Original article

Accuracy of various diagnostic tests and antimicrobial sensitivity pattern of *Helicobacter pylori* infection in a tertiary care centre in Nepal

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Abstract:

Introduction: *Helicobacter pylori* infection is the most important cause of peptic ulcer disease as well as other gastrointestinal conditions. Most widely used diagnostic tests for detection of *Helicobacter pylori* include rapid urease test, histopathology, culture/sensitivity and serology. The objective of this study was to determine accuracy of standard diagnostic tests and the prevailing local antibiotic susceptibility patterns.

Methods: This cross sectional study, conducted from May to October 2009 at Tribhuvan University Teaching Hospital. 3 pieces of biopsy pieces were obtained from the antral mucosa which were subsequently used for Rapid Urease Test (RUT), histopathology and culture/sensitivity. Then, 2 ml of venous blood was drawn from the antecubital vein for serological examination.

Results: *H. pylori* positivity status was reflected as 32%, 32% and 47% by rapid urease test, culture and histology. The seroprevalence of *H. pylori* was 63%. A patient was considered to be positive with respect to *H. pylori* infection when at least two of three tests namely rapid urease test, culture and histology gave positive results. Hence, proportion of patients with positive H. pylori infection was 36%. Only 30 from 32 culture positive cases were efficiently subcultured and further processed for antibiotic susceptibility testing. Resistance to metronidazole was found to be the highest securing 53.3% and lowest to levofloxacin (0%).

Conclusions: Detection of *Helicobacter pylori* infection using serology was the most effective among the diagnostic tests performed. More than half of the patients were resistant metronidazole while levofloxacin was sensitive in all the cases.

Keywords: *Helicobacter pylori*, diagnostic tests, antimicrobial susceptibility.

Introduction

The discovery of *Helicobacter pylori* by Warren and Marshall not only introduced a whole new group of bacteria to science but also revolutionized the concept of gastroduodenal pathology and diverted the worldwide attention from pH to Hp. [1] *H. pylori* is a key factor in the etiology of various gastrointestinal diseases, ranging from chronic active gastritis without clinical symptoms to peptic ulceration, gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue (MALT) lymphoma (MALToma), B-cell mucosa-associated lymphoid tissue (MALT) lymphoma and Menetrier's disease.

H. pylori is the first formally recognized bacterial carcinogen and is one of the most successful human pathogens, as over half of the world's population is colonized with this bacteria.[2] Unless treated,

colonization usually persists lifelong. The prevalence of the infection is greater in developing countries and is influenced by socioeconomic conditions, ethnic background and age.[3,4]

Currently, there are several popular methods for detecting the presence of *H. pylori* infection, each having its own advantages, disadvantages, and limitations.[5,6] They may broadly be divided into tests that indirectly determine the presence of the microorganism (antibody tests in blood, urine, or saliva) or direct tests that detect the intact organism (histology and culture), antigens shed from the organism (stool antigen test), or metabolic functions of the organism (rapid urease test and urea breath test). The choice of a specific test for an individual patient depends on local experience and the clinical setting.[7,8] For routine diagnostic purposes, histology, urea breath testing, and culture are currently most often used, whereas the use of serology is most appropriate for large epidemiological studies. In hospital-based care, many patients undergo endoscopy, which is then combined with an invasive test for *H. pylori*. Otherwise, breath tests and serology are commonly used. For children, fecal antigen tests offer the opportunity to assess *H. pylori* status without the need for endoscopy or vena puncture.[2]

The Maastricht consensus report recommends starting with clarithromycin triple therapy, following with quadruple therapy in the case of failure, and using levofloxacin triple therapy (consisting of a proton-pump inhibitor, amoxicillin, and levofloxacin) if the first two strategies fail.[9] With this approach, a 90% overall eradication rate can be achieved.[10] In contrast, the triple-therapy regimen suggested by McColl (consisting of a proton pump inhibitor, amoxicillin, and metronidazole) had a poor eradication rate, (76%) and is a less satisfactory alternative.[11]

Antibiotic resistance has increasingly been recognized as a major cause of treatment failure for *H. pylori* infection. Primary antimicrobial resistance against clarithromycin and metronidazole is now commonplace in several countries.[12-18] Regional variations in susceptibility and resistance patterns may be ascribed to differences in local antibiotic prescription practices, antibiotic usage in the community and mass eradication programs for *H. pylori* infection as part of gastric cancer prevention strategies. These factors may well be expected to influence success of eradication therapy.[19,20]

Information regarding the prevalence of *H. pylori* is

truly restricted in Nepal. In Nepal, histopathology is unquestionably the first and foremost choice for the diagnosis of *H. pylori* infection. Nevertheless rapid urease test is also in application.[21,22] Very few seroprevalence studies have also been reported.[23]

Realizing the increasing prevalence of antimicrobial resistance and its potential negative impact on the efficacy of many treatment eradication regimens, it is important in clinical practice to determine the prevailing local antibiotic susceptibility patterns.[3] unfortunately; authentic data pertaining to this global issue is still countable in Nepal.

Methods

This cross sectional study conducted from May to October, 2009 at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Approval was taken from Institutional Review Board of Institute of Medicine, Tribhuvan University. Informed consent was taken from each patient before each procedure. Endoscopy was executed by expert gastroenterologists using Olympus video endoscope. The endoscopic findings were noted as normal, gastritis (acute or mild and chronic) and peptic ulcer (gastric and duodenal ulcer). 3 pieces of biopsy pieces were obtained from the antral mucosa which were subsequently used for Rapid Urease Test (RUT), histopathology and culture/sensitivity. Then, 2 ml of venous blood was drawn from the antecubital vein for serological examination. First biopsy piece for RUT was directly inoculated into the in-house RUT tubes. The second was placed into a sterile container containing 10 % formalin for histology. The third one was placed into a sterile eppendorff tube containing 0.5 ml of normal saline In the laboratory, specimens were vortexed for the formation of a homogenate. In the laboratory, culture and gram-staining was performed. The bacterial isolates tested for sensitivity for amoxycillin, clarithromycin, metronidazole, tetracycline and levofloxacin. The collected blood was centrifuged at 3000 rpm for 10 minutes and serum was separated.

Results

H. pylori positivity status was reflected as 32%, 32%, 47% and 63% by RUT, culture, histology and serology methods respectively among the 100 patients of this study. The male to female ratio for *H. pylori* infection

was 1.22:1. The age of the subjects ranged from 11 to 77 years. The mean age of the patients was 38.9. The prevalence of *H. pylori* infection was highest in the age group of 20-29 years. The overall prevalence of *H. pylori* infection was higher in duodenal ulcer cases whereas the normal cases from endoscopic findings also demonstrated remarkable *H. pylori* infection.

For the definition of a positive *H. pylori* status, the results of a RUT, histological examination and microbiological cultures were used. A patient was considered to be positive with respect to *H. pylori* infection when at least two of these three tests gave positive results.[24] Accordingly, proportion of patients with positive *H. pylori* infection has been depicted in table 1.

Table 1. Proportion of Endoscopic findings with positive *H. pylori*

Endoscopic findings	Number of patients	*H. pylori* positive (%)
Antral gastritis	49	14 (28.6)
Fundal gastritis	6	3 (50)
Pangastritis	3	1 (33.3)
Gastroduodenitis	7	3 (42.9)
Gastric ulcer	6	1 (16.6)
Duodenal ulcer	7	5 (71.4)
Normal	22	8 (36.3)
Total	100	36

The seroprevalence of *H. pylori* was 63%. Then efficacy of the serological test with other three tests i.e. RUT, culture and histology were compared by applying chi-square test in which result was found to be significantly associated. Only 30 from 32 culture positive cases were efficiently subcultured and further processed for antibiotic susceptibility testing.
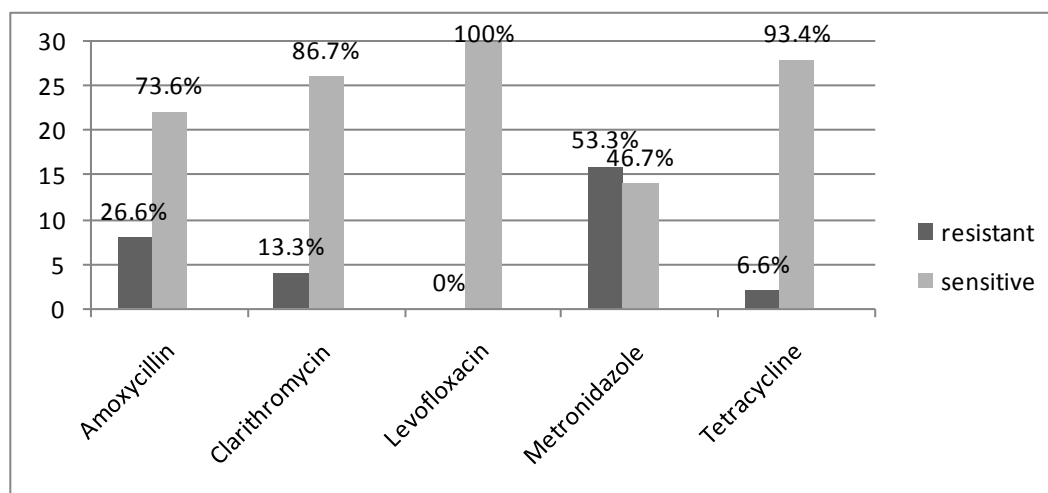

Figure 1. Antibiotic susceptibility pattern

Resistance to metronidazole was found to be the highest securing 53.3%, followed by amoxycillin (26.6%), clarithromycin (13.3%) and tetracycline (6.6%) while all isolates were sensitive to levofloxacin. Multi drug resistance was also observed in which amoxycillin and metronidazole resistance was 16.6% and clarithromycin and metronidazole 6.6%. two patients had resistance to four drugs namely amoxycillin, clarithromycin, metronidazole and tetracycline.

Discussion

With the goal of assessing the accuracy of various diagnostic tests for detection of H.pylori infection in patients presenting with gastrointestinal symptoms in Nepal and antibiotic susceptibility of the isolates to contemporary antibiotics, this study was completed proficiently by employing the four diagnostic tests: rapid urease test, culture, histology and serology.

Higher detection rate with serology compared to other methods could be explained by the fact that in most cases IgG antibodies remain detectable for years after eradication.[25] Numerous studies have applied different diagnostic tests to evaluate the prevalence of H.pylori infection. Certain studies carried out in Nepal presented the prevalence of H. pylori infection as 56.8% using serology, 33.9% using histopathology, 29.5% using RUT, culture and PCR. [26,23,21]

Metronidazole, clarithromycin, amoxycillin and tetracycline are regularly prescribed for the treatment of H. pylori infection in Nepal. Resistance to metronidazole in Europe and the USA is between 20% and 40%, but wide use of metronidazole for parasitic infections increases resistance in the developing countries up to 80%.[27,28] In developing countries, the prevalence of metronidazole resistance among H. pylori isolates was much higher, 50 to 80%.[29-31] In Japan, exceptionally low resistance rates are seen (12.4%), again with local differences. Women more often harbour resistant strains than men, most probably due to therapies for gynaecologic infections.[27,32]

Amoxycillin resistance was not considered important until recently identified in the United States, Canada and Italy.[33,34] Amoxycillin is one of the most commonly used antimicrobial agents in Nepal in recent years for respiratory tract infections, urinary tract infections etc. Amoxycillin resistance develops due to structural alterations in one of the penicillin-binding proteins or changes in other proteins involved in cell wall synthesis. The reported prevalence of resistance to clarithromycin ranges between 0-15% in most countries[35,36] Previous use of macrolides has been shown to be associated with H. pylori resistance to clarithromycin. Resistance to tetracycline is very low, less than 1% in western countries.[17,37] whereas resistance ranges between 5 and 59% in Asian countries.[38,39] Several studies have demonstrated that most of the patients with both metronidazole and clarithromycin resistant are cured with second line regimens with levofloxacin.[40,41]

Antibiotic resistance is undisputedly an emerging problem for the treatment of H. pylori infected patients in Nepal. The present study also demonstrates the need for continuous monitoring of the antimicrobial susceptibility in H. pylori that will certainly guide the clinicians for determination of optimal treatment regimens as multi drug resistant strains are already in the act.

Conclusion

Detection of Helicobacter pylori infection using serology was the most effective among the diagnostic tests performed. However, serology is an indirect test which may also be positive long after eradication. Histopathology was the most accurate among the direct tests. More than half of the patients were resistant metronidazole while levofloxacin was sensitive in all the cases. Considering the rising incidence of antimicrobial resistance, indiscriminate use of antibiotics should be avoided.

Conflict of interest: None declared

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