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Original Article

Comparison of Stool Antigen Test and CLO Test in Diagnosis of *Helicobacter pylori* Infection

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ABSTRACT

Helicobacter pylori (H. pylori) is a major cause of gastrointestinal diseases. Effective antimicrobial treatment depends on accurate diagnostic approaches. Stool antigen tests have recently been welcomed with great expectations as they are convenient for patients and can be easily performed even in small laboratories. The objective of the study was to explore the diagnostic usefulness of stool antigen test for H. pylori infection in dyspeptic patients. This cross-sectional study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet, from July 2012 to June 2013. A total of 150 dyspeptic patients were enrolled. Exclusion criteria were chronic liver disease, carcinoma stomach, coagulopathy, treatment with antibiotics within the last 4 weeks, and taking NSAIDs. Upper GI endoscopy was performed using a video endoscope, and biopsy materials were taken from the antrum and body of the stomach and tested for presence of H. pylori infection by a rapid urease test (CLO test). Stool was collected for detection of H. pylori antigen by an immunochromatographic tests (ICT) kit. The mean age of the patients were 43.35±16.30 years, and male to female ratio was 1.63:1. CLO and stool antigen tests were positive in 64.7% and 48.7% of patients, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the stool antigen test in the diagnosis of H. pylori infection was 72.7%, 94.3%, 95.9%, 64.9% and 80%, respectively. CLO test (Invasive) is still superior to the stool antigen (Non-invasive) in the diagnosis of H. pylori infection in dyspeptic patients.

Keywords: *CLO test, Helicobacter pylori, Stool antigen test.*

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INTRODUCTION

Helicobacter pylori (*H. pylori*) colonisation of the human gastric mucosa potentially leads to chronic gastritis that may progress to peptic ulcer disease[1]. *Helicobacter pylori* infection is one of the most common infections worldwide[2]. In Bangladesh, the reported prevalence of *Helicobacter pylori* infections in adults is high (>90%)[3]. Consequently, many histopathologists and microbiologists are being asked to detect infection with this organism, but there is no commonly acknowledged "Gold standard" method for diagnosing *Helicobacter pylori* infection.

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There are several diagnostic tools, which include invasive and non-invasive methods, for the diagnosis of *Helicobacter pylori* infection. But all of the tests have their pitfalls and limitations. Invasive tests, such as culture, histopathology, and Campylobacter like organism test (CLO test) also known as urease test, require an endoscopic biopsy of gastric tissue. The sensitivity and specificity of the rapid urease test are 83.3-86.9% and 95.1-97.2%, respectively. Histological examination has a sensitivity of 83-95% and a specificity of 90-100%, respectively. For culture, the reported sensitivity and specificity are 80-90.8% and 97.2-98.8%, respectively[4-6].

Non-invasive methods for the diagnosis of *Helicobacter pylori* infection include the urea breath test, serology, and stool antigen test. Urea breath test is costly and not available in every corner of Bangladesh. Anti-*Helicobacter pylori* immunoglobulin G (IgG), quantified by ELISA, was previously validated and adapted for use in US, Latin American, and Asian populations[7]. But serology, with its inherent weakness as indirect evidence, is unlikely to provide perfect information. Rapid faecal *Helicobacter pylori* antigen (HpSA) tests are becoming more widely used due to their low equipment requirements and ease of use. The *Helicobacter pylori* stool antigen (HpSAg) test is a simple, non-invasive test for detecting antigens in *Helicobacter pylori* from a small amount of stool sample. This test is generally based on immunochromatographic reactions between monoclonal antibodies to *Helicobacter pylori* and *Helicobacter pylori* antigens that may be present in the patient's stool sample[8]. Faecal antigen detection and urea breath test (UBT) are recommended noninvasive approaches for confirmation of *Helicobacter pylori* infection in paediatric patients because serologic tests are less reliable (Especially in children younger than 5 years) and are useful only for screening in this population[9]. The detection of *H. pylori* antigens in stools using polyclonal anti-*H. pylori* antibodies (HpSA) had a sensitivity and specificity of 88.8% and 94.5%, respectively[10]. Gisbert and Pajares[11] summarised 89 studies, including 10,858 untreated patients. The weighted mean sensitivity and specificity of HpSA were 91% and 93%, respectively.

The accurate diagnosis of a subject's *Helicobacter pylori* status is important for correct therapy and additionally, for the eradication programme. A literature search failed to reveal any relevant study carried out in the Bangladeshi population, and no study has been done on stool antigen tests in the detection of *Helicobacter pylori* infection in Sylhet M.A.G. Osmani Medical College Hospital, Sylhet. So, this study compared the non-invasive test for antigen detection in stool with one of the invasive tests (CLO test) in the diagnosis of *Helicobacter pylori* infection.

MATERIALS AND METHODS

This cross-sectional study was conducted in the department of microbiology at Sylhet M.A.G. Osmani Medical College, Sylhet, between July 2012 and June 2013. A total of 150 dyspeptic patients subjected to endoscopy of the upper GIT in the department of gastroenterology, aged 18 to 80 years, irrespective of sex, were enrolled by consecutive sampling. Exclusion criteria were chronic liver disease, carcinoma stomach, coagulopathy, and patients were on antibiotics within the last 4 weeks or NSAIDs. Prior to the commencement of this study, approval of the research protocol was obtained from the Ethical Committee of Sylhet M.A.G. Osmani Medical College, Sylhet, and written informed consent was obtained from each patient after explaining the aims and objectives of the study. The samples were collected from the patients attending the department of gastroenterology for an upper GI tract endoscopy according to inclusion and exclusion criteria. The clinical histories of the patients were noted. Each patient was examined thoroughly. The patient with clinical features suggestive of dyspepsia was selected for upper GI endoscopy. The endoscopic procedures were carried out by a qualified gastroenterologist using a video endoscope (Pentax-EPK-100). Biopsy materials were taken from the antrum and body of the stomach and tested for the presence of *Helicobacter pylori* infection by a rapid urease test (CLO test). A stool sample was collected from the patient for the detection of *Helicobacter pylori* antigen by a ICT device. Stool samples are diluted in specific diluents and subsequently applied to a support matrix. A positive test for *Helicobacter pylori* infection is indicated by the appearance of both a control line and a test line on the support matrix. A negative test was indicated by the appearance of only the control line. Any other combination or lack of lines on the matrix indicated an invalid result. All the findings, previous history and reports of investigations were recorded in a structured questionnaire. After collection, data were processed, and analysis was performed using SPSS (Statistical Package for Social Science) for windows version 21.0. Quantitative data were expressed as mean and standard deviation and qualitative data as frequency and percentage. Sensitivity, specificity, positive and negative predictive value, and accuracy of the stool antigen test were measured.

RESULTS

The age of the patients ranged from 18 to 80 years, with a mean age of 43.35±16.30 years. There were 32 (21.3%) patients in the age group of 21 to 30 years, 31 (20.7%) patients in the age group of 31 to 40 years, 30 (20%) patients in the age group of 41-50 years, 26 (17.3%) patients in the age group of 51-60 years, 21 (14%) patients in the age group of above 60 years and 10 (6.7%) patients in the age group up to 20 years. There were 93 (62%) males and 57 (38%) females with a ratio of males to females of 1.63:1 (Table-I). Endoscopic findings were normal in 85 (56.7%) patients, duodenal ulcer in 26 (17.3%) patients, gastritis in 23 (15.3%) patients, and gastric ulcer in 16 (10.7%) patients (Figure-1). CLO test was positive in 97 (64.7%) patients and negative in 53 (35.3%) patients (Figure-2). The stool antigen test was positive in 73 (48.7%) patients and negative in 77 (51.3%) patients (Figure-3). Using the CLO test as the gold standard, the sensitivity and specificity of the stool antigen test in the diagnosis of *Helicobacter pylori* infection were 72.7% and 94.3%, respectively. Positive and negative predictive values were 95.9% and 64.9%, respectively. The overall accuracy was 80% (Figure-4).

Table-I: *Distribution of demographic characteristics of the patients, N=150*

Demographic characteristics	Frequency	Percentage
Age		
≤20 years	10	6.7
21 to 30 years	32	21.3
31 to 40 years	31	20.7
41 to 50 years	30	20
51 to 60 years	26	17.3
≥ 60 years	21	14
Sex		
Male	93	62
Female	57	38

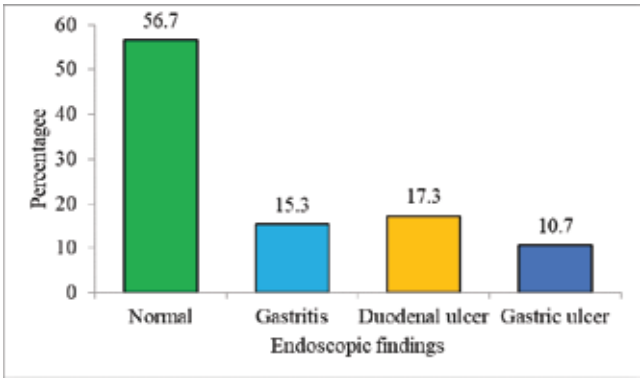

Figure-1: *Distribution of patients according to endoscopic findings, N=150*

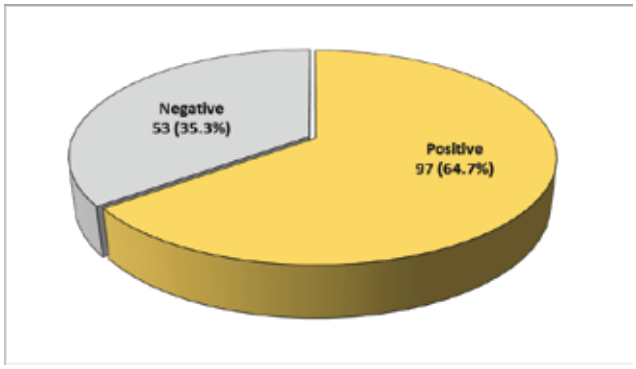

Figure-2: *Distribution of patients according to CLO test, N=150*

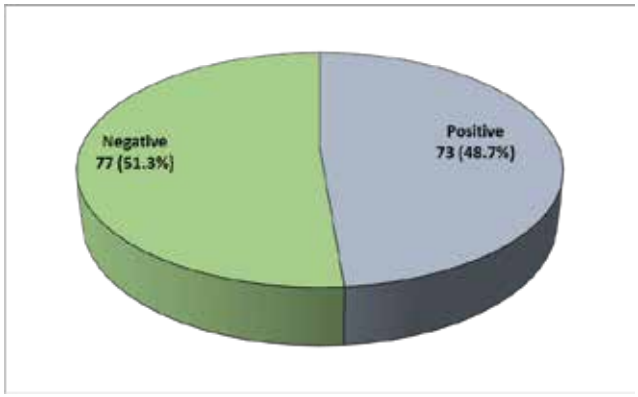

Figure-3: *Distribution of patients according to stool antigen test, N=150*

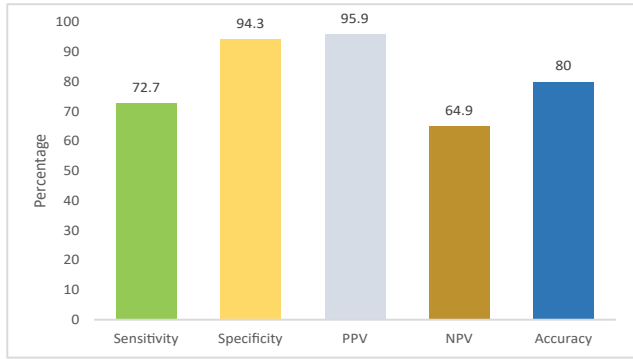

Figure-4: *Validity of stool antigen test in the diagnosis of H. pylori infection in patients with dyspepsia*

DISCUSSION

Helicobacter pylori infection has been established firmly with the development of peptic ulcer, chronic active gastritis, chronic persistent gastritis, atrophic gastritis, and gastric neoplasia, including gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphomas[9]. Recent studies have shown an association

between long-term infection with *Helicobacter pylori* and the development of gastric cancer, the second most common cancer worldwide[12]. In a developing country like Bangladesh, overcrowding, bad sanitation, and unhealthy practices favour the high prevalence of *Helicobacter pylori* in the population[13]. The risk of ulcer recurrence and associated complications is not diminished unless the *Helicobacter pylori* infection is cured. Effective antimicrobial treatment depends on sensitive and accurate diagnostic approaches. There are several invasive and noninvasive methods for the diagnosis of *Helicobacter pylori* infection. Invasive methods requiring endoscopic evaluation include bacteriologic culture, histopathologic studies, cytological examination of smears, rapid urease testing or CLO (Campylobacter like organism) testing and molecular studies. Non-invasive approaches include serologic testing, faecal antigen detection, and urea breath testing[9]. This cross-sectional study was conducted to explore the diagnostic usefulness of the stool antigen test compared with the CLO test in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients.

In this study, the age of the dyspeptic patients ranged from 18 to 80 years, with a mean age of 43.35±16.30 years. This result was supported by Syam et al[14]. They found that the mean age was 42.4±15 years. However, a higher mean age (47.6±14.0 years) was found in a study by Rahman et al.[15], whereas a lower mean age (37.98 years) was reported in another study by Mundey et al[8].

In the current study, there were 62% of patients in the age group of 21 to 50 years. In this regard, Islam et al.[16] reported that there were 74.74% of patients in the age group of 21 to 50 years.

In the present study, 62% of patients were male, and 38% of were female with a ratio of male to female of 1.63:1. This result was correlated with several studies[1,15,17]. But a nearly equal male-to-female ratio was reported in other studies[14,16].

Endoscopic findings in this study were normal in 56.7% of patients, duodenal ulcers in 17.3% of patients, gastritis in 15.3% of patients, and gastric ulcers in 10.7% of patients. Rahman et al.[15] reported that endoscopic diagnoses were normal in 24.4%, gastritis in 29.3%, duodenitis in 9.8%, peptic ulcer in 30.5%, gastric carcinoma in 4.8% and reflux esophagitis in 1.2% of their series of dyspeptic patients. The difference may be due to the exclusion of the gastric carcinoma in present study.

In this study, the CLO test was positive in 64.7% of patients and negative in 35.3% of patients. Nearly similar results were reported in several studies[16,18,19]. But Rahman et al.[15] found 48.8% of patients had rapid urease test positive.

In the present study, the stool antigen test was positive in 48.7% of patients and negative in 51.3% of patients. Alim et al.[20] reported that *Helicobacter pylori* stool antigen was detected in 29.6% of stool and Rahman et al.[16] found 71.9% positive stool antigen. The difference may be due to differences in the methods of diagnosis for *Helicobacter pylori* infection as the gold standard.

Using the CLO test as the gold standard, the present study showed that the sensitivity and specificity of the stool antigen test in the diagnosis of *Helicobacter pylori* infection were 72.7% and 94.3%, respectively. Positive and negative predictive values were 95.9% and 64.9%, respectively. The overall accuracy was 80%. In this regard, using rapid urease test (RUT) and histopathology as gold standard methods for the diagnosis of *Helicobacter pylori* infection, the diagnostic accuracy of simple *Helicobacter pylori* cassette tests was 87.5%[18]. Rahman et al.[16] found that the sensitivity and specificity of ICT in the diagnosis of *Helicobacter pylori* infection were 90.2% and 81%, respectively. Positive and negative predictive values were 93.2% and 73.9%, respectively. The overall accuracy was 82.9%.

CONCLUSION

The CLO test (Invasive) is still superior to the stool antigen test (Non-invasive) in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients. So, stool antigen can be used as the initial screening test. However, further studies with a larger sample size involving multicenters should be carried out to determine the validity of the stool antigen compared the gold standard test in the diagnosis of *Helicobacter pylori* infection among dyspeptic patients.

LIMITATIONS

The limitations of the study were that this was a single-centre study, the sample size was small due to time constraints and budgetary limitations, and the CLO test was taken as the gold standard in the diagnosis of *Helicobacter pylori* infection.

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