

Diagnosis of *Helicobacter pylori* Associated Acid Peptic Disease by Serology and Rapid Urease Test

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H. pylori has been recognized as the principle cause of peptic ulcer disease and the main risk factor for development of gastric cancer. Up to 95% of patients with duodenal ulcer, and 80% of the patients with gastric ulcer are infected by *H. pylori*. The diagnosis of *H. pylori* infection can be made by using several invasive (culture, histological stains and rapid urease tests) and non-invasive techniques (serology, urea breath test or stool antigen detection). Eradication of this organism leads to ulcer healing and markedly lowers the incidence and recurrences. This study was conducted to compare, the different diagnostic methods in the local population of Kanpur to select the most effective test for the diagnosis of *H. pylori*. Biopsy specimens were taken from antral mucosa of 100 patients reported to endoscopic unit with complaints of APD. The biopsy specimens were tested for *H. pylori* infection by RUT. Serum IgG against *H. pylori* (sIgGHP) was detected by ELISA. Forty six per cent cases were positive by serology test and 45% cases were positive by RUT. Serology and RUT had the best sensitivity and specificity. Non-invasive techniques like serology can be as sensitive as invasive technique like RUT. Serology and RUT can be used to confirm clinical diagnosis of duodenal erosion and ulcer.

Key words: *Helicobacter pylori*, peptic ulcer, serology, urease.

Helicobacter pylori is now recognized as one of the most common bacterial infections in humans¹. It is estimated that more than half of the world's population is currently infected with this organism¹. It has been recognized as the principle cause of peptic ulcer disease and the main risk factor for development of gastric cancer². Up to 95% of patients with duodenal ulcers, and 80% of the patients with gastric ulcers are infected by *H. pylori*¹. Earlier peptic ulcers were believed to be

caused by stress, strains and dietary factors and the injurious effects of gastric acid were blamed for ulcer diseases. Antacids became the main stay of therapy. Subsequently, H₂ receptor appeared to be the principle mediator of the gastric acid secretions and recently inhibitors of the proton pump in gastric parietal cells have proved to be rapidly effective against acid peptic disease (APD). Despite these therapeutic agents, the recurrence of ulcer remained a problem even after complete healing. In 1983 Warren and Marshall³ provided the first insight into another important pathogenic factor in peptic ulcer disease. They isolated a spiral or curved urease-producing organisms in the narrow interface between gastric epithelial cell surface and the overlying mucous gel and it was named as *H. pylori*⁴. The diagnosis of *H. pylori*

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infection can be made by using several invasive or non-invasive techniques. Invasive diagnostic methods such as culture, histological stains, and urease tests require an endoscopic biopsy of gastric mucosa. Serology, urea breath test or stool antigen test are current non-invasive tests. Eradication of this organism leads to ulcer healing and markedly lowers the incidence and recurrence¹. This study was conducted to compare, the different diagnostic methods like culture, histopathology, rapid urease test and serology in the local population of Kanpur at Rama Medical College, Hospital and Research Centre, Kanpur, to select the most sensitive, specific, rapid, reliable and cost effective test for the diagnosis of *H. pylori*.

MATERIAL AND METHODS

Method of collection of samples

This study was conducted in the Kanpur at Rama medical College, Hospital and Research Center, Kanpur from June 2011 to May 2012 with the support of Departments of Microbiology, Pathology and Gastroenterology. The study group consisted of 100 patients, varying in different age and sex and 20 controls that were referred for endoscopy. All patients had the symptoms and signs like upper abdominal pain, vomiting, epigastric pain immediately after food, in empty stomach and two hours after food suggestive of duodenal ulcer, gastric ulcer, gastritis, gastric malignancies and non-ulcer dyspepsia. The patients were instructed to report to the endoscopic unit empty stomach. Upper gastrointestinal endoscopy was performed under aseptic precautions.

Four antral biopsies were collected from each patient with an Olympus endoscope. The mucosal biopsies were taken from the antrum, 1-2 cms from the pylorus, and processed as follows-

1. One biopsy tissue was put into Christensen's urea broth.
2. Two biopsies were transported in Brucella broth with supplements for culture, which were immediately kept at 2°C- 4°C before being processed.
3. One biopsy tissue was submitted for histopathological analysis.

Culture

The biopsies for culture were

homogenized and inoculated on brain heart infusion agar (BHIA). Difco supplement and incubated in 10% CO₂ and 98% humidity in CO₂ incubator¹⁴. Earlier, the culture was tried with BA, CA with Skirrow's supplement without Isovitalex for few samples in a candle jar, but showed no growth. The procedure was standardized using standard strain of *H. pylori* (26695) obtained from National Institute of Cholera and Diarrhoeal diseases (NICED). The media were observed for growth. Catalase test, oxidase test and urease test were performed for biochemical confirmation as per recommended procedures (CLSI)¹⁵.

Histopathology

Biopsy specimens were assessed for the presence of inflammation as well as for *H. pylori* using haematoxylin & eosin and Giemsa stains. The histopathological grading of gastritis, and the presence of *H. pylori* was scored according to the Sydney system.

Rapid urease test

A Biopsy tissue from antrum is placed in 0.5ml Christensen's urease broth and incubated at 37°C, the tube was examined after 15mins, 30 mins, 60 mins, 4hrs. A colour change from yellow to red or magenta was read as positive.

Serology

About 5ml of blood was drawn from each patient using sterile syringe and the serum was separated by centrifugation and stored at -20°C, until it was used for the detection of SIgGHP (VIRION/SERION Serion Elisa classic) as per manufacturer's instructions.

Statistical Methods

Chi square test/Fisher Exact test has been used to find the significant association of findings. Statistical calculation was done by CDC EpiInfo 7 software.

RESULTS

A correlational descriptive study with 100 Acid peptic disease (APD) patients is undertaken to study correlation of findings of Rapid Urease Test (RUT) and serology for the detection of *H. pylori* infection. There were 79 males and 21 females. Patients were uniformly distributed in 3rd to 6th decades and only 4 each in the age groups below 20 and above 70yrs presented with APD

Table 1. Age and sex distribution of patients with Acid peptic disease

Age in years	Number	Male	Female
≤20	04	03	01
21-30	17	14	03
31-40	16	13	03
41-50	20	10	10
51-60	22	14	08
61-70	17	13	04
71-80	04	04	00
Total	100	71	29

Mean ±SD 46.51±16.24

(control n=20): Mean ±SD 51.45±16.21

(Table-1). Twenty six percent of cases were positive by culture with a ($p = 0.04$), 45% of cases were positive by RUT, 36% of cases were positive by histopathology, and 49% of cases were positive by serology ($p = < 0.001$). The most common endoscopic finding in *H. pylori* positive cases were antral gastritis (26.6%), erosive duodenitis and duodenal ulcer (26.6%), followed by erosive gastritis (11.5%), antral erythema (7.7%) and Ca stomach (3%). In 21 patients endoscopy finding was normal mucosal study and four of them had positive serological results ($p=0.004$). For patients with Antral erythema ($n=19$), Antral gastritis ($n=26$), Ca stomach ($n=1$) and others such as antral sessile polyp, duodenal polyp, diffuse pangastritis, deep

Table 2. Correlation of Endoscopic findings with serology

Endoscopic Diagnosis	sIgGHp +ve	sIgGHp -ve	χ^2	Fisher's exact OR	2 tailed corrected P value
Normal mucosal study	4	17	8.0862	0.0405-0.6221	0.004
Antral erythema	9	10	0.0094	0.2972-2.8306	0.9228
Antral gastritis	12	14	0.012	0.3157-2.3052	0.9128
Ca. stomach	1	0	0.0004	0.0267- -1.000	0.9839
Erosive duodenitis and Duodenal ulcer	15	4	7.0037	1.4549-22.995	0.008
Others	8	6	0.1361	0.4042-5.5639	0.712
Total	49	51			

Table 3. Correlation of Endoscopic findings with Rapid urease test

Endoscopic Diagnosis	RUT +ve	Rut -ve	χ^2	Fisher's exact OR	2 tailed corrected P value
Normal mucosal study	4	17	7.5774	0.0416-0.6417	0.00591
Antral erythema	8	11	0.0007	0.2715-2.6538	0.9795
Antral gastritis	12	14	0.0084	0.3911-2.8653	0.9269
Ca. stomach	1	0	0.0102	0.0313- -1.000	0.9195
Erosive duodenitis and Duodenal ulcer	14	5	7.5304	1.4874-19.0467	0.006
Others	6	8	0.0134	0.237-3.2672	0.9077
Total	45	55			

antral ulcer ($n=14$), there was no significant correlation with serology. For 15 of the 19 cases of erosive duodenitis and duodenal ulcer serological correlation was significant ($p=0.008$) (Table-2). In 21 patients with normal mucosal study 4 had positive RUT results ($p=0.0059$). For patients with Antral erythema ($n=19$), Antral gastritis ($n=26$), Ca stomach ($n=1$) and others ($n=14$), there was no

significant correlation with serology. For 15 of the 19 cases of erosive duodenitis and duodenal ulcer serological correlation was significant ($p=0.006$) (Table-3). Taking RUT as standard, sensitivity, specificity, positive predictive value and negative predictive value of serology are 97.8%, 96.4%, 95.7% and 98.1% respectively (Table-4).

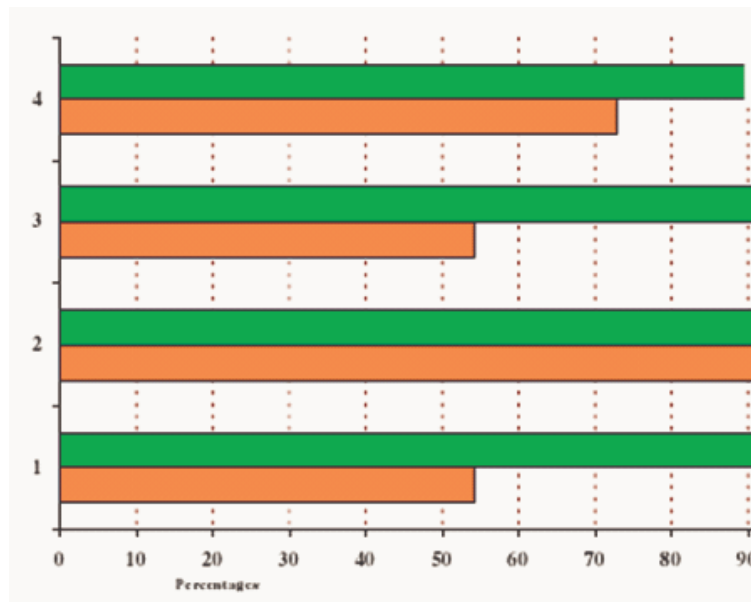


Fig. 1. RUT findings compared with other tests

DISCUSSION

H. pylori are a pathogen of the gastric mucosa and a major cause of peptic ulcer disease and chronic gastritis⁵. Various diagnostic tests for *H. pylori* infection may have false negative results and the use of multiple tests may help to provide a more accurate diagnosis of *H. pylori*.

Infection⁶ the present study was undertaken to compare the different invasive and non-invasive diagnostic methods for detection of *H. pylori* infection in patients with acid peptic disease. Though *H. pylori* infection is acquired during childhood, clinical manifestations are predominantly seen at the age of 50-60 years^{7, 8}. The most common endoscopic finding in *H. pylori* culture positive cases were antral gastritis (26%), erosive duodenitis and duodenal ulcer (19%), antral erythema (19%) and Ca stomach (1%). Only 26% of the samples yielded growth of *H. pylori*. The low yield of culture could be attributed to patients being treated with proton pump inhibitors, H₂ blockers and antibiotics prior to investigations; patchy distribution of the organism in the antrum, not sampling mucosa of the body of stomach, not storing biopsied sample at room temperature in a semisolid agar before culturing and chances of overgrowth of other bacterial flora in spite of using selective agents like polymyxin B, trimethoprim and

Vancomycin and Skirrow's supplement. Even though the culture has shown low sensitivity, it helps in studying the antimicrobial susceptibility and molecular study of the isolates. In the present study, when culture is compared with other tests it shows a sensitivity and specificity of 100% and 70.3% with RUT and RUT has the distinct advantage of rapidity, while culture has an advantage of allowing antimicrobial susceptibility and strain typing, in spite of taking 4 to 5 days for growth⁹. Culture with histopathology had a sensitivity and specificity of 61.5% and 72.9% correlates with other studies¹, it had a sensitivity and specificity of 100% and 65.2% with serology which correlates with other studies^{1,10}. The low specificity may be due to the demonstration of IgG alone against *H. pylori*. If IgM and IgA isotypes are also tested, the specificity may improve further. In this study, 45% and 49% of cases were positive by RUT and serology respectively. RUT is rapid and simple for detecting *H. pylori* infections but indicate only the presence or absence of infection, however, the sensitivity of RUT test is often higher than that of other biopsy based methods, because the entire biopsy specimen is placed in the media, thus avoiding additional sampling or processing error associated with histology or culture⁶. RUT is useful when read at the end of 4 hrs, and result of this study corroborates to earlier other studies¹¹.

With serology it shows a sensitivity and specificity of 97.8% and 96.4% respectively. In the present study, 49% of cases were positive by serology. ELISA is characterized by its sensitivity and is widely used method of diagnosis since infected subjects develop elevated levels of SIgGHP in symptomatic stage. Serology for SIgGHP may play an important role in decreasing the need for endoscopy provided the cut-off value for assay is set based on the prevalence of antibodies in the population. It has been shown that serological diagnosis of *H. pylori* infection is capable of reducing the endoscopy workload by 23%^{12, 13}. In the present study, serology, a non-invasive test, as specific as RUT and has a positive predictive value of 95.7% and negative predictive value of 98.1%. RUT and Serology showed a good correlation with normal mucosal study and with duodenal involvement in endoscopic study but there was no significant correlation with non-duodenal APDs. Therefore, more than one method may be required for the definitive diagnosis of *H. pylori* associated non-duodenal APDs. This is in agreement with other investigators who recommended a combination of two tests to increase the sensitivity¹.

Disclosure

The authors declare that they have no conflict of interests.

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