Original Article

Study of invasive and non-invasive methods to detect h pylori infection

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Abstract

Introduction: As per epidemiology at least half the world's population is infected by H.pylori making it the most widespread infection in the world. Actual infection rates vary from nation to nation. The Third World has much higher infection rates than the West (Western Europe, North America, Australia), where the rates are estimated to be around 25%. **Aims and Objectives:** To Study of Invasive and Non-Invasive Methods to Detect H Pylori Infection. **Methodology:** The present study was carried out in the Department of Pathology in a tertiary care hospital in Tamil Nadu. This was a Prospective study. Total 105 cases were studied, Detection of Helicobacter pylori done by Rapid Urease Test, Serological tests, Histopathological examination**. Result:** Out of 105 patients, 86 [81.9%] patients were positive for H.pylori by rapid urease test. Out of the 105 cases, 90 [85.7%] patients were positive for H.pylori by serological method. The observed cut-off value was 0.516. Histopathologically, 60 cases (57.1%) showed evidence of chronic active gastritis, of which 58 cases (96.7%) were H.pylori positive. The sensitivity, specificity and accuracy of rapid urease test are 93.24%, 45.16% and 79.05% respectively. The sensitivity, specificity and accuracy of serologic testing are 98.65%, 45.16% and 82.86% respectively. **Conclusion:** Histopathological examination is the gold standard test for H.pylori detection against which the sensitivity, specificity and accuracy of serologic testing are 98.65%, 45.16% and 82.86% respectively and The sensitivity, specificity and accuracy of rapid urease test are 93.24%, 45.16% and 79.05% respectively.

Keywords: Serological test for H. Pylori, Rapid Urease test, Histopathological examination of H.Pylori.

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INTRODUCTION

As per epidemiology at least half the world's population is infected by H.pylori making it the most widespread infection in the world[1]Actual infection rates vary from nation to nation. The Third World has much higher infection rates than the West (Western Europe, North America, Australia), where the rates are estimated to be around 25%.[2]H.pylori is contagious, although the exact route of transmission is not known.[3,4] Person-to-person transmission by either the oral-oral or fecal-oral route is most likely.[5] Consistent with these transmission routes, the bacteria have been isolated from feces, saliva and dental plaque of some infected people.[5]Helicobacter pylori is a spiral shaped organism associated with gastrointestinal disease in humans. It causes a chronic gastric infection that usually is lifelong and many epidemiologic studies have shown that this is probably one of the most common bacterial infection throughout the world, involving 40% to 50% of the population in developed countries and 80% to 90% of the population in developing regions.[6]Over 80% of individuals infected with this bacterium are asymptomatic. Infections are usually acquired in early childhood in all countries.[7]However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions. It causes a chronic low-level inflammation of the stomach. The clinical outcomes associated with H.pylori infection

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include duodenal ulcer, gastric ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The bacterium has been classified as a class I definite gastric carcinogen to human.[8] H.pylori infection can be diagnosed by invasive (i.e., requiring endoscopy) and noninvasive techniques (i.e., techniques that do not require endoscopy with biopsy sampling).[9]Eradication of H.pylori improved markedly the inflammatory cell infiltration characteristic of H.pylori related gastritis, inhibited recurrence of peptic ulcer[10] and also led to regression of MALT lymphoma.[11]H.pylori is a spiral to curved, rod shaped, gram negative microaerophilic bacterium about 3 microns long with a diameter of about 0.5 micron. It has 4 to 7 polar sheathed flagella, which enable the bacterium to move freely in viscous environments such as gastric mucus.[12]This bacterium is the human-adapted helicobacter primarily found in the gastric mucosa and areas of gastric metaplasia in the duodenum and occasionally in Meckel's diverticulum and rectum.[13,14]H.pylori is urease, catalase and oxidase positive. The urease activity is striking, and the amounts produced have allowed accurate diagnosis in patients by direct detection of the enzyme in gastric biopsy specimens and by breath tests using carbon isotopes labelled with urea. Many roles have been proposed for urease enzyme. It is known to be important for colonization and survival of the bacterium in the gastric environment.[15] The hydrolysis of urea to ammonia by urease could have a buffering effect, protecting the bacterium from acidity.[16] In vitro studies have shown that helicobacter pylori cannot survive in acidic condition without the presence of urea, and urea inhibits its growth in alkaline conditions.[7] Urease also has been proposed as an important virulence factor. There are two main two categories for the diagnosis **Noninvasive techniques:** Serologic testing, Urea breath tests, Stool tests **Invasive techniques:** Urease tests**,** Biopsy**,** Culture, Polymerase chain reaction.

AIMS AND OBJECTIVES

To Study of Invasive and Non-Invasive Methods to Detect H Pylori Infection.

MATERIAL AND METHODS

The present study was carried out in the Department of Pathology in a tertiary care hospital in Tamil Nadu. During the two year study period, 105 gastric biopsy materials and 105 serum samples were collected for Helicobacter pylori study in patients presenting with dyspepsia. Patients were clinically diagnosed as suffering from dyspepsia based on the following symptoms: nausea, vomiting, anorexia, heartburn, flatulence, regurgitation, early satiety, fullness and bloating in addition to pain or discomfort.[18] This was a Prospective study. Patients of both sex above 12 years of age who were found to have peptic ulcer, gastritis, duodenitis and normal on endoscopy on evaluation of dyspepsia were taken up for the study. History of antibiotic ingestion in the previous 4 to 6 weeks, history of ingestion of antacids or H_2 blockers or proton pump inhibitors and Non-steroidal anti-inflammatory drugs over the past 4 to 6 weeks were excluded from the study. Informed consent was obtained from all patients included in this study. The relevant history and clinical details were recorded using a structured proforma. Three gastric biopsy materials, one from corpus and two from antrum of stomach were obtained. One antral specimen was used for urease test in the endoscopic room itself. Remaining biopsy materials were used for histopahological examination. 2ml blood was collected by venipuncture for IgG ELISA serology investigation. Detection of Helicobacter pylori done by **Rapid Urease Test**: One gastric antral biopsy specimen was taken and placed immediately in 5ml of freshly prepared solution of 10% urea containing 1% phenol red as pH indicator. Change of colour from yellow to pink was observed in the next 24 hrs. **Histopathological examination**: Two gastric biopsy materials from corpus and antrum of the stomach were taken for histopahological examination and fixed in 10% neutral buffered formalin. The tissues were processed, paraffin blocked, 5 microns thin sections were cut and stained with Hematoxylin and Eosin and Giemsa stains. H and E staining was used for the histological diagnosis of activity of H.pylori infection, mucosal inflammation, glandular atrophy and intestinal metaplasia. Giemsa staining was used for the histological diagnosis of H.pylori infection. Being active was signified by the presence of neutrophils within the glandular and surface epithelial layer. Glandular atrophy was identified when the gastric glands were correspondingly decreased in amount and/or widely separated. An increase in lymphocytes and plasma cells in lamina propria categorizes the gastritis as chronic. As an arbitrary guideline, infiltration involving up to 1/3 of the gastric pits and surface are designated mild; between 1/3 and 2/3 moderate and more than this as severe[19]. Lymphoid aggregates were defined as accumulation of lymphocytes without germinal center formation.[20] **Serologic testing:** Collected blood by venipuncture was allowed to clot and the serum was separated by centrifugation at a speed of 3000 rpm for 5 min at room temperature. The serum samples were stored at -20^0C. Using DEMEDITEC H.pylori IgG antibody ELISA test kit, detection and quantitative determination of specific IgG antibodies against H.pylori in serum was done.

RESULT

Table 1: Rapid urease test (RUT)

Sr. No	No of cases	Positive cases	Percentage
1	105	86	81.9

Out of 105 patients, 86 [81.9%] patients were positive for H.pylori by rapid urease test.

Table 2: Serological assay

Sr. No	No of cases	Positive cases	Percentage
1	105	90	85.7

Out of the 105 cases, 90 [85.7%] patients were positive for H.pylori by serological method. The observed cut-off value was 0.516.

Table 3: Histopathology

Sr. No	No of cases	Positive cases	Percentage
1	105	74	70.5

Table 4: Histopathological features

Sr. No	Hp features	No of cases	Positive cases	Percentage
1	Chronic active gastritis	60	58	96.7
2	Normal gastric mucosa	15	5	33.3
3	Mild chronic gastritis	13	10	76.7
4	Intestinal metaplasia	10	-	-
5	Chronic gastritis with dysplastic glands	5	1	20
6	Chronic atrophic gastritis	2	-	-

Histopathologically, 60 cases (57.1%) showed evidence of chronic active gastritis, of which 58 cases (96.7%) were H.pylori positive. Mild gastritis was evidenced in 13 cases (12.4%), of which 10 cases (76.7%) were H.pylori positive. 15 cases were normal (14.3%) of which 5 cases (33.3%) were H.pylori positive. Ten cases of intestinal metaplasia (9.5%) and two cases of atrophy (1.9%) were detected, all were H.pylori negative. Five cases of dysplasia (4.8%) were found, of which one case (20%) was H.pyloripositive. Lymphocyte infiltration was more prominent in the antrum.

Table 5: Correlation of Rapid urease test, Histopathological and Serological methods

Sr. No	Tests	No of Cases	Positive Cases
1	Rapid urease test	105	86
2	Histopathology	105	74
3	Serological assay	105	90

Out of 86 positive cases in rapid urease test, only 69 cases showed H.pylori by Giemsa staining. Out of 90 positive cases in serological assay, only 73 cases showed H.pylori by Giemsa staining. The sensitivity, specificity and accuracy of rapid urease test are 93.24%, 45.16% and 79.05% respectively. In cases of Intestinal metaplasia and atrophic gastritis, there are no demonstrable H.pylori organisms. The sensitivity, specificity and accuracy of serologic testing are 98.65%, 45.16% and 82.86% respectively. The H.pylori positivity rate for peptic ulcer is 89.74% (duodenal ulcer is 93% and gastric ulcer is 77.8%).

DISCUSSION

In the present study, H.pylori is positive in 86 (81.9%) cases by RUT and in 90 (85.7%) cases by serological assay. It correlates with a study done by U Arora*et al*[21]. They studied 75 gastric biopsy specimens and 75 serum samples of same patients complaining of dyspepsia. H.pylori is positive in 52 cases (72%) by RUT and in 57 cases (76%) by serological testing. Gill *et al*[22] have shown that antibodies to H.pylori in serum are present in about 80% of Indian subjects with upper gastrointestinal symptoms. In our study also the positivity rate is 85.7% which is very well correlated with the previous mentioned study. Some of those methods are based on the high urease activity of *H.pylori*,[23] but because they detect all enzyme activity of urease regardless of its origin, there is the possibility that urease derived from other bacterial species, such as *Proteus mirabilis* or *Klebsiellapnuemoniae*, will confound the result. Serological identification of anti-HP antibodies is a non-invasive method. However, the antibody titers preserve their levels even after the eradication of the bacteria by antibacterial therapy. The prevalence of H.pylori in the present study is 70.5%. It is similar to a study by Abdul Rahman E Fakhro*et al*[24] and a study by Basic H, Katic V *et al*[25]. In their study the prevalence rates are 79.4% and 71.8% respectively. Histopathologically, 60 cases out of 105 patients (57.1%) showed evidence of chronic active gastritis, of which 58 cases (96.7%) were H. pylori positive. Mild gastritis is evidenced in 13 cases (12.4%), of which 10 cases (76.7%) are H.pylori positive. 15 cases are normal (14.3%) of which 4 cases (26.7%) are H.pylori positive. Ten cases of intestinal metaplasia (9.5%) and two cases of atrophy (1.9%) are detected, all were H.pylori negative. Five cases of dysplasia (4.8%) are found, of which two cases (40%) are H. pylori positive. Abdul Rahman E Fakhro*et al*[24] studied 102 gastric biopsies in dyspeptic patients. In their study, 66 cases out of 102 patients (64.7%) showed evidence of chronic active gastritis, of which 65 cases (98.5%) are H.pylori positive. Mild gastritis is evidenced in 15 cases (14.7%), of which 9 cases (60%) are H.pylori positive. 16 cases are normal, of which 3 cases (18.8%) are H.pylori positive. Our study results are comparable to this study.

CONCLUSION

Histopathological examination is the gold standard test for H.pylori detection against which the sensitivity, specificity and accuracy of serologic testing are 98.65%, 45.16% and 82.86% respectively and The sensitivity, specificity and accuracy of rapid urease test are 93.24%, 45.16% and 79.05% respectively. Serological identification of anti-HP antibodies is a non-invasive method. However, the antibody titres preserve their levels even after the eradication of the bacteria by antibacterial therapy. Methods based on the high urease activity of *H. pylori* detect all enzyme activity of urease regardless of its origin, there is the possibility that urease derived from other bacterial species, such as *Proteus mirabilis* or *Klebsiellapnuemoniae*, will confound the result.

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