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Original Research Article

The Diagnostic Usefulness of Stool Antigen Test with Serum *Helicobacter pylori* Antibody and CLO Test in the Diagnosis of *Helicobacter pylori* Infection in Dyspeptic Patients

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Abstract

Introduction*: Helicobacter pylori* colonization of the human gastric mucosa potentially leads to chronic gastritis that may progress to peptic ulcer disease. Additionally, this micro-organism has been identified as a risk factor for the development of gastric carcinoma and gastric lymphoma. Its relation with non-ulcer dyspepsia has not been clear. ***Objective:*** To assess the diagnostic usefulness of stool antigen test with serum *Helicobacter pylori* antibody and CLO test in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients. ***Materials and Methods*:** This was a cross sectional study was conducted in the Department of Microbiology, Sylhet M.A.G. Osmani Medical College in collaboration with the Department of Gastroenterology Sylhet M.A.G. Osmani Medical College, Sylhet from July 2012 to June 2013. The calculated sample size was 196 but in this study we took 150 patients due to financial constrain and time limitation. Patients upper GI tract for dyspepsia and whoever fulfilled the inclusion and exclusion criteria were considered as study sample. All the findings, previous history and reports of investigations were recorded in a preformed data collection sheet prepared for this purpose. The patient with clinical features suggestive of dyspepsia was selected for upper GI endoscopy. ***Results:*** For this purpose, 150 dyspeptic patients fulfilling the inclusion and exclusion criteria were enrolled. The age of the patients ranged from 18 to 80 years with the mean age of 43.35 (SD ± 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 (20.0%) patients in the age group of 41-50 years, 26 (17.3%) patients in the age group of 51-60 years, 21 (14.0%) 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 (62.0%) male and 57 (38.0%) female with a ratio of male to female was 163:1. Showed the distribution of patients according to endoscopic findings. Endoscopic findings was 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. The distribution of patients according to CLO test. CLO test was positive in 97 (64.7%) patients and negative in 53 (35.3%) patients. The patients according to serum *Helicobacter pylori* antibody by ELISA. Serum *Helicobacter pylori* antibody was positive in 101 (67.3%) patients and negative in 49 (32.7%) patients. Stool antigen test was positive in 73 (48.7%) patients and negative in 77 (51.3%) patients. Using CLO test as the gold standard the sensitivity and specificity of stool antigen test in the diagnosis of *Helicobacter pylori* infection was 72.7% and 94.3% respectively. Positive and negative predictive values were 95.9% and 64.9% respectively. The overall accuracy was 80.0%. There was moderate agreement between the two test (Kappa, k=0.603; p<0.001). Using CLO test as the gold standard in the diagnosis of *Helicobacter pylori* infection the sensitivity and specificity of serum *Helicobacter pylori* antibody was 89.7% and 82.2% respectively. Positive and negative predictive values were 86.1% and 79.6% respectively. The overall accuracy was 84.0%. There was a substantial agreement between the two test (Kappa, k=0.644; p<0.001). ***Conclusion:*** The overall accuracy was 80.0%. There was good agreement between the two test (Kappa, k=0.603). Using CLO test as the gold standard in the diagnosis of *Helicobacter pylori* infection this study showed that the sensitivity and specificity of serum *Helicobacter pylori* antibody was 89.7% and

82.2% respectively. Positive and negative predictive values were 88.7% and 100.0% respectively. The overall accuracy was 84.0%. In conclusion comparing CLO test (invasive) is still the superior to stool antigen and serology (non-invasive), both of which is going very close to each other in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients.

Keywords: Stool Antigen Test, Serum *Helicobacter Pylori*, Antibody, CLO Test, *Helicobacter Pylori* Infection.

INTRODUCTION

Helicobacter pylori colonization of the human gastric mucosa potentially leads to chronic gastritis that may progress to peptic ulcer disease. Additionally, this micro-organism has been identified as a risk factor for the development of gastric carcinoma and gastric lymphoma [1]. Its relation with non-ulcer dyspepsia has not been clear [2]. *Helicobacter pylori* infection is one of the most common infections worldwide [3]. *Helicobacter pylori* is a Gram negative spiral-shaped bacterium that is characterized by its many unipolar flagella which give it corkscrew-like motility, and its prodigious production of urease. *Helicobacter pylori* produces large amount of enzyme urease, which breaks endogenous urea to carbon dioxide and ammonia. Carbon dioxide and water then produces bicarbonate and neutralizes gastric acid, thus ensures survival in acidic environment. The other product ammonia encircles the bacteria and helps to move easily through the thick mucin protection layer and causing toxicity to stomach epithelium. *Helicobacter pylori* also produces toxins, catalase, protease, phospholipase; all of them damage epithelium. Catalase protects them from free radical injury. Individual infected with *Helicobacter pylori* showed a reduction in gastric juice ascorbic acid levels and may also impair the protective role of carotenoids in the stomach [4, 5]. Human being is the only reservoir of *Helicobacter pylori* and infection is predominantly acquired by oral-oral or fecal-oral and iatrogenic transmission by endoscopy [6]. Person to person transmission is most commonly implicated with feacal/oral or oral/oral or gastric/oral pathways each have supportive biologic as well as epidemiologic evidence [7]. Like many common gastrointestinal infections, infection is associated with conditions of crowding and poor hygiene and with intrafamilial clustering [8]. The organism has been recovered most reliably from vomitus and from stools during rapid gastrointestinal transit [9]. These findings raise the hypothesis that gastroenteritis episodes provide the opportunity for *Helicobacter pylori* transmission. *Helicobacter pylori* produce a protease that reduces the ability of acid to diffuse through the mucus. A majority of *Helicobacter pylori* infections are acquired during early childhood in particular, in developing countries [10, 11]. It is thought that *Helicobacter pylori* infections may persist throughout life, if not treated, and that spontaneous eradication of *Helicobacter pylori* infection is uncommon [12]. In Bangladesh, the reported prevalence of *Helicobacter pylori* infections in adults is high (>90%) [13] and recently 50%–60% of the children from a poor setting in Dhaka were infected with *Helicobacter pylori* at 2 years of age [11]. A typical seasonality, peaking in spring and autumn, was observed for the acquisition of *Helicobacter pylori* infection, coinciding with peak rates of infection with enterotoxigenic *E. coli* in the same setting [14]. The rate of reinfection with *Helicobacter pylori* in industrialized countries is usually low (0.5% to 2.0% per year) [15, 16], whereas it is 10% per year in Bangladesh [13, 17]. Poor socioeconomic status, lack of education, poor hygiene and sanitation, over-crowding were associated with a higher prevalence of *Helicobacter pylori* infection [18]. Densely populated developing country like Bangladesh has all these factor to create favorable environment for *Helicobacter pylori* infection. In Bangladesh the prevalence of *Helicobacter pylori* was 42% already by 2 years of age with a rapid increase to 67% by 10 yrs of age and that as many as 84% of 6 to 9 years old children were *Helicobacter pylori* positive in any areas where sanitary conditions were very poor [19]. Consequently many histopathologists and microbiologist are being asked to detect infection with this organism, but there is no commonly acknowledged "Gold standard" method for diagnosing *Helicobacter pylori* infection. There are several diagnostic tools, which include invasive and non-invasive methods, for the diagnosis of *Helicobacter pylori* infection, are available. But all of the tests have their pitfalls and limitations. Invasive tests, such as culture, histopathology and biopsy urease test require endoscopic biopsy of gastric tissue. Culture allows testing for susceptibility of antimicrobials and its sensitivity and specificity is 77-95% and 100% respectively. Rapid urease test is a qualitative assay for the detection of urease and its sensitivity and specificity are 89-98% and 93-98% respectively. Histopathology by haematoxylin-eosin and modified Giemsa staining is important to detect *Helicobacter pylori* under light microscope and its sensitivity and specificity are 93-98% and 95-98% respectively [20]. In a study at BIRDEM, the positivity of culture is 59.3%, for rapid urease test it is 60.4%, for histopathology it is 34.4% [21]. Culturing also has a low sensitivity, therefore a combination of the tests is recommended as gold standard [22]. Non-invasive methods include the Urea breath test (costly and not available in Bangladesh), Serology and Stool antigen test. Anti– *Helicobacter pylori* immunoglobulin G (IgG), quantified by ELISA previously validated and adapted for use in US, Latin American, and Asian populations [8]. This novel rapid test is based on monoclonal antibody immunochromatography of stool samples. The test has been reported to be very specific (98%) and sensitive (94%). The results are positive in the initial stages of infection and can be used to detect eradication after treatment [23]. Direct fecal antigen detection of

Helicobacter pylori has been approved by the US Food and Drug Administration for diagnosis and follow-up testing. Meridian Bioscience (Cincinnati, OH) developed a commercial kit (Premier Platinum HpSA) for the rapid, noninvasive detection of *Helicobacter pylori* antigens by enzyme immunoassay. *Helicobacter pylori* antigens from fresh human fecal specimens are detected by polyclonal antibodies adsorbed to microwells. The sensitivity and specificity of fecal antigen detection were approximately 89% and 94% to 95%, respectively, in multiple studies [24]. To explore the diagnostic usefulness of stool antigen test with serum *Helicobacter pylori* antibody and CLO test in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients.

MATERIALS AND METHODS

This was a cross sectional study was conducted in the Department of Microbiology, Sylhet M.A.G. Osmani Medical College in collaboration with the Department of Gastroenterology Sylhet M.A.G. Osmani Medical College, Sylhet from July 2012 to June 2013. The calculated sample size was 196 but in this study we took 150 patients due to financial constrain and time limitation. Patients upper GI tract for dyspepsia and whoever fulfilled the inclusion and exclusion criteria were considered as study sample.

Inclusion Criteria

1. All dyspeptic patients subjected to endoscopy of upper GIT in the Department of gastroenterology and aged between 18-80 years irrespective of sex.

Exclusion Criteria

1. Patients who refused endoscopy
2. Failed endoscopy
3. Severely ill patients
4. Chronic liver disease
5. Carcinoma stomach
6. Coagulopathy
7. Patients who received antibiotic within last 4 weeks.
8. Patients taking NSAIDs.

The samples were collected from the patients attending the Department of Gastroenterology for upper GI tract endoscopy according to inclusion and exclusion criteria. The clinical histories of the patients were noted. Each patient was examined thoroughly. All the findings, previous history and reports of investigations were recorded in a preformed data collection sheet prepared for this purpose. The patient with clinical features suggestive of dyspepsia was selected for upper GI endoscopy. All the instruments were sterilized properly prior to perform endoscopy. The endoscopic procedures were carried out by qualified gastroenterologist. Biopsy materials were taken from antrum of the stomach and were tested for presence of *Helicobacter pylori* infection by Rapid urease test (CLO test). With all aseptic measure 5 ml venous blood was collected from patient using a disposable syringe. Serum was separated and was preserved at -20^{0}C till serological analysis. Estimation of anti *Helicobacter pylori* IgG antibody in the serum was determined by ELISA using commercially available kits manufactured by DRG international (Lot No. RN-46145). Stool was collected from patient for detection of *Helicobacter pylori* antigen by 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.

Statistical Analysis

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 and serum *Helicobacter pylori* IgG antibody were done.

RESULTS

This cross-sectional study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet during the period from July 2012 to June 2013 with a view to explore the diagnostic usefulness of stool antigen test and serum *Helicobacter pylori* antibody comparing with CLO test in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients. For this purpose, 150 dyspeptic patients fulfilling the inclusion and exclusion criteria were enrolled. The age of the patients ranged from 18 to 80 years with the mean age of 43.35 (SD ± 16.30) years. Table I showed the age distribution of the patients. The distribution of the patients on the basis of age group. 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 (20.0%) patients in the age group of 41-50 years, 26 (17.3%) patients in the age group of 51-60 years, 21 (14.0%) 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 (62.0%) male and 57 (38.0%) female with a ratio of male to female was 163:1. Showed the distribution of patients according to endoscopic findings. Endoscopic findings was 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.

Table 1: Demographic profile, Clinical details of the patients (N=150)

	Frequency	Percentage
Age Group		
≤20 years	10	6.7
21-30 years	32	21.3
31-40 years	31	20.7
41-50 years	30	20.0
51-60 years	26	17.3
>60 years	21	14.0
Mean± (SD) Range	43.35±16.30(18 to 80)	
Sex		
Male	93	62.0
Female	57	38.0
endoscopic findings		
Gastric ulcer	16	10.7
Duodenal ulcer	26	17.3
Gastritis	23	15.3
Normal	85	56.7

Figure 1 showed the distribution of patients according to CLO test. CLO test was positive in 97 (64.7%) patients and negative in 53 (35.3%) patients.

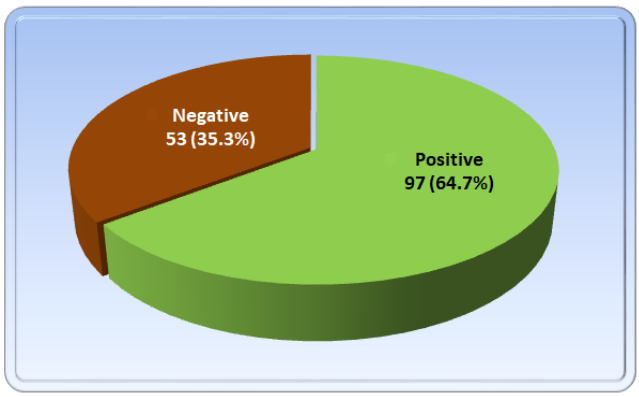

Figure 1: Distribution of patients according to CLO test (n=150)

Figure 2 showed the distribution of patients according to serum *Helicobacter pylori* antibody by ELISA. Serum *Helicobacter pylori* antibody was positive in 101 (67.3%) patients and negative in 49 (32.7%) patients.

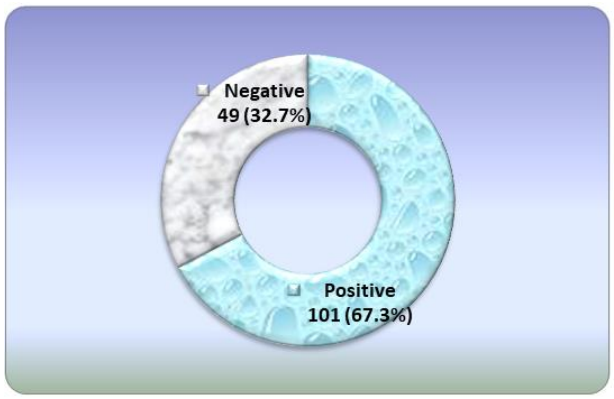

Figure 2: Distribution of patients according to serum *Helicobacter pylori* antibody by ELISA (n=150)

Figure 3 showed the distribution of patients according to stool antigen test. Stool antigen test was positive in 73 (48.7%) patients and negative in 77 (51.3%) patients.

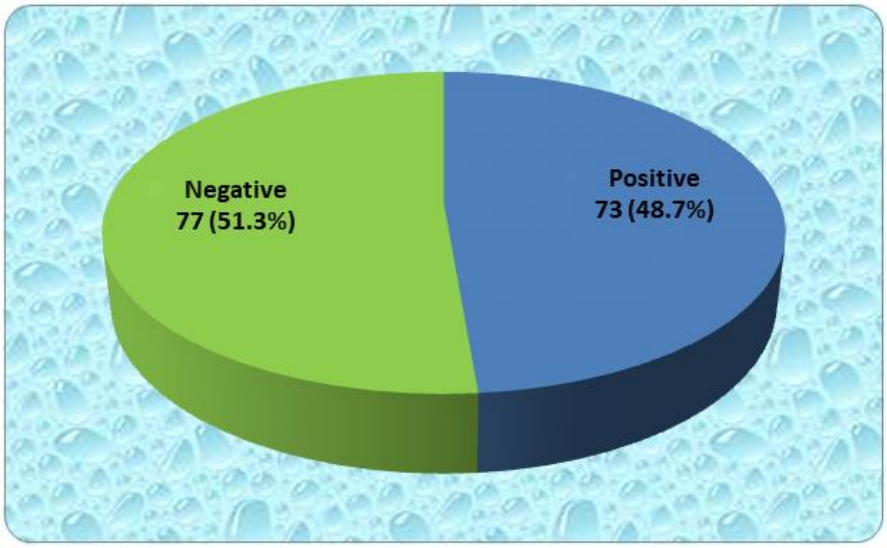

Figure 3: Distribution of patients according to stool antigen test (n=150)

Table-2 showed the cross tabulation of stool antigen test and *Helicobacter pylori* infection. Using CLO test as the gold standard the sensitivity and specificity of stool antigen test in the diagnosis of *Helicobacter pylori* infection was 72.7% and 94.3% respectively. Positive and negative predictive values were 95.9% and 64.9% respectively. The overall accuracy was 80.0%. There was moderate agreement between the two test (Kappa, k=0.603; p<0.001).

Table-2: Cross tabulation of stool antigen test and *Helicobacter pylori* infection

Stool antigen test	*H pylori* infection (CLO test)		Total
	Positive	Negative	
Positive	70 (a)	3 (b)	73
Negative	27 (c)	50 (d)	77
Total	97 (a+c)	53 (b+d)	150

Sensitivity=a/(a+c)X100=70/(70+27) X100=72.7%
Specificity=d/(b+d)X100=50/(3+50) X100=94.3%
Positive predictive value=a/(a+b) X 100=70/(70+3) X100=95.9%
Negative predictive value=d/(c+d) X 100=50/(27+50) X100=64.9%
Accuracy= (a+d)/(a+b+c+d)=(70+50)/(70+3+27+50) X100=80.0%

Table-3 showed the cross tabulation of serum *Helicobacter pylori* antibody by ELISA and *Helicobacter pylori* infection. Using CLO test as the gold standard in the diagnosis of *Helicobacter pylori* infection the sensitivity and specificity of serum *Helicobacter pylori* antibody was 89.7% and 82.2% respectively. Positive and negative predictive values were 86.1% and 79.6% respectively. The overall accuracy was 84.0%. There was a substantial agreement between the two test (Kappa, k=0.644; p<0.001).

Table-3: Cross tabulation of serum *Helicobacter pylori* antibody by ELISA and *Helicobacter pylori* infection

Serum *H. pylori* antibody	*H pylori* infection (CLO test)		Total
	Positive	Negative	
Positive	87 (a)	14 (b)	101
Negative	10 (c)	39 (d)	49
Total	97 (a+c)	53 (b+d)	150

Sensitivity=a/(a+c)X100=87/(87+10) X100=89.7%
Specificity=d/(b+d)X100=39/(10+39) X100=82.2%
Positive predictive value=a/(a+b) X 100=87/(87+10) X100=86.1%
Negative predictive value=d/(c+d) X 100=39/(10+39) X100=79.6%
Accuracy= (a+d)/(a+b+c+d)=(87+39)/(87+14+10+39) X100=84.0%

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 [25]. Recent studies have shown an association between long term infection of *Helicobacter pylori* and the development of gastric cancers, the second most common cancer worldwide. Peptic ulcer disease has shown a very high prevalence in Bangladesh and is important among the major chronic problems encountered by the physicians and surgeons [26]. In a developing country like Bangladesh, overcrowding, bad sanitation and unhealthy practice favor high prevalence of *Helicobacter pylori* in the population [27]. The association of *Helicobacter pylori* with gastritis, duodenal ulcer and gastric cancer has been reported by investigators from different countries all over the world. Similar studies have been done in our country and high association of *Helicobacter pylori* in peptic ulcer and gastric cancer has been reported by Ahmed *et al*.,; Vakil *et al*., and Sultana *et al*., [27-29]. The risk of ulcer recurrence and associated complications are not diminished unless *Helicobacter pylori* infection is cured. Effective antimicrobial treatment depends on sensitive and accurate diagnostic approaches. There are several invasive and non invasive methods for diagnosis of *Helicobacter pylori* infection. Invasive methods requiring endoscopic evaluation include bacteriologic culture, histopathologic studies, cytological examination of smear, rapid urease test or CLO (Campylobacter like organism) test and molecular studies. Non invasive approaches include serologic testing, fecal antigen detection and urea breath testing [25]. Although culture is the gold standard, but it is very difficult to perform, requires an enriched transport medium, is expensive and results are delayed. Histology also takes at least 3-4 days and is costly. A further limitation of uses of histology with regard to sensitivity and specificity is the quality of biopsies. If the biopsy is too small, is poorly oriented or is inappropriately fixed or stained, detection of *Helicobacter pylori* may not be possible. Among the noninvasive methods, *Helicobacter pylori* antibody can be detected in serum by Enzyme Linked Immuno Sorbent Assay (ELISA) and also by Immuno Chromatographic (ICT) rapid spot test [27]. This cross-sectional study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet during the period from July 2012 to June 2013 with a view to explore the diagnostic usefulness of stool antigen test and serum *Helicobacter pylori* antibody comparing with CLO test in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients For this purpose, 150 dyspeptic patients fulfilling the inclusion and exclusion criteria were enrolled. The outcome of the study was discussed below: In this study the age of the dyspeptic patients ranged from 18 to 80 years with the mean age of 43.35 (SD ± 16.30) years. This result was supported by Syam *et al*., [30] found that the mean age of their patients was 42.4±15 years and ranging 16-73 years. In this regards Rahman *et al*., [31] found that the age of patients ranged 18 to 75 years with a mean age of 47.6 years and standard deviation 14.0 years. Rahman *et al*., [1] in their another study found that age of their dyspeptic patients were aged between 18 to 75 years with a mean age of 47.4 years and standard deviation ±13.7 years. Islam *et al*., [22] reported the age of the dyspeptic patients was between 16 to 70 years with a mean age of 37.98 years. In the current study 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 (20.0%) patients in the age group of 41-50 years, 26 (17.3%) patients in the age group of 51-60 years, 21 (14.0%) patients in the age group of above 60 years and 10 (6.7%) patients in the age group up to 20 years. In this regards Rahman *et al*., [31] found that there were 11 (13.4%) patients in the age group up to 30 years, 15 (18.3%) patients in the age group of 31 to 40 years, 21 (25.6%) patients in the age group of 41-50 years, 22 (26.8%) patients in the age group of 51-60 years and 13 (15.9%) patients in the age group of above 60 years. Islam *et al*., [22] reported that there were 28 (34.57%) patients in the age group of 21 to 30 years, 20 (24.69%) patients in the age group of 31 to 40 years, 11 (13.58%) patients in the age group of 41-50 years, 11 (13.58%) patients in the age group of 51-60 years, 6 (7.41%) patients in the age group of 61-70 years and 5 (6.17%) patients in the age group up to 20 years. In the present study there were 97 (62.0%) male and 57 (38.0%) female with a ratio of male to female was 163:1. This result was correlated with the study of Rahman *et al*., [31] that there were 49 (59.8%) male and 33 (40.2%) female of their patients with dyspepsia. In another study Rahman *et al*., [1] found that 53 (58.9%) were males and 37 (41.1%) were females with a male female ratio of 1.4: 1. Jekarl *et al*., [32] found that 59.3% of their patients were male and 40.7% of their patients were female. Islam *et al*., [22] reported that 42 (51.85%) were males and 39 (48.15%) were females with a male female ratio 1: 92.86. Syam *et al*., [30] found that 31 (49.2%) patients were males and 32 (50.8%) patients were females. Endoscopic findings in this study was 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. Rahman *et al*., [31] reported that endoscopic diagnoses was normal in 20 (24.4%), gastritis in 24 (29.3%), duodenitis in 8 (9.8%), peptic ulcer in 25 (30.5%), gastric carcinoma in 4 (4.8%) and reflux esophagitis in 1 (1.2%) patients among their series of dyspeptic patients. In another study Rahman *et al*., [1] found that At 20 (22.2%) patients had normal gastro-duodenal mucosa, 27 (30.0%) had gastritis, 9 (10.0%) had duodenitis, 28 (31.1%) had peptic ulcer disease, 4 (4.4%) had gastric carcinoma and 2 (2.2%) had reflux oesophagitis at endoscopy. Jekarl *et al*., [32] found the endoscopic

results with respect to the gastric mucosa were as follows: normal, 27 (12.9%); atrophic gastritis, 60 (28.7%); erosive gastritis, 50 (23.9%); erythematous gastritis, 25 (11.9%); combination of atrophic and erosive gastritis, 26 (12.4%); combination of erosive and erythematous gastritis, 7 (3.3%); and combination of atrophic and erythematous gastritis, 14 (6.7%). Ulcers with or without scars were observed in 41 patients (19.7%), gastric polyps in 43 (20.6%), and adenoma in 4 (1.9%). Syam *et al*., [30] found that endoscopic findings were gastric cancer 1.6%, peptic ulcer 4.8%, duodenal ulcer 7.9%, esophagitis 6.3%, gastritis 77.7% and gastroduodenitis 4.8%. In this study CLO test was positive in 97 (64.7%) patients and negative in 53 (35.3%) patients. Demiray *et al*., [33] found nearly similar result of rapid urease test of 68.18% among their of series of patients. Baqai *et al*., [34] found CLO test was positive in 26 (60.5%) cases. Islam *et al*., [6] found that 63 (73.26%) dyspeptic patients were rapid urease test positive. In another study Islam *et al*., [22] reported that 61 (75.31%) dyspeptic patients were rapid urease test positive. Rahman *et al*., [31] found 40 (48.8%) were rapid urease test positive among their dyspeptic patients. In the present study serum *Helicobacter pylori* antibody was positive in 101 (67.3%) patients and negative in 49 (32.7%) patients. Similar results observed in the study of Alim *et al*., [35] that *Helicobacter pylori* IgG antibody was found positive in 487 (69.0%) of the blood serums of 705 patients taken into their study, whereas found negative in 218 (31.0%). Baqai *et al*., [34] found *Helicobacter pylori* antibody titer was positive in 33 (76.7%) cases. In the present study stool antigen test was positive in 73 (48.7%) patients and negative in 77 (51.3%) patients. Alim *et al*., [35] reported that *Helicobacter pylori* stool antigen was detected in stool in 209 (29.6%) of total 705 persons, whereas antigen could not be detected in 496 persons (70.4%). Demiray *et al*., [33] found that rapid *Helicobacter pylori* stool antigen was detected in stool in 209 (29.6%) of total 705 persons, whereas antigen could not be detected in 496 persons (70.4%). Difference may be due to difference in the methods of diagnosis *Helicobacter pylori* infection as gold standard. Rahman *et al*., [31] found that 59 (71.9%) patients were ICT positive for *Helicobacter pylori* infection. Using CLO test as the gold standard the present study showed that the sensitivity and specificity of stool antigen test in the diagnosis of *Helicobacter pylori* infection was 72.7% and 94.3% respectively. Positive and negative predictive values were 95.9% and 64.9% respectively. The overall accuracy was 80.0%. There was moderate agreement between the two test (Kappa, k=0.603). In this regards using 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% [33]. Rahman *et al*., [31] found that the sensitivity and specificity of ICT in the diagnosis of *Helicobacter pylori* infection was 90.2% and 81.0% respectively. Positive and negative predictive values were 93.2% and 73.9% respectively. The overall accuracy was 82.9%. Using CLO test as the gold standard in the diagnosis of *Helicobacter pylori* infection this study showed that the sensitivity and specificity of serum *Helicobacter pylori* antibody was 89.7% and 82.2% respectively. Positive and negative predictive values were 86.1% and 79.6 respectively. The overall accuracy was 84.0%. There was substantial agreement between the two test (Kappa, k=0.644). In this regards Baqai *et al*., [34] found that using CLO test as the gold standard in the diagnosis of *Helicobacter pylori* infection the sensitivity and specificity of serum *Helicobacter pylori* antibody was 81.0% and 29.0% respectively. Positive and negative predictive values were 64.0% and 50.0% respectively. The overall accuracy was 60.0%. Performance of the ELISA kit varies in different populations. Laheij *et al*., [36] reviewed a range of sensitivity of 57%-100% and a range of specificity of 31%-100% for different commercial kits in different populations. A study in the Netherlands evaluated eight commercial ELISA tests and found sensitivities of 93%-98% and specificities of 95%-98% [37]. Using another commercial kit, a study from Bangladesh found sensitivity, specificity, PPV, and NPV of 100%, 13.6%, 54.8%, and 100%, respectively [38].

CONCLUSION

Using CLO test as the gold standard the present study showed that the sensitivity and specificity of stool antigen test in the diagnosis of *Helicobacter pylori* infection was 72.7% and 94.3% respectively. Positive and negative predictive values were 95.9% and 64.9% respectively. The overall accuracy was 80.0%. There was good agreement between the two test (Kappa, k=0.603). Using CLO test as the gold standard in the diagnosis of *Helicobacter pylori* infection this study showed that the sensitivity and specificity of serum *Helicobacter pylori* antibody was 89.7% and 82.2% respectively. Positive and negative predictive values were 88.7% and 100.0% respectively. The overall accuracy was 84.0%. In conclusion comparing CLO test (invasive) is still the superior to stool antigen and serology (non-invasive), both of which is going very close to each other in the diagnosis of *Helicobacter pylori* infection in dyspeptic patients.

Limitation of the study

This study was not without limitations. The limitations were:

- This study was conducted in a single centre among dyspeptic patients which may not represent the overall picture prevailing in different hospitals and geographical locations.
- Sample size was small due to time constraint and budgetary limitations.
- CLO test was taken as gold standard in the diagnosis of *Helicobacter pylori* infection.

RECOMMENDATION

The following recommendations are made:

One of the two non invasive tests (stool antigen and anti- *Helicobacter pylori* antibody) can be used as the initial screening test especially when large number of patients needs to be screened. Stool collection appears to be easy then blood collection especially in children in our setup.

Further studies with larger sample size involving multicenter should be carried out to determine the validity of stool antigen and anti-*Helicobacter pylori* antibody comparing gold standard test in the diagnosis of *Helicobacter pylori* infection among dyspeptic patients.

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