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Original Article

Efficacy of Different Laboratory Tests to Diagnose *Helicobacter pylori* Infection

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Abstract :

Helicobacter pylori is a Gram negative bacteria which causes chronic gastritis, peptic ulcer disease, primary B-cell gastric lymphoma, and adenocarcinoma of the stomach. There are a set of laboratory tests to diagnose *H. pylori* infection with a variable accuracy, they are divided into non-invasive tests and invasive tests. Non-invasive tests include serology, urea breath test (UBT) and stool antigen test (SAT). Invasive tests include rapid urease test (RUT), histology and culture. This cross sectional study was carried out in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University (BSMMU) and *H. pylori* laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from July 2008 to September 2009 to evaluate the efficacy of RUT, SAT and Culture as a diagnostic tool for *H. pylori*. Dyspeptic patients were collected from outpatient department of BSMMU. Out of 224 dyspeptic patients 149 patients had ulcers or erosions in the stomach or duodenum. Stool sample could be collected from 139 patients. RUT has sensitivity of 100%, specificity 80.28%, positive predictive value 85% and negative predictive value 100%. Regarding culture, sensitivity is 100%, specificity 94.37%, positive predictive value 95% and negative predictive value 100%. Stool antigen test has sensitivity 95.94%, specificity 92.31%,positive predictive value 93% and negative predictive value 95%.

Key words: *Helicobacter pylori*, laboratory test, Rapid Urease Test, Culture, Stool Antigen Test.

Introduction :

Helicobacter pylori (H. pylori) is a slow growing micro-aerophilic, highly motile, Gram negative spiral bacteria that chronically infects stomach[1]. In most patients *H. pylori* does not cause symptoms and the infection often persists without any clinically evident disease. However, only 10-20% of *H. pylori* infected patients develop severe diseases during their lifetime including chronic gastritis, peptic ulcer disease(PUD), primary B-cell gastric lymphoma, and adenocarcinoma of the stomach[2,3]. A study conducted on Bangladeshi children by ICDDR,B scientists has shown that 60% are infected by the age of 3 months and 80% are infected by 3 years of age[4]. In adult, about 92% have been found to be sero-positive for *H. pylori* antibody[5]. PUD has been a major cause of morbidity and rarely mortality for more than a century. Approximately 10% of individual in western countries develop PUD at some point in their life time[6]. In a survey conducted in a defined population aged 15 years and above in Bangladesh, the prevalence of duodenal and gastric ulcer was estimated to be 11.98% and 3.58% respectively, found to be much higher than in western countries[7]. To diagnose *H. pylori* no single test can stand alone as "gold standard" as none is 100% accurate[8]. Diagnostic tests are divided into Non-invasive tests and invasive tests. Non-invasive tests include serology, urea breath test and stool antigen test. Invasive tests includes rapid urease test (RUT), histology and culture[8]. Urea breath test is not available in our country and serology is a nonspecific test as nearly 100% of adult are positive for this. So this study was conducted to compare the diagnostic efficacy of rapid urease test, stool antigen test and culture.

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Materials and Methods:

This cross sectional study was carried out in the Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University (BSMMU) and *H. pylori* laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) from July 2008 to September 2009. Dyspeptic patients aged between 15 to 60 years attended to outpatient department (OPD) of BSMMU who had not taken Proton Pump Inhibitor (PPI), H_2 receptor blocker or antibiotics in the preceding 2 weeks and had no contraindication for endoscopy were initially enrolled for the study. Every ethical issue was discussed with the patients regarding the study and informed written consents were obtained. Detailed clinical history was taken and through physical examination was done. After that, patients were underwent upper GI endoscopy in the department of Gastroenterology, BSMMU by an experienced endoscopist under topical lignocaine anesthesia. Between patients the endoscopes was carefully cleaned and disinfected by first keeping the tube immersed in cidex (2.2-2.4% activated glutaraldehyde solution) solution for 10 minutes and then rinsing it with sterile distilled water, biopsy forceps were also cleaned in same manner. Patients found to have ulcers or erosions anywhere in the stomach and duodenum at endoscopy, were included in the study. During endoscopy two biopsy sample ware taken from the antrum within 2 cm of the pylorus and two sample ware taken from the body of the stomach within 8 cm of the cardia along the greater curvature[9,10]. One sample from each site was incubated to RUT kit and results were recorded in data sheet. Another two samples were stored in 1ml BHIA containing 25% glycerol for *H. pylori* culture. Stool was collected by patients from home in a previously supplied container with proper labelling. Then, stool and biopsy samples were transported to *H. pylori* laboratory of ICDDR,B. In the laboratory prepared seeded plates from biopsy sample were incubated for 3 to 6 days at 37^{o}C in a double gas incubator with 5% O_2, 10% CO_2, and 85% N_2. The incubated plates were periodically examined from 2 days onwards for the growth of *H. pylori*. Presumptive *H. pylori* colonies were propagated on new plate for confirmation and stock for storage at -86^{o}C. Monoclonal stool antigen test were done from stool samples. All data were recorded in a printed data sheet, statistical analysis were done by commercially available SPSS software.

Results:

A total 224 dyspeptic patients were initially enrolled for upper GI endoscopy. Of them, 149 patients had ulcers or erosions anywhere in the stomach or duodenum up to second part. They were included in this study. Out of 149 patients 92 (61.7%) were RUT positive and 57 (38.3%) were RUT negative. Stool sample can be collected from 139 patients, among them 76 (51%) were positive and 63 (42.3%) were negative for monoclonal antigen. Total 82 patients (55%) were positive for H pylori culture and rest 67 (45%) samples were negative (Table I).

All three modalities of tests were done in 139 patients among them all tests were positive in 71 (51.08%) patients. Out of three laboratory tests 82 patients were positive for at least any two of them. But as a diagnostic gold standard, two biopsy based tests should be positive for *H. pylori* diagnosis; here we found 78 (52.35%) patients were positive for both RUT and culture. On the basis of this gold standard, a total of 78 of the patients were *H. pylori* positive.

Table I: Efficacy of different laboratory tests (N=149)

Test Name	Positive No (%)	Negative No (%)
Rapid Urease Test	92 (61.7%)	57(38.3%)
Stool Antigen Test	76 (51%)	63 (42.3%)
Culture	82 (55%)	67 (45%)

RUT: According to gold standard, 78 patients were true positive, 14 patients were false positive, 57 were true negative and none were false negative. So, sensitivity (Sn) of RUT is 100%, specificity (Sp) 80.28%, positive predictive value (PPV) 85% and negative predictive value (NPV) 100%.

Culture: Here also according to gold standard, 78 patients were true positive, 4 were false positive, 67 were true negative and none were false negative. So, sensitivity is 100%, specificity 94.37%, positive predictive value 95% and negative predictive value 100%.

Stool antigen test: Here 71 patients were true positive, 5 patients were false positive, 60 were true negative and 3 were false negative. So, sensitivity is 95.94%, specificity 92.31%, positive predictive value 93% and negative predictive value 95% (Table II).

Table II: Diagnostic accuracy of different tests

Test	Sn (%)	Sp (%)	PPV (%)	NPV (%)
RUT	100	80.28	85	100
Culture	100	94.37	95	100
SAT	95.94	92.31	93	95

Discussion:

There is no single test that can be considered the gold standard for the diagnosis of *H. pylori*. Diagnostic testing for *H. pylori* can be divided into those that do and those that do not require endoscopy.

Endoscopic/Invasive Diagnostic Tests

There are presently four biopsy-based diagnostic methods for *H. pylori* infection. These include the Rapid Urease Test (RUT), histology, culture, and molecular testing.

Rapid Urease Testing

The RUT identifies active *H. pylori* infection through the organism's urease activity. Gastric biopsies are obtained and placed into an agar gel or on a reaction strip containing urea, a buffer, and a pH-sensitive indicator. In the presence of *H. pylori*'s urease, urea is metabolized to ammonia and bicarbonate leading to a pH increase in the microenvironment of the organism. A change in color of the pH sensitive indicator signifies the presence of active infection. Commercially available kits yield results in 1-24 h. There are a number of commercially available RUT kits including the CLOtest, HpFast, HUT-test, Pronto Dry, and Pyloritek with overall pretreatment sensitivities of >90% and specificities of >95%[11,12]. Medications that reduce the density and/or urease activity of *H. pylori*, such as bismuth-containing compounds, antibiotics, or PPIs, can decrease the sensitivity of the RUT by up to 25%[11]. In this study sensitivity is 100% but with a bit low specificity (80.28%), which may be due to more false positive results. The simplicity, low cost, and relatively rapid results make the RUT a practical and cost-effective means of testing for *H. pylori* in patients not taking antibiotics, bismuth, or PPIs who require uppers endoscopy.

Histology

Histology has been considered by some to be the gold standard for detection of *H. pylori*[55]. Unfortunately, histology is an imperfect gold standard as the detection of *H. pylori* relies upon a number of issues including the site, number, and size of gastric biopsies, method of staining, and the level of experience of the examining pathologist[13]. Warren used the Warthin-Starry silver stain for the visualization of *H. pylori*. Detection is also possible with a number of other stains such as modified Giemsa, haematoxylineosine, Genta, toluidine blue, Romanouski and immunochemical methods. Although widely available and capable of achieving sensitivity and specificity of >95%, the cost and need for properly trained personnel are limitations of histology in clinical practice. Histology was not done in this study.

Culture:

Culture is highly specific method for identifying active *H. pylori* infection. Conceptually, culture is attractive because it not only provides a means by which to identify infection, but also allows characterization of antimicrobial sensitivities[14]. Isolation of *H. pylori* from gastric biopsy samples is difficult and not always successful. Cultures should be inspected from day 3 to day 14. *H. pylori* forms small (1-mm), translucent, smooth colonies[15]. Upon successful sub-culturing, *H. pylori* isolates tend to adapt to the growth conditions used in the laboratory. Subsequently, good growth can generally be achieved following 1 to 3 days of incubation when reference strains and laboratory-adapted isolates of *H. pylori* are used. Culturing techniques for *H. pylori* are demanding and costly and as a consequence, only available in a limited number of clinical laboratories. Study by Doorn LJV[16] in their study shows sensitivity of 89.5% and specificity of >98%. In this study sensitivity and specificity both are >94%.

Molecular methods

PCR was found to be as sensitive as culture in detecting *H. pylori* in gastric biopsies[17]. Real-time PCR is now becoming more popular. But in our country PCR is not available for *H. pylori*.

Nonendoscopic Diagnostic Tests

Antibody Tests

Antibody testing relies upon the detection of IgG antibodies specific to *H. pylori* in serum, whole blood, urine or even saliva. IgG antibodies to *H. pylori* typically become present approximately 21 days after infection and can remain present long after eradication[18]. Antibodies to *H. pylori* can be quantitatively assessed using Enzyme-Linked Immunosorbent Assay (ELISA) and latex agglutination techniques or qualitatively assessed by using office-based kits. Unfortunately, several factors limit the usefulness of antibody testing in clinical practice. A meta-analysis evaluated the performance characteristics of several commercially available quantitative serological assays and found their overall sensitivity and specificity to be 85% and 79%, respectively, with no differences between the different assays[19]. Tests that detect active infection, although more expensive, are preferable to serology as these reduce the number of patients inappropriately treated for presumed *H.pylori* infection[20,21]. Serology was done in our patients.

Urea Breath Tests

Urea Breath Test like Rapid Urease Test identifies active *H. pylori* infection by way of the organism's urease activity.

In the presence of *H. pylori*, the ingestion of urea, labeled with either the non-radioactive isotope ^{13}C or the radioactive isotope ^{14}C, results in production of labeled CO_2, which can be quantitated in expired breath[22,23]. Overall, the performance characteristics of both tests are similar with sensitivity and specificity typically exceeding 95% in most studies[22,23]. The UBT also provides an accurate means of post treatment testing[24]. This test is also not available in our country and was not done in present study.

Stool Antigen Test

The Stool antigen test identifies *H. pylori* antigen in the stool by enzyme immunoassay with the use of polyclonal anti-*H. pylori* antibody. Recently, a stool test utilizing a monoclonal anti-*H. pylori* antibody has been evaluated[25,26]. As both tests detect bacterial antigen(s) suggestive of ongoing infection, they can be used to screen for infection and as a means of establishing cure following therapy. The Stool antigen test has been approved by the United States Food and Drug Administration (USFDA) and endorsed by the European Maastricht II and subsequently Maastricht III Consensus Report as an alternative means of establishing *H. pylori* cure to urea breath testing[27]. When a gold standard based on at least two diagnostic methods (different from stool antigen tests) was used, the accuracy of the stool antigen test pretreatment was confirmed to be very high (sensitivity 91%, specificity 94%, positive predictive value 92%, and negative predictive value 86%). In the present study we found sensitivity 95.9%, specificity 92.3%, positive predictive value 93% and negative predictive value 95%.

Conclusion :

There are a set of laboratory test to diagnose *H. Pylori* infection. We discussed three of them : RUT. Culture and SAT. Of them culture and SAT has got sensitivity, specificity, PPV and NPV more than 90%.

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