

Original Article

Validation of diagnostic tests and epidemiology of *Helicobacter pylori* infection in Bangladesh

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Abstract

Introduction: *Helicobacter pylori* infection is associated with gastritis, peptic ulcer, and gastric cancer. We conducted a cross-sectional study to compare five diagnostic tests for *H. pylori* infection and studied the epidemiology of the infection in Bangladesh.

Methodology: Bangladeshi patients with dyspeptic symptoms referred for endoscopic examination were enrolled in this study. Each patient underwent upper endoscopic examination and four gastric biopsy specimens were taken. We used 5 tests for the diagnosis of *H. pylori*; culture, histology confirmed by immunohistochemistry, rapid urease test (RUT), urinary and serological test. Demographic and environmental variables were collected.

Results: A total of 133 patients participated in the study, 61 males and mean age 37.3 ± 12.3 years. We used the culture and/or histology results as the gold standard to estimate the sensitivity, specificity, positive and negative predictive values for the studied diagnostic tests. RUT, culture and histology had high sensitivity and specificity with moderate positive and negative likelihood ratio, whereas urine test and serology showed a good sensitivity and specificity but poor likelihood ratio. The overall prevalence of *H. pylori* among study subjects was 47% with no difference between gender and age groups.

Conclusions: The invasive tests showed better performance than noninvasive tests among Bangladeshi population. The overall prevalence of *H. pylori* was less than the previously reported in the region with no difference among all age groups.

Key words: Bangladesh; *Helicobacter pylori*; prevalence; diagnosis test.

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Introduction

Helicobacter pylori (*H. pylori*) is a well-recognized pathogen that chronically infects more than half of the world's population and is associated with gastritis and the gastritis-associated diseases including peptic ulcer and gastric cancer [1-4]. Gastric cancer is the third leading cause of cancer death worldwide with substantial geographical variation of its incidence [5]. According to the age-standardized incidence rate (ASR) of the malignancy, specifically for gastric cancer, countries can be categorized as high risk (e.g. Japan, China, Korea, Bhutan), intermediate risk (e.g. Vietnam) or low risk (e.g. Northern America, Southern and Southeastern Asia) [6]. Despite the strong association between *H. pylori* infection and gastric cancer, a high

prevalence of *H. pylori* infection in a population does not always follow a high incidence rate of gastric cancer. This observation exists in Africa and South Asia, the so-called African and Asian enigma [7-10].

Bangladesh is incorporated in Asian Enigma. The ASR of gastric cancer in Bangladesh is 5.7 per 100,000 [5] whereas the prevalence of *H. pylori* infection is more than 90% in asymptomatic adult and 80% in children diagnosed by serum ELISA and Urea Breath Test (UBT), respectively [11,12]. The reason behind the low incidence of gastric cancer in Bangladesh remains still unanswered. It could be due to the genetic diversity of the infecting *H. pylori* strains, differences in the host genetic background, or due to

environmental factors and dietary habits of Bangladeshi population.

There are several tests available for the diagnosis of *H. pylori* infection, consisting of invasive tests, i.e. rapid urease, histology, bacterial culture and PCR; and noninvasive tests, i.e. serology, ¹³C-urea breath test, stool antigen, and *H. pylori* antibody in urine. A wide variation between the diagnostic performance of invasive and noninvasive methods has been documented [13-17]. The discrepancies might be due to the use of different methodology and gold standard in the respective study. To date there is no universally accepted single test for diagnosis of *H. pylori* infection, and often a combination of tests is recommended as gold standard [18-21]. Despite limitations serology is a popular test for mass screening for *H. pylori* infection. Currently there is no consensus about the gold standard method for diagnosis of *H. pylori* infection in Bangladesh and a scanty information regarding validation of various diagnostic tests. Therefore, we aimed to compare five diagnostic tests for *H. pylori* infection (rapid urease, culture, histology confirmed by immunohistochemistry (IHC), serum antibody, and urinary antibody). In addition, we studied the epidemiology and risk factors of *H. pylori* infection in relation with socio demographic factors.

Methodology

Study population

Patients with dyspeptic symptoms who referred for endoscopic examination from Gastroenterology outpatient department of Dhaka Medical College were enrolled in this study. Patients who received *H. pylori* eradication therapy or treatment with antibiotics, bismuth-containing compounds, H₂-receptor blockers, or proton pump inhibitors within 4 weeks prior to the examination as well as patients with history of partial gastrectomy were excluded. Each patient underwent upper endoscopic examination and four gastric biopsy specimens were taken (three from the antrum and one from the corpus). The specimens from the antrum were used for *H. pylori* culture, rapid urease test and histological examination while the specimens from the corpus were only used for histological examination. Biopsy specimens for culture were kept at –20 °C on the same day of collection and later transferred to –80 °C until culture was performed. The culture of *H. pylori* had been performed by our experienced microbiologist (PS). Endoscopic findings were applied for the diagnosis of peptic ulcers and gastric cancer. Gastric cancer was further confirmed by histopathology.

Gastritis was defined as *H. pylori* gastritis in the absence of both peptic ulcer and gastric cancer

Demographic information, occupation, family size living in the same household, consumption of betel nut, and aspects household environment were collected. The study started in October 2015 and ended November 2015. Written consent form was obtained from each participant before going under endoscopic examination. The protocol was approved by the Ethics Committee of Bangladesh Medical Research Council, Bangladesh and Oita University Faculty of Medicine, Japan.

Diagnostic tests of H. pylori infection

We used 5 different tests for the diagnosis of *H. pylori* infection.

Culture

To culture *H. pylori*, one biopsy specimen from the antrum was homogenized in saline and inoculated onto Mueller Hinton II Agar medium (Becton Dickinson, Sparks, MD, United States) supplemented with 7% horse blood (Nippon Biotest Laboratories Inc., Tokyo, Japan) without antibiotics. The plates were incubated for up to 10 days at 37 °C under microaerophilic conditions (10% O₂, 5% CO₂ and 85% N₂). *H. pylori* were identified based on colony morphology, Gram staining and positive reactions for oxidase, catalase, and urease.

Histology confirmed by IHC

All biopsy materials were fixed in 10% buffered formalin for 24 hours and then embedded in paraffin for histological examination. Serial sections were stained with hematoxylin and eosin and with May-Giemsa stain. The status of the gastric mucosa was evaluated according to the updated Sydney system [22]. To confirm to determine the *H. pylori* status, IHC was performed as described previously [23]. Briefly, after antigen retrieval and inactivation of endogenous peroxidase activity, tissue sections were incubated with the α -*H. pylori* Ab (DAKO, Glostrup, Denmark) overnight at 4 °C. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG (Nichirei Co., Tokyo, Japan), followed by incubation with a solution of avidin-conjugated horseradish peroxidase (Vectastain Elite ABC kit; Vector Laboratories Inc., Burlingame, CA, United States). Peroxidase activity was detected using a H₂O₂/diaminobenzidine substrate solution. The status of the degree of the bacterial load was evaluated according to the updated Sydney system; classified into four grades: 0, “normal”; 1, “mild”; 2, “moderate”; and

3, “marked” [22]. A bacterial load grade greater than or equal to 1 was defined as *H. pylori* positive.

Rapid Urease Test

CLO (Kimberly Clark Ballard Medical Products, Atlanta, Georgia, United States) was used as a rapid urease test to detect the presence of *H. pylori* urease.

Urinary *H. pylori*

Urinary *H. pylori* status was evaluated by rapid urine test (RAPIRUN *H. pylori* antibody, Otsuka Pharmaceutical Co., Tokyo, Japan) according to the manufacturer’s instructions. Urine was collected before endoscopy and immediately after collection was tested for *H. pylori* antibodies.

Serum *H. pylori*

Serum *H. pylori* antibody was evaluated with a commercially available ELISA kit (Eiken Co., Ltd., Tokyo, Japan) and according to the manufacturer’s instructions. Blood samples were collected from each patient before the endoscopy and, serum was separated and cryopreserved at -20 °C until it was subjected to assay of antibody titer in serum. Determination of the cutoff value was set to a titer equal or more than 10 U/mL and was labeled as *H. pylori* positive according to the manufacturer’s instructions.

Statistical analysis

Standard methods were used to calculate sensitivity, specificity, and positive predictive value (PPV), negative predictive value (NPV), the likelihood ratio (LR) and accuracy with 95% confidence interval.

Each method was tested against predefined gold standard. Agreement between different diagnostic tests was evaluated by calculating the Cohen’s kappa coefficient. Sub-analyses with receiver operating curves (ROCs) were performed to determine the optimal cut point for serological test. The differences between the groups were evaluated by the nonparametric Mann–Whitney U-test. The crude and age adjusted measures of the association between single possible risk factor and *H. pylori* infection was expressed as odds ratio (OR) and 95% confidence interval (CI). Logistic regressions were used subsequently is used to assess the relative importance of the risk factors by controlling the confounding factors. All statistical testing were 2-tailed, and the level of significance was $P < 0.05$. The IBM SPSS 21 statistical software package was used for statistical analyses.

Results

A total of 133 patients participated in the study, 61 males and mean age 37.3 ± 12.3 years old (range 18-65 years). Endoscopy diagnosed duodenal ulcer in 3 (2.3%), gastric ulcer in 2 (1.5%), gastric and duodenal ulcer in 2 (1.5%). We defined “gastritis” as “*H. pylori* gastritis confirmed by histology. When culture and/or histology results yielded positive results, patients were defined as *H. pylori* positive. Following the criteria, the prevalence of *H. pylori* infection was 33.3% with duodenal ulcer, 50% with gastric ulcer, 50% with both gastric and duodenal ulcer and 47.6% (60/126) in patients without ulceration.

Table 1 Performance of different diagnostic tests for *H. pylori* infection where each method is tested against culture and or histology as gold standard.

Diagnostic test	RUT	Urine test	Histology	Serology	Culture
<i>H. pylori</i> -positive number	58	63	54	40	56
(%)	(43.6%)	(47.4%)	(40.6%)	(30.1%)	(42.1%)
Sensitivity	90.5%	79.4%	85.7%	54.0%	92.1%
(95%CI)	(79.7%-6%)	(67.3% - 88.5%)	(74.6% - 93.3%)	(40.9% - 66.6%)	(82.4% - 97.4%)
Specificity	98.5%	81.2 %	100 %	91.4 %	100 %
(95%CI)	(91.0% -99.9%)	(69.9% -89.6%)	(94.9% - 100%)	(82.3% - 96.8%)	(94.9% - 100%)
PPV	98.5%	79.4%	100%	85.0%	100.0%
(95%CI)	(91.0% -99.9%)	(67.3% - 88.5%)	(93.4% -100%)	(70.2% - 94.3%)	(93.8% -100%)
NPV	92.0%	81.2 %	88.6 %	68.8 %	93.3 %
(95%CI)	(82.7% - 96.7%)	(69.9% - 89.6%)	(79.5% - 94.7%)	(58.4% - 78.0)	(85.1% - 97.8%)
Likelihood ration (+)	63.3	4.21		6.30	
(95%CI)	(9.03-444)	(2.54 -6.98)		(2.83 -13.99)	
Likelihood ration (-)	0.10	0.25	0.14	0.50	0.08
(95%CI)	(0.05- 0.21)	(0.15 - 0.42)	(0.08 - 0.26)	(0.38 -0.66)	(0.03 - 0.18)
Accuracy	94.7%	80.3%	93.2%	73.6%	96.2%
(95%CI)					

RUT, rapid urease test, PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Comparison of diagnostic tests of H. pylori infection

We used the culture and/or histology results as the gold standard to estimate the sensitivity, specificity, positive and negative predictive values for the studied diagnostic tests. We found that RUT, culture and histology had high sensitivity and specificity with moderate positive and negative likelihood ratio, whereas urine test and serology showed a good sensitivity and specificity but poor likelihood ratio (Table 1). Table 2 shows the concordance between the results of five tests those were used in this study. The kappa coefficient was best between culture and RUT: 0.90, was 0.85 between histology and RUT and 0.78 between culture and histology. Sub analysis with ROC curve demonstrated that using the new cut points 5.50, the sensitivity of the serology test became more than 75% from without decreasing test specificity (Figure 1).

Prevalence of H. pylori infection and its associations with the studied variables among the studied patients

H. pylori infection was defined by the detection of the presence of *H. pylori* on histological and/or culture examination of gastric biopsies. The overall prevalence of *H. pylori* among the 133 participants was 47% with no difference between males and females ($p = 0.12$). There was no significant difference between all age groups; however, the peak was at age group 30-39 (59%). Age adjusted ORs were calculated for *H. pylori* positivity in relation to the study variables (Table 3). The overall prevalence of *H. pylori* infection among people reside in the villages of Bangladesh was slightly lower than those reside in the cities (42%. Vs. 50.6% $p = 0.07$); however, the difference did not reach a significant level due the small sample size. The prevalence of *H. pylori* infection was examined in relation to the number of family members living in the same household which reflects crowding living condition, type of occupation, marital status, smoking, and consumption of betel nut that showed no association with *H. pylori* prevalence. The results did not alter after age adjusted analysis was applied with all the variables in the model.

Discussion

Our current study is the first study to evaluate three invasive diagnostic tests (RUT, culture, histology) and two non-invasive tests (serology, *H. pylori* antibody in urine) in Bangladeshi population. The culture showed the highest performance with high sensitivity and specificity similar to other studies [15-19]. However, culture has technical difficulties in isolation and culturing the bacteria that discourage its use as gold

Table 2 Agreement between the results of the diagnostic tests for *H. pylori* infection among the studied patients.

Diagnostic test	Kappa coefficient
RUT-Culture	0.908
RUT-Rapid Urine test	0.558
RUT-Histology	0.846
RUT-Serology (ELISA)	0.398
Culture-Rapid Urine test	0.558
Culture-Histology	0.784
Culture-Serology (ELISA)	0.430
Rapid Urine test-Histology	0.526
Urine antibody-Serology	0.460
Histology-serology	0.447

standard [13]. The sensitivity and specificity of histology in the current study were in consistent with previous reports [15-21]. To optimize the yield of histology a number of issues including the site, number and size of gastric biopsies, method of staining and the expertise of the examining pathologist have to be considered [24]. The advantages of histologic examination are detecting *H. pylori*, and providing the status of mucosal changes such as inflammation, atrophy, intestinal metaplasia, and malignancy [22].

We found that the RUTs had > 90% sensitivity, specificity, positive predictive value, negative predictive value, accuracy with high positive and low negative likelihood ratio in this study. Morshed et al. reported similar results earlier [25]. The important issues to be taken into account that good accuracy of

Figure 1. ROC curve of serum *H. pylori* antibody in 133 patients.

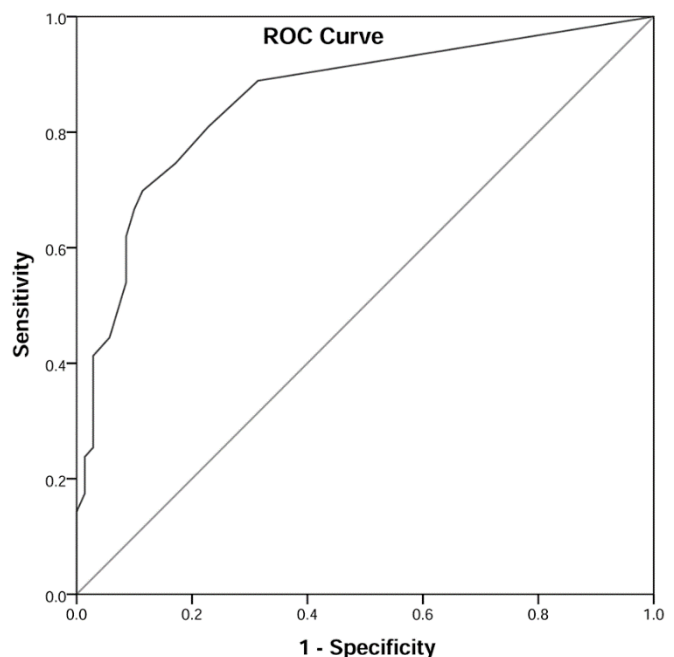


Table 3. Distribution of *H. pylori* (HP) infection according to the study variables among 133 Bengali.

Variable		Total (+Hp %)	Crude OR 95% CI	Age Adjusted OR 95% CI
Age	< 20	7 (43%)	Ref	
	20-29	32 (47%)	1.2 (0.66 - 4.3)	
	30-39	34 (59%)	2.0 (0.8-6.5)	
	40-49	32 (44%)	1.0 (0.7-4.4)	
	50-59	20 (45%)	1.0 (0.7-4.8)	
	≥ 60	8 (25%)	0.3 (0.05 -5.93)	
	Total	133 (47.3%)		
Gender	Males	61 (47.5%)	Ref	Ref
	Females	72 (47.2%)	0.98 (0.49-1.95)	0.91 (0.36-4.30)
Residence place	City	83 (50.6%)	Ref	Ref
	Village	50 (42%)	0.8 (0.69 -2.89)	0.6 (0.47- 2.66)
# of people in the household	1-3 persons	24 (37.5%)	Ref	Ref
	4-6 persons	82 (50%)	1.66 (0.66-4.37)	1.14 (0.51-6.28)
	> 6 persons	27 (48.1%)	1.54 (0.50-4.84)	1.08 (0.40-8.13)
Chew betel nut	No	96 (47.9%)	Ref	Ref
	Yes	37 (45.9%)	0.92 (0.42 -1.97)	0.81 (0.50-3.60)
Occupation	House wives	55 (45.5%)	Ref	Ref
	Laborer	30 (50%)	1.2 (0.49-2.94)	1.4 (0.36- 5.79)
	Service	26 (46.2%)	1.02 (0.39-2.62)	1.86 (0.42-8.33)
	Others	22 (50%)	1.2 (0.44-3.25)	1.2 (0.73-14.77)
Marital status	Single	24 (41.7%)	Ref	Ref
	Married	109 (48.6 %)	1.32 (0.64- 3.31)	1.03 (0.55-6.17)
Smoking	No	106 (46.2 %)	Ref	Ref
	Yes	27 (51.9 %)	1.25 (0.53-3.94)	1.074 (0.34 -2.35)

RUT is adversely affected by reduced density of *H. pylori* and or urease activity resulting from drugs (bismuth, proton pump inhibitors, antibiotic) as well as acute bleeding ulcer [26-31].

The noninvasive rapid urine test was introduced for the first time in Bangladeshi population in this study. This kit detects antibody in the urine requires only 20minutes for obtain the status of *H. pylori* result. The sensitivity, specificity, and accuracy of the kit in Japanese population have been reported to be 92%, 93.1%, and 92.3%, respectively [32] which is much higher than our findings. The insufficient antibodies in the urine or difference of the *H. pylori* strain as antigen might be the possible reason for the low performance of the test.

Serological tests are mostly IgG based, have been validated in Western populations against invasive methods with acceptable sensitivity and specificity [33]. Burucoa et al compared 29 commercial serological tests kits and reported sensitivities 55.6%-100%, specificities 59.6%-97.9%, positive predictive values 69.8%-100% and negative predictive value 68.3%-100% [34]. When culture was used as a gold standard, the sensitivities and the specificities of serological tests were 80–95% and 80–95% respectively [15] and with histology as a gold standard the sensitivities were ranging from 76% to 84% and the

specificities were ranging from 79% to 90% [35]. The overall performance of serology against predefined gold standard in our study was not satisfactory. It differs from previous study conducted on 45 Bangladeshi patients where the sensitivity, specificity, and positive and negative predictive values were 100%, 13.6%, 54.8% and 100% for serological test against culture [25]. One possible explanation is the type of antigen used in the test kits was not similar. Serological kits developed from *H. pylori* strains from one region may not perform well in patients in other region due to difference in strain specific antigenicity [36-40]. Moreover, host immune response, duration of exposure, nutritional status, cross antigenicity with other prevalent antigenically related bacteria e.g. *Campylobacter* etc. in endemic area also influence the antibody based diagnostic tests [41]. The performance of ELISA is also directly related with the antibody concentration. Several studies have emphasized the use of local strain for preparing test kits and adjusted cutoff level for optimum performance [39-42]. In this study, both sensitivity and specificity were improved with new cut off point at 5.50 rather than at 10 by manufacturer. Therefore, noninvasive tests demand local validation prior to their use in clinical settings.

We found that the overall prevalence of *H. pylori* was lower than the previous studies from Bangladesh.

Matsuhisa et al found 60.2% [43] and Ahmed et al reported more than 90% [11]. These discrepancies could be due to the use of different detecting methodology. Moreover, the noncompliance of the participant with not using proton pump inhibitor two weeks prior to endoscopic examination might produce a false negative result with RUT, culture and histology. The use of antibiotics for various infections may contribute in false negative result too. Recently, several studies from Asian and Middle East countries reported declining trend in *H. pylori* infection with improvement of hygienic condition [44-46]. Bangladesh is also improving its water sanitation sector under United Nation's Millennium Development Goals (MDG) program since last decades that might partially explain low prevalence in our study. We could not detect any significant risk factor(s) for *H. pylori* infection in our study. Several studies checked the risk factors for acquiring *H. pylori* infection; such as age, gender, socioeconomic status, smoking, alcohol drinking, dietary habits for *H. pylori* infection, ended with a lot of controversy [47-55].

Conclusions

In conclusion, the invasive tests showed better performance than noninvasive tests for Bangladeshi population. There is little difference between the accuracy of different invasive tests. RUT may be recommended as first choice due to its advantages followed by either culture or histology. Rapid Urine test and serology require to be validated locally. Further studies are required to estimate the prevalence rate in general population in addition to symptomatic group with risk factor analysis by using a validated noninvasive to invasive diagnostic tests for *H. pylori*

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