control indicated the test kit was not functioning properly and is invalid.

Gastric mucosa obtained from biopsy was taken during UGI endoscopy from each patient. Two specimens were collected from the gastric antrum and the first part of duodenum. One among these samples is used for detection of *H. pylori* infection through RUT and another one is sent for histopathology examination.

The H-P Test kits (Lenus Medicare and Research, OPC Private Ltd Kolkata) which were used for RUT is flow immunochromatographic assay used for the detection of *H. pylori* qualitatively. The RUT is based on the production of urease enzymes by *H. pylori* bacteria and the presence of this enzyme in the gastric mucosa obtained with UGI endoscopy and biopsy. The *H. pylori* produces urease which hydrolyses urea into ammonia, raising pH of the medium, due to which color of the specimen changes form yellow which is negative (inferring absence of *H. pylori* infection) to red which means positive results which confirms *H. pylori* infection.

Microsoft Excel sheet 2016 and IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA), were used for data record and analysis respectively. Mean frequency was used for descriptive analysis along with percent and standard deviation. Chi-square test for categorical variables to test the level of significance. Significance level was observed at p-value less than 0.05. Sensitivity was calculated using the positive cases from both UGI Endoscopy and stool antigen (true positive) divided by the total positive cases from UGI Endoscopy. The specificity was calculated from the negatives obtained from both UGI Endoscopy and stool antigen (true negative) divided by the total negative cases from UGI Endoscopy.

RESULTS

The mean age of the total 155 participants in the study was 42.7 years. Almost half (75, 48.4%) of the respondents were between the age 30 years and 49 years. More than half (83, 53.5%) of the participants were female. More than one-quarter (42, 27.1%) had service as occupation followed by housewife (41, 26.5%) and day labour (36, 23.2%) respectively. More than one-quarter (42, 27.1%) of the participants were illiterate in this study and almost one-fifth (30, 19.4%) had completed primary education (Grade: 1-5). Equal proportion of the participants smoked and took alcohol in this study, that is; 27 (17.4%).

The clinical characteristics of the participants was more than two-thirds (111, 71.6%) had abdominal pain, 114 (73.5%) had nausea, and 98 (63.2%) of the participants had water brash. The mean systolic blood pressure (BP) and diastolic BP were 116.25 ± 13.6 mm Hg and 74.2 ± 8.8 mm Hg respectively (Table 2).

The mean body mass index (BMI) of the participants was 24.8 ± 4.0 kg/m^2 and more than one-third (59, 38.1%) were overweight (Figure 1). More than two-thirds (105, 67.7%) were positive in UGI endoscopy and nearly two-thirds (100, 64.5%) had positive stool antigen.

The sensitivity and specificity of stool antigen and its association with UGI endoscopy shows the sensitivity of Stool Antigen was 62 (59%) and the specificity was 12 (24%) (Table 3). The association between UGI endoscopy and stool antigen was statistically significant (p = 0.039).

The diagnosis of *H. pylori* infection was defined as positive for SAT positive results alone or RUT positive results in gastric mucosa biopsy specimens obtained from UGI endoscopy, considered as gold standard methods. A patient was considered as *H. pylori* negative when RUT and SAT were both negative. A total 155 symptomatic gastritis cases visited in medicine OPD, and those who were admitted in Birat Medical College and Teaching Hospital. Socio-demographic and behavioral characteristics of the participants were, the mean age of the participants in the study was 42.7 years. Almost half (75, 48.4%) of the respondents were between the age 30 to 49 years. The clinical characteristics of the participants in this study were, more than two-thirds (111, 71.6%) had abdominal pain, 114 (73.5%) had nausea, and 98 (63.2%) had water brash. More than two-thirds (105, 67.7%) was positive in UGI endoscopy and nearly two-thirds (100, 64.5%) had positive SATs.

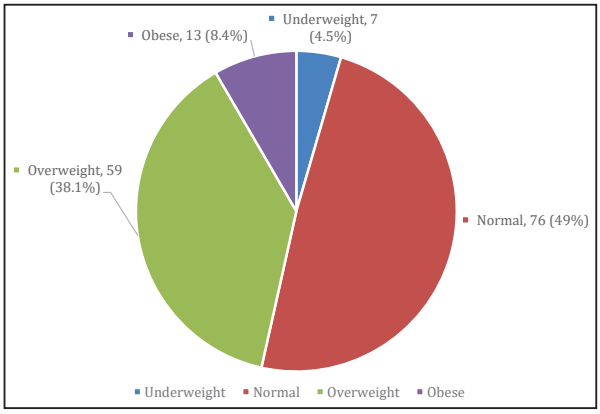

Figure 1: Body mass index of the participants (Mean ± SD= 24.8±4.0 kg/m^2)

Table 1: Clinical characteristics of the participants

Characteristics	Frequency (%)
Abdominal pain	
Yes	111 (71.6)
No	44 (28.4)
Nausea	
Yes	114 (73.5)
No	41 (26.5)
White brash	
Yes	98 (63.2)
No	57 (36.8)

Table 2: Blood pressure and other test results of the participants

Systolic BP (Mean±SD= 116.25±13.6 mm Hg)	Frequency (%)
Less than 120	81 (52.3)
120-139	60 (38.7)
140-159	12 (7.7)
160 or more	2 (1.3)
Diastolic BP (Mean±SD= 74.2±8.8 mm Hg)	
Less than 90	136 (87.7)
90-99	18 (11.6)
100 or more	1 (0.6)
Upper gastrointestinal endoscopy	
Positive	105 (67.7)
Negative	50 (32.3)
Stool antigen	
Positive	100 (64.5)
Negative	55 (35.5)

Table 3: Sensitivity and specificity of stool antigen and its association with upper gastrointestinal endoscopy

Stool Antigen	Upper gastrointestinal endoscopy		p-value
	Positive	Negative	
Positive	62 (59.0)	38 (76.0)	0.039
Negative	43 (41.0)	12 (24.0)	

DISCUSSION

This study was done in a tertiary health care centre to compare SAT with RUT in a symptomatic patient of PUD. This study showed that majority of PUDs was common in age group of 30 - 49 years which was slightly different from two cross-sectional studies published recently with most frequent group being above age 51 years and 52 years respectively.[1,2] In other similar single centre based study mean age was 44.7 years which is similar to this study.[3] This could be due to variation in the socio-demographic aspects of the two places or limited number of enrollment. This study showed disease common in house wives followed by daily wages labourer which in contrast was businessperson in a cross-sectional study.[1]

Among the participants most of them had not received primary level (five years of school) education which was not much studied and could be one of the areas to study. This research also stated that the PUD was more common in females than male as in other similar studies.[1–4]

The UGI endoscopy showed positive RUT on biopsy in 105 (67.7%) of participants which was 67%, 66.1% similar to other monocentric prospective studies.[4,5] And which is not 100% mostly because for RUT to come positive bacterial load need to be 10^4 per biopsy.[6] But the result was in difference to similar study conducted in Nepal,[7] where the positive RUT was only 17% and one of the other cross-sectional study where it was 43%.[8] Similarly stool antigen for *H. pylori* came positive in 100 (64.5%) cases which could be due to high sensitivity and specificity of the test in active disease.[9] Whereas it is high in comparison to 17% seen in a similar study done in a cross sectional study done at the tertiary health centre,[7] and 45% seen in other cross-sectional study in Cameroon.

Sensitivity and specificity of SAT in patients with RUT positive status post UGI endoscopy biopsy status when compared to similar studies were low.[1–4,7,9] However the data must have low cause the specificity and sensitivity always depends upon the sample obtained. Samples for RUT have various sensitivity according to the site of biopsy within the stomach.[10] With the above results we could not rule out the possibility of alternate diagnosis in the patient who were clinically considered peptic ulcer disease but had both RUT and SATs for *H. pylori* negative.

In an observational retrospective cross-sectional study, the sensitivity and specificity of SAT was comparable,[11] which could be helpful for choice of management with minimal invasive tests.

The limitations of the current study is that it was a single centre study in a private set up with low sample size.

CONCLUSION

This was a hospital-based retrospective study. This study showed the *H. pylori* SAT is a quick, simple, and non-invasive way to find out *H. pylori* infection. This test demonstrated great specificity and sensitivity. There was no significant difference between stool antigen tests and rapid urease tests sensitivity and specificity. In conclusion, stool antigen tests (SAT) can be considered as a routine diagnostic tool for surveying clinical significance as well as eradication of *H. pylori* in symptomatic gastritis cases in PUD with *H. pylori* infection. The authors recommend further studies regarding the *H. pylori* diagnosis and treatment in Nepal.

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