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Original Article

Urine-Based ELISA for the Detection of *Helicobacter pylori* IgG Antibody and Comparison with Other Invasive Methods.

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Abstract

The present study was conducted in the department of Microbiology, Dhaka Medical College, Dhaka during the period of January, 2007 to December, 2007. Urine samples were collected from 86 dyspeptic patients undergoing upper Gastrointestinal Tract (GIT) endoscopy to determine anti-*H. pylori* IgG antibody by an ELISA method. Gastric biopsy tissues were tested for culture, rapid urease test and H&E/Giemsa stain. Out of 86 endoscopic biopsy specimens, 45 (52.33%) were culture positive, 63 (73.26%) were rapid urease test positive and 64 (74.42%) were H&E/Giemsa stained positive for *H. pylori.* According to operational standard definition, among the 86 study population, 66 (76.74%) were *H. pylori* infected, 16 (18.60%) were uninfected and 4 (4.65%) were indeterminate. Among 66 *H. pylori* infected cases, 63 (95.45%) were urine ELISA positive and among 16 uninfected cases 3 (18.75%) were urine ELISA positive. Out of 86 study population, 66 (76.74%) were urine ELISA positive. The sensitivity, specificity, PPV, NPP and accuracy of urine ELISA were 95.45%, 81.25%, 95.45%, 81.25% and 92.68% respectively. The result of the study shows that *H. pylori* infection can be rapidly and reliably diagnosed by detecting anti-*H. pylori* IgG from urine.

Key words: *H. pylori*; ELISA; GIT; IgG.

Introduction

Helicobacter pylori is one of the most common bacterial pathogens in human & it seems to be the only bacterium that can survive in the harsh condition in the stomach. *H. pylori* causes type B or non-immune chronic gastritis[1], peptic ulcer disease[2], gastric adenocarcinoma[3] and gastric MALT lymphoma[4].

Human being is the only reservoir and infection is predominantly acquired by oral-oral or fecal-oral and iatrogenic transmission by endoscopy[5]. Presence of *H. pylori* infection does not mean clinical disease. Majority of infected individuals remain asymptomatic with histological gastritis. Only 10-20% of the infected individuals develop *H. pylori* associated diseases.

H. pylori infection occurs worldwide. Approximately 50% of the world population is infected with this organism.[6] In developing countries, its prevalence is 80% to 90% and in developed countries, the prevalence of infection is <40%.[7] High rate of infection is associated with low socioeconomic status and high densities of living.[6]

H. pylori infection typically acquired in childhood & persists throughout life unless specifically treated.[8] In developing countries, the prevalence of *H. pylori* infection begins in first few month of life, exceeds 50% by 5 years and by adult hood, it exceeds 90%.[5,6] In Bangladesh, 60% of children aged <5 years are infected.[9] In developed countries, there is age related increase in prevalence and overall prevalence of *H. pylori* infection ranges from 10% in children to 60% in 60 years old.[8]

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Although a number of diagnostic tools, which include invasive such as culture, histology and biopsy urease test and non-invasive methods for diagnosis of *H. pylori* infection, are available. But no single test has yet been proved accurate enough to be used as the gold standard. Invasive tests, require endoscopic biopsy of gastric tissue and has its expense, inconvenience and risk of complications. As a result, a simple, rapid, reliable, and non-invasive diagnostic test has become essential in clinical practice. Among non-invasive methods, serology,^{13}C –urea breath test, detection of *H. pylori* antigen in stool are now in practice. *H pylori* specific IgG antibodies can also be detected in saliva and in urine by using an ELISA kit. In Bangladesh, different tests have been evaluated for diagnosis of *H. pylori* infection. Though non-invasive, easy and simple, there is no study in Bangladesh on diagnosis of *H. pylori* infection from urine sample. So, this study has been designed and carried out to diagnose *H. pylori* infection by detecting anti-*H. pylori* IgG from urine sample.

Methods

This study was carried out in the Department of Microbiology of Dhaka Medical College, in collaboration with Department of Gastroenterology of Dhaka Medical College and Laboratory Sciences Division of ICDDR,B. A total of 86 adult patients with dyspeptic symptoms, referred for upper gastrointestial endoscopy were studied. Excluded patients from the study were, (i) Patients who had partial or complete gastrectomy or gastro-jejunostomy, (ii) Patients who had ever received *H. pylori* eradication therapy, (iii) Patients who had taken any antibiotic, colloidal bismuth compound, proton pump inhibitor in last one month, (iv) Patients with bleeding peptic ulcer, (v) Chronic use of corticosteroids or immunosuppressant drugs.[9]

From each selected patient, three gastric biopsies were taken with biopsy forceps. Two biopsies, one from antrum and one from corpus, were taken for both culture and for rapid urease test and were put in two separate microcentrifuge tubes. These samples were then transported to the *H. pylori* laboratory of ICDDR,B within 6 hours in a cool box. Another biopsy from antrum was taken for H&E and Giemsa staining and was preserved in a separate tube containing 10% formalin. Around 3-5 ml of randomly voided urine specimen was collected from each patient. Sodium azide was added (0.001 w/v% at final concentration) with the urine and was preserved at 4^0C for maximum 60 days.[10]

In the laboratory, tubes containing gastric biopsy specimens, were vortexed vigorously for 5 minutes. From each tube 200 µl of brain heart infusion (BHI) broth were taken and plated on brain heart infusion (BHI) agar plates containing 7% sheep blood, 0.4% IsovitaleX and *H. pylori* selective (Dent) supplement. The plates were incubated at 37^0C in a CO_2 water jacketed incubator for 3-7 days. Positive cultures were identified by-colony morphology, Gram stain morphology, positive catalase test, positive oxidase test, strong urease activity. From two separate transport media containing gastric biopsy specimens, 100µl BHI broth were taken and inoculated into Christensen's urea broth media separately. Tubes were kept at room temperature. Change of colour from yellow to pink within 24 hours was considered as positive rapid urease test. One gastric biopsy specimen from the antrum was stained H&E and modified Giemsa. Stained slide were examined under microscope for find out curved spiral shaped *H. pylori* bacilli.

Definition of *H. pylori* infection : patients with positive culture results were considered as infected. In case of negative culture, patients positive by both biopsy urease test and histology were considered as infected. Patients negative by all three gastric biopsy-based tests i.e. culture, urease test and histology were considered as uninfected. Patients negative by culture and positive by either biopsy urease test or histology were considered as indeterminate.[11] Detection of Anti *H. pylori* IgG in urine by ELISA was done among infected patients by commercial test kit (URINELISA *H. pylori* antibody test, Otsuka Pharmaceutical Co, Ltd, Japan).

Results:

Of the 86 patients, 45 (52.33%) were culture positive, 63 (73.26%) were rapid urease test positive (RUT), 64 (74.42%) were H&E/Giemsa stained positive and 66 (76.74%) were urine IgG ELISA positive for *H. pylori.* According to Gold standard definition, total number of infected cases were 66. 16 were uninfected and 4 were indeterminate. (Fig.1)

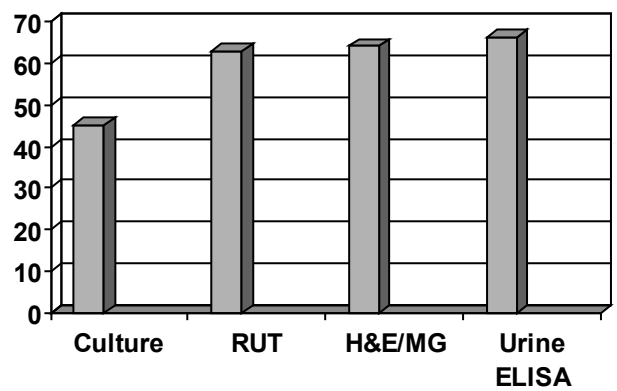

Fig.1: Bar diagram showing percentage of total patients positive for *H. pylori* by different methods.

Among the 45 culture positive cases, 43 (95.54%) were urine IgG ELISA positive. Among 41 culture negative cases 23 (56.09%) were urine IgG ELISA positive. In rapid urease test, among 63 rapid urease test positive cases, 60 (95.23%) were urine IgG ELISA positive. And among 23 rapid urease test negative cases, 6 (26.08%) were urine IgG ELISA positive. Of the 64 H&E/Giemsa stained positive cases, 59 (92.18%) were urine IgG ELISA positive and among the 22 negative cases, 7 (31.81%) were urine IgG ELISA positive. (Table 1)

Table 1: Comparison of culture, rapid urease test and histology with Urine IgG ELISA.

Test results	Urine IgG ELISA	
	Positive	Negative
Culture		
Positive (n=45)	43 (95.54)	2 (04.56)
Negative(n=41)	23 (56.09)	18 (43.91)
RUT		
Positive (n=63)	60 (95.23)	3 (04.77)
Negative(n=23)	6 (26.08)	17 (73.92)
Histology		
Positive (n=64)	59 (92.18)	5 (07.82)
Negative(n=22)	7 (31.81)	15 (68.19)
Total	66	20

Among 66 *H.pylori* infected cases, 63 (95.45%) were urine IgG ELISA positive. Among 16 uninfected cases, 3 (18.75%) were urine IgG ELISA positive and all the 4 indeterminate cases were urine IgG ELISA negative. (Table 2)

Table 2: Comparison of *H. pylori* infection with urine IgG ELISA.

H. pylori	Urine IgG ELISA	
	Positive	Negative
Infected (n=66)	63 (95.45)	3 (4.55)
Uninfected (n=16)	3 (18.75)	13 (81.25)
Indeterminate (n=4)	0 (00.0)	4 (100.0)
Total (n=86)	66 (77)	20 (23)

Figures in parentheses indicate percentage.

Discussion

Bangladesh is a developing country with high prevalence of peptic ulcer disease and *H. pylori* infection.

At present, many tests are available for diagnosis of *H. pylori* infection. Invasive tests, such as culture, histology and biopsy urease test require endoscopic gastric tissue. Some non-invasive tests, such as urea breath test, stool antigen detection, antibody detection in serum and saliva. Present study was aimed to observe the urine IgG response of adult dyspeptic patients to *H. pylori* antigens to find a rapid, reliable, non-invasive method.

In this study, a total of 45 (52.33%) out of 86 patients were positive by culture. In Taiwan , Kuo *et al*. (2005) reported 55.6% of culture positive cases among dyspeptic patients.[12] The less number of culture positivity might be due to the fact that distribution of *H. pylori* in stomach may be patchy, a few sq. mm biopsy tissue may not contain it.

In this study, 63 (73.26%) out of 86 patients were rapid urease test positive. Similarly in Bangladesh, Rahman (2005) reported 60.4% & in a study in USA, Alemohammad *et al* (1993) reported 67% rapid urease test positive among dyspeptic patients.[14,13] Among 86 study cases, 64 (74.42%) were H&E/ Giemsa stained positive for *H. pylori* (Fig. 1) in our study. Similarly in a study in USA, *H. pylori* was identified in stained biopsy specimen among 75% of the patients attending for endoscopy.[13] In this study, according to the standard definition, among 86 dyspeptic study populations, 66 (76.74%) were infected with *H. pylori*

In this study, among 66 *H. pylori* infected cases, 63 (95.45%) were urine IgG ELISA positive (Table 2). Similar results were reported by, Alemohammad *et al.* (1993) in USA where among *H. pylori* infected cases, 96.18% were urine IgG ELISA positive. In the present study, among 16 uninfected cases, 3 (18.75%) were urine IgG ELISA positive. Alemohammad *et al.* (1993) in USA also reported that among *H. pylori* uninfected cases, 22.85% were urine IgG (ELISA) positive in the same study in USA. These results are similar to the findings of the present study.[13]

Though urine ELISA (IgG) tests based on anti-*H. pylori* IgG detection can not differentiate present and past infection even then urine-based ELISA test is preferable because: (1) Urine can be obtained easily, and its collection requires little skill, (2) Urine does not require centrifugation, (3) Absolutely non-invasive and easy to handle, (4) Urine tests can be simply formatted for use in doctor's offices or even for home testing, (5) *H. pylori* specific IgG antibodies in urine are stable for at least 60 days at 4^0C, (6) Low levels of anti-*H. pylori* antibodies in urine can be detected and (7) Random single voided urine specimens without preliminary preparation can be used for test.[12]

Laboratory-based urine testing using ELISA technology to detect IgG antibody is inexpensive, non-invasive, and it may be included in primary health care practice. In conclusion, URINELISA test is rapid, reliable, inexpensive and easy to use and its high degree of diagnostic accuracy warrants its use as a first line-screening tool for the diagnosis of *H. pylori* infection in untreated patients.

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