

Use of five different tests for *Helicobacter pylori* infection in Nepal

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Abstract

Introduction: The prevalence of *Helicobacter pylori* (*H. pylori*) has declined rapidly in Asia. This has been shown in both sero-prevalence-based and endoscopy-based studies. The present study was conducted to determine the current prevalence of *H. pylori* infection in Nepali population referred for upper gastrointestinal endoscopy and its relation with gastro-duodenal diseases.

Methods: Total of 146 patients (71 males and 75 females with the mean age of 42.43yrs, range 16-90 years) underwent endoscopy at endoscopy services in Tribhuvan University Teaching Hospital (TUTH), Nepal. The diagnosis of *H. pylori* infection was determined non-invasively by serology and invasively by rapid urease test, culture, histology and immunohistochemistry.

Results: The overall prevalence of *H. pylori* infection was 48.6%. The sero-prevalence was 43.8%. Gastritis was the predominant finding (76.7%), followed by duodenal ulcer (4.1%), gastric ulcer (4.8%), gastric adenocarcinoma (3.4%) and normal mucosa (10.9%).

Conclusion: The present study revealed the prevalence of *H. pylori* infection measured by serology was higher than that with other invasive tests. In contrast to other studies conducted in Nepal, the present study is the first study to use several tests at the same time to determine *H. pylori* prevalence.

Keyword: *Helicobacter pylori*, infection, test, Nepal.

Introduction

Helicobacter pylori is a micro-aerophilic spiral-shaped, gram-negative bacillus which was discovered more than two decades ago by Warren and Marshall. *H. pylori* organisms colonize approximately half of the world's human population. It is considered to play a major role in the pathogenesis of several gastro-duodenal diseases, including gastric ulcer, duodenal ulcer, gastric mucosa-associated lymphoid tissue and gastric adenocarcinoma. *H. pylori* is regarded as class I carcinogen by WHO. Previous sero-epidemiologic studies indicated that about 50% of adults in developed countries and nearly 90% of adults in

developing countries were positive for serum antibodies against *H. pylori*.^{3,4} High rates of *H. pylori* infection have been significantly associated with low socioeconomic status and poor living conditions during childhood. There are many reports describing a significant difference in the prevalence of *H. pylori* infection, between and within countries due to variation in geographical locations and ethnicity of each population.^{6,7}

Various diagnostic tests for *H. pylori* have been developed and they can be broadly classified into invasive and non-invasive tests. Invasive tests utilize endoscopic biopsy

samples for histology, culture, rapid urease test (RUT) and polymerase chain reaction. All these tests have been found to have sensitivity and specificity that are well above 90%.

The non-invasive tests do not require endoscopy. These include urea breath test (UBT), immune-globulin G and M serology, stool antigen test, saliva antibody test and urinary antibody test.⁸ In Nepal, the non-invasive tests are not generally available except for immunoglobulin G (IgG) serology. The value of serological tests in an endemic area like Nepal is limited, because of their low discriminatory power between past and current infection. The study aimed to determine the prevalence of *H. pylori* among dyspeptic patients referred to endoscopy services of TUTH, the tertiary care referral center of Nepal, and its association with gastro-duodenal pathologies using several diagnostic tests at the same time including histology, culture, immunohistochemistry (IHC), rapid urease test and serology.

Methods

We recruited a total of 146 patients with dyspeptic symptoms (75 females and 71 males; mean age of 42.43 years) during July to September, 2012. The survey was conducted in Endoscopy services of the Gastroenterology department, TUTH Nepal. Written informed consent was obtained from all participants, and the study was approved by the Institutional Review Board (IRB) of Institute of Medicine.

Patients who were previously treated for *H. pylori* infection or who had received antibiotics in preceding 4-weeks or proton pump inhibitors or H₂ blockers in the preceding 2-weeks before endoscopy were excluded. Other exclusion criteria were patients with history of gastrectomy and patients with diffuse gastric tumor (Linitis plastica). During each endoscopy session, four gastric biopsy specimens were obtained (three from the antrum and one from the corpus). The three specimens from the antrum were used for *H. pylori* culture, rapid urease test and histological examination. The specimen from the corpus was used for histological examination. Peptic ulcers and gastric cancer were identified by endoscopy, and gastric cancer was further confirmed by histopathology. Gastritis was identified as *H. pylori* gastritis in the absence of a peptic ulcer or gastric malignancy.

To maximize the diagnostic accuracy, 5 different methods were combined for the diagnosis of *H. pylori* infection, including culture, histology, IHC, the rapid urease test and serum *H. pylori* antibody. For the *H. pylori* culture, one biopsy specimen from the antrum was homogenized in saline and inoculated onto Mueller Hinton II Agar medium (Becton Dickinson, NJ, United States) supplemented

with 7% horse blood 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* was identified on the basis of colony morphology, Gram staining and positive reactions for oxidase, catalase, and urease. Isolated strains were stored at -80°C in Brucella Broth (Difco, NJ, United States) containing 10% dimethyl sulfoxide and 10% horse serum. For histology, all biopsy materials were fixed in 10% buffered formalin for 24 h and then embedded in paraffin. Serial sections were stained with hematoxylin and eosin and with May-Giemsa stain. The state of the gastric mucosa was evaluated according to the updated Sydney system. The degree of the bacterial load was classified into four grades: 0 “normal”; 1 “mild”; 2 “moderate”; and 3 “marked”. A bacterial load grade greater than or equal to 1 was defined as *H. pylori* positive. *H. pylori* sero-positivity was evaluated with a commercially available ELISA kit (Eiken Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. Patients were considered to be *H. pylori*-negative when all five tests were negative, and a *H. pylori*-positive status required at least one positive test result.

Immunohistochemistry

IHC was performed as described previously. Briefly, after antigen retrieval and inactivation of endogenous peroxidase activity, tissue sections were incubated with the anti *H. pylori* antibody (DAKO, Denmark) overnight at 40°C. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG (Nichirei Co., 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. For all cases, we performed Giemsa staining using a serial section to identify the presence of *H. pylori*. If the *H. pylori* identified by Giemsa staining were found to be positively immunostained, we judged the case to be positive.

Statistical analyses

The collected data were entered in Excel and statistical analysis is performed using SPSS software (version 16.0, SPSS Inc., Chicago, USA). Statistical analysis was done using χ^2 test, Fisher’s exact test as applicable and p-value less than 0.05 was considered as significant at 95% level of confidence.

Results

A total of 146 subjects were recruited, comprising 21.9% who were < 29 years old, 23.3% who were 30-39 years old, 23.3% who were 40-49 years old, 17.1% who were 50-59 years old, and 14.4% who were > 60 years old. Table 1 shows the *H. pylori*-positive rate for each test.

Table 1: Prevalence of *H. Pylori* infection in each diagnostic test n (%)

Diagnostic test	Age (years)					Total (n=146)
	≤29 years (n=32)	30 to 39 years(n=34)	40 to 49 years(n=34)	50 to 59 years(n=25)	≥60 years (n=21)	
Serum	13(40.6%)	13(38.2%)	18(52.9%)	11(44.0%)	9 (42.9%)	64 (43.8%)
CLO Test	8 (25.0%)	10 (29.4%)	10 (29.4%)	9 (36.0%)	6 (28.6%)	43 (29.5%)
Culture	12 (37.5%)	9 (26.5%)	12 (35.3%)	11 (44.0%)	7 (33.3%)	51 (34.9%)
Histology	12 (37.5%)	11 (32.4%)	13 (38.2%)	11 (44.0%)	8 (38.1%)	55 (37.7%)
IHC	12 (37.5%)	11(32.4%)	13 (38.2%)	11 (44.0%)	8 (38.1%)	55 (37.7%)
Final	13 (40.6%)	15(44.1%)	20 (58.8%)	13 (52.0%)	10 (47.6%)	71 (48.6%)

The serological test showed higher positive rate compared with invasive tests (CLO, culture, histology and IHC). The prevalence of *H. pylori* infection by the serological test was as follows: 40.6% (13/32) for the ≤ 29 years old group, 38.2% (13/34) for the 30-39 years old group, 52.9% (18/34) for the 40-49 years old group, 44% (11/25) for the 50-59 years old group, and 42.9% (9/21) for the ≥ 60 years old group. When the subjects were considered to be *H. pylori* positive in the case of at least one positive test, the prevalence of *H. pylori* was 40.6 % for the ≤29 years old group, 44.1% for the 30-39 years old group, 58.8% % for the 40-49 years old group, 52% for the 50-59 years old group, and 47.6% for the ≥60 years old. The prevalence of *H. pylori* infection was higher in patients aged 40-49 yrs. The prevalence of *H. pylori* infection in females and males was 30.1% and 18.5%, respectively, so when gender was considered the *H. pylori* was significantly more prevalent in female ($p = 0.013$).

In the endoscopic diagnosis, gastritis was the most common finding (76.7%). GU and DU were found in (4.8%) and (4.1%) cases, respectively. Gastric cancer was found in 5 cases (3.4%). A high infection rate was detected among patients with duodenal ulcer (100%). Table 2 shows the prevalence *H. pylori* infection in each diagnosis. When gastric and duodenal ulcers were defined as peptic ulcers, the prevalence of *H. pylori* infection in peptic ulcer was higher than that in gastritis (69.2% vs 43.8%). Among the symptoms of dyspepsia, the most common symptoms were epigastric pain(90%), epigastric burning(70%) and post prandial fullness(50%). None of our patients had upper GI bleeding.

Table 2: Prevalence of *Helicobacter pylori* infection in each diagnosis (n %)

Disease	n	Positive
Gastritis	112	49 (43.8%)
gastric ulcer	7	3 (42.9%)
duodenal ulcer	6	6 (100%)
gastric cancer	5	4 (75%)
normal	16	9 (56.2%)

The sensitivity of serology was found highest i.e. 90%. The lowest sensitive test was CLO i.e. 61%. The chronological findings are shown in table-3.

Table 3: Accuracy of diagnostic Tests

Tests	Sensitivity	Specificity	PPV	NPV
Serology	90.1%	100%	100%	91.5%
CLO	61.0%	100%	100%	72.8%
IHC	77.5%	100%	100%	82.4%
Culture	71.8%	100%	100%	78.9%
Histology	77.5%	100%	100%	82.4%

Discussion

The overall prevalence of *H. pylori* infection in Nepal was 48.6%. The *H. pylori* prevalence in developing countries is much higher than the developed countries, which may be due to lower socioeconomic status and poorer hygienic practices.

In contrast with developed countries, *H. pylori* infections occur earlier in life and with a higher frequency in the developing world. The prevalence of infection with *H. pylori* infection exceeds 50% by 5 years of age, and by adulthood, infection rates exceeding 90% are not unusual in developing countries. Although the prevalence of infection has dropped significantly in many parts of North America, Western Europe and Asia, no such decline has been noted in the developing world. Age distribution of *H. pylori* infection did not show any trend towards increase or decrease in infection with the advancing age. Though maximum percent of *H. pylori* positivity was seen in the age group 40-49 yrs (58.8%) and minimum number were seen in ≤ 29 yrs group. This data shows that prevalence of *H. pylori* infection could be in declining trend in Nepal (although not statistically significant) since the prevalence is low in younger age group. Improvement in the personal hygiene due to improved living conditions of the Nepali population could have contributed to lower prevalence of *H. pylori*. Widespread use of antisecretory drugs and antibiotics could be a reason for false negative results contributing to low prevalence rate observed. Previous studies conducted in various parts of Nepal in which patients were investigated for *H. pylori* with the use of either histology and or RUT showed prevalence rates of 29.4% to 50.47%.^{13,14,15,16,17} Sero-prevalence studies conducted in Nepal showed prevalence rates of 56.8%. In contrary to previous studies, this study has utilized many different tests to improve diagnostic accuracy.

Several clinical tests have been developed to diagnose *H. pylori* infection. However, there is still no established "gold standard" for the diagnosis of *H. pylori*, and thus the combination of two or more tests should be applied to determine the accurate prevalence of infection. In this study, we combined 5 different tests and considered *H. pylori*-positivity to be at least one positive test among the five tests. In this study, the serological test showed the highest positive rate compared with the other 4-tests. Although the serological test is widely used in epidemiological studies and not affected by local changes in the stomach that could lead to false-negatives, as in the other tests, this test cannot distinguish between current and past infections because *H. pylori* IgGs persist even after the disappearance of this bacterium. Therefore results that are positive in the serological test and negative in the endoscopic tests may indicate a past infection. Culture from biopsy specimens has the potential of leading to a high sensitivity, given that only one bacterium can multiply and provide billions of bacteria. However, both strict transport conditions and careful handling in the laboratory are necessary.²⁰ Histopathological positivity depends on the density of *H. pylori* biopsy sites; thus, these tests can occasionally

show false negative results.²⁰ In addition, the histological diagnosis of *H. pylori* infection is very much dependent on the expertise of the pathologists. The rapid urease test, such as the CLO test, can be useful as a rapid diagnostic method. However, these results can also be affected by the bacterial load.²⁰ A high proportion of the elderly population develops gastric atrophy and intestinal metaplasia, even gastric carcinoma²¹, which can lead to a hostile environment for *H. pylori* and thus fewer bacteria and potentially a negative result. A detailed study in histological scoring is necessary for further study. Moreover, endoscopic tests, including the CLO test, culture and histological examination, can be affected in bleeding patients with peptic ulcers. However, although the reason is not clear, all peptic ulcer cases in our study were not in the active bleeding phase, indicating that we do not need to consider any effects of bleeding.

Conclusion

The study revealed that the prevalence of *H. pylori* infection measured by serology higher compared with other invasive tests. In contrary to other studies conducted in Nepal, the present study utilized many tests at the same time to determine *H. pylori* prevalence. As per this study, the prevalence of *H. pylori* infection in peptic ulcer disease is higher than in gastritis. Because most cases of gastritis were *H. pylori* negative in this study, there needs to be studies of etiological factors for gastritis. However, it is at least clear that in clinical practice, empirical treatment for *H. pylori* is not warranted in patients with gastritis without clear evidence for the presence of this infection.

Conflict of interest: None declared

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