

ROLE OF H. PYLORI IN GASTRODUODENAL DISEASESB. Shanti Reddy¹, P. Venkateswarlu², B. Naga Jyothi³, A. Renuka Devi⁴**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: PURPOSE: The study was conducted to compare the different methods of detection of H.pylori in Gastroduodenal diseases. **MATERIAL & METHODS:** Endoscopic biopsy specimens & serum were collected from 110 cases suffering from gastroduodenal diseases. Biopsy specimens were subjected to rapid urease test, Grams staining & culture. Serum samples were used to detect anti H.pylori antibodies of IgG type by ELISA method. **RESULTS:** H. pylori was identified in 56 Grams staining smears (50.9%), rapid urease test was positive in 64 cases (58.1%), H. pylori was isolated from culture in 22 cases(20%), IgG of H. pylori was detected in 62 cases (56.3%) by ELISA method. **CONCLUSION:** A Combination of Grams staining smear examination, rapid urease test & serum IgG detection appear to be highly sensitive & specific for detection of H. pylori infection in patients undergoing endoscopy.

KEYWORDS: H. pylori, gastro-duodenal disorders, endoscopic biopsy, rapid urease test, Grams staining, culture & serology IgG-ELISA.

INTRODUCTION: The study of gastric bacteriology has gained significance since the isolation of H. pylori from gastric mucosal biopsies in 1983 by Barry Marshall & Robin Warren in Perth, Australia.⁽¹⁾ H. pylori is a Gram negative curved motile bacilli found in the deeper position of the mucosal gel coated gastric mucosa. H. pylori can cause a group of disorders of upper gastro intestinal tract involving principally stomach & most proximal part of duodenum.

International agency for Research on cancer has declared this pathogen as independent carcinogen in addition to association with cardiovascular disease⁽²⁾ & metabolic syndromes⁽³⁾ therefore it is important to detect the H. pylori infection & pursue with eradication therapy & follow-up.

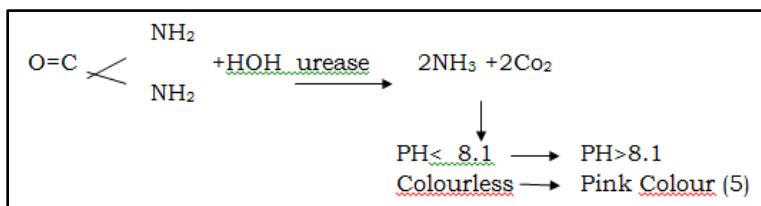
Now there is a battery of tests to diagnose H. pylori by using endoscopic biopsy specimens—⁽¹⁾ Rapid urease test⁽²⁾ Grams staining⁽³⁾ culture – most difficult yet gold standard with high specificity.⁽⁴⁾ Serological method of detecting IgG anti-bodies in serum samples by ELISA.⁽⁵⁾ PCR- used to detect H. pylori cag A & Vac A virulence genes in gastric biopsy samples but costly, so only few labs run this PCR.

MATERIALS & METHODS: The study was conducted to know the role of H. pylori in gastro-duodenal disorders among patients of age group 20 – 70 years at the department of Microbiology in collaboration with Gastro-enterology department, Kurnool Medical College, Kurnool.

Endoscopic biopsy samples were collected from patients who were allowed to fast for 12hrs before endoscopy. From each patient, 3 biopsy specimens 1mm in diameter were taken from pyloric antral mucosa within 5cms of pylorus with a sterile Olympus fiber optic endoscope. These 3 specimens were used for bacteriological study.

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RAPID UREASE TEST: The first biopsy specimen was inoculated in Stuart's urease broth⁽⁴⁾ and incubated at 37°C. Result were read at 1st hr, 2nd hr & 3rd hr by observing the color change from yellow to bright pink color throughout the medium indicating production of urease.



GRAMS STAINING: The second biopsy specimen was kept on a clean, new microscopy glass slide & compressed with another glass slide. The smears prepared were air dried and fixed in methanol for 1hr. Grams staining was done & examined by light microscopic under oil immersion for Gram negative 'S' or 'U' shaped bacilli arranged in groups.⁽⁶⁾

CULTURE: The third biopsy specimen was gently teased and rubbed on a freshly prepared selective medium BHI with lysed defibrinated horse blood & antibiotics like vancomycin, trimethoprim & Nalidixic acid⁽⁷⁾ then incubated under microaerophilic conditions in a dynamic jar at 37°C for 4-5 days which contained gas generating envelop that gives constant gas mixture of 6% Oxygen 10% Carbondioxide & 84% Hydrogen.

The growth found on the selective medium were identified as *H. pylori* on basis of their growth characters like slow growing, 5 to 2mm diameter, circular, non - hemolytic and grey translucent colonies showing Gram negative curved bacilli in Grams staining.

OXIDASE TEST: when colony material was inoculated on filter paper disc soaked in 1% tetramethyl paraphenelene-diamine-dihydrochloride, immediately a violet colour was observed as positive reaction. Negative control was put up by *E.coli*.

CATALASE TEST: A colony was taken by a wooden stick & dipped into the test tube with 0.5ml of H₂O₂. The evolution of bubbles indicates positive reaction. Negative control was put up by enterococci.

UREASE TEST: A Colony was taken with a straight wire and inoculated on urease medium. After 1 hr development of pink colour indicated positive reaction. Negative control was put up by *E.coli*.

SEROLOGY: Serum samples of patients were taken & IgG against *H.pylori* are detected by RIDASCREEN Helicobacter test which is an enzyme immunoassay.

PROCEDURE OF ELISA: On the surface of the microtitre wells purified specific antigens of *H.pylori* are bound. Diluted serum samples & controls were pipetted into the wells and incubated for 30 min at 37° C in a humid chamber. The present antibodies are bound to the immobilized antigens. The unbound material was removed in 4 washing steps by washing buffer. In the second step a peroxidase conjugate anti-human antibodies are added. After incubation for 30 min at 37° C, the

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unbound conjugate was removed by washing step. In the third step substrate (H₂O₂/TMB) was added to the wells & incubated for 30 min at 37° C. The enzyme bound in the wells convert the colorless substrate in blue colour. The results were confirmed by the change in colour from blue to yellow in microwells after addition of stop solution and also by measuring the absorbance at 450nm in the ELISA reader.

RESULTS: Endoscopic biopsy specimens from 110 cases of study group (82 males & 28 females) suffering from gastro duodenal disease & 20 cases of control group from normal healthy individuals were studied to detect H. pylori infection.

The maximum number of patients in this study was in the age group of 26 years to 50 years who were showing a gradual increase of positivity & decline thereafter.

H. pylori has predilection towards gastric cells in antral mucosa so antral gastritis (54 case) were predominant & followed by duodenal ulcers (26 cases) gastric ulcers (15 cases), gastro duodenitis (12 cases) & carcinoma stomach (3 cases) were studied to detect H. pylori. Rapid urease test, Grams staining, culture & serology IgG detection were done to all these cases & observed that H. pylori were detected more in antral gastritis case followed by duodenal ulcers & then other cases of the study group. The control group was negative for all the above tests.

Table showing Positivity of different laboratory tests employed to detect H. pylori

Sl. No	Laboratory tests	No. of Positivity	Percentage of Positivity	Sensitivity	Specificity	Positive predictive Value
1	Rapid urease test	64	58%	93.55%	87.50%	90.63%
2	Grams Staining	56	51%	76.47%	90.48%	92.86%
3	Culture	22	20%	68.75%	100%	100%
4	Serology IgG	62	56%	75.75%	72.72%	80.65%

Rapid urease test, Grams staining, serology IgG have given high positivity's.

DISCUSSION: In the present study of 110 symptomatic patients 82 were males & 28 were females (3:1) with an age group between 21-50 years. In the study conducted by Nair et al (8) showed males were more affected than females.

RAPID UREASE TEST: Urease testing of biopsy tissue is the single best indirect method for identification of H. pylori. The strong urease activity of H. pylori was indicated by placing biopsy material in urea broth & observing the change of colour within 3hrs. The positivity of rapid urease test was 58.1% which correlates with the positivity of Maimoona et al (65.8%)⁽⁹⁾ & Mohamed & Md. Yoosuf et al (70.67%).⁽¹⁰⁾

GRAMS'S STAINING: Observation of spiral or curved Gram negative bacteria in Grams stained smears of biopsy material has been played an important role in the diagnosis of H. pylori infection. The use of this simple direct Gram stained smear is an important test for detection of H. pylori from biopsy specimens which has 100% sensitivity & 96% specificity with low false positivity as in study of the Montgomery et al⁽¹¹⁾ & Kelkar Rohini et al.⁽¹²⁾ In our study, presence of small curved gram

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negative rods with typical spiral or gull wing appearance were observed in 56 cases (51%) which is closely related to the percentage of this test done by Maimoona et al⁽⁹⁾ & SR Gaval et al.⁽¹³⁾

CULTURE: The gold standard in detecting *H. pylori* infection is its isolation from biopsy material by appropriate culture methods. The fastidious nature of *H. pylori* made culture problematic. Many workers optioned that though culture of endoscopic samples are highly specific, time consuming, technically demanding are unnecessary unless antibiotic sensitivities are required. SR Gaval et al⁽¹³⁾ reported 11% culture positivity which correlated with the present study where *H. pylori* was isolated from 22 cases ie 20% culture positivity because of the fastidious nature of organism and laboratory problems.

SEROLOGY: The easiest way to diagnose *H. pylori* infection in a patient is testing for antibodies to it. IgG sero conversion occurs in 22-23 days after infection Morris et al.⁽¹⁴⁾ Serological test for detection of *H. pylori* infection are noninvasive & better to identify when organisms are scanty. In the present study anti *H. pylori* antibodies of IgG are detected in 62 cases with seropositivity of 56.3% which correlates with Nair et al,⁽⁸⁾ Siva Prakash et al⁽¹⁵⁾ & Shanjan Sharma et al.⁽¹⁶⁾

Many methods for identification of *H. pylori* are now available. Some of them being direct methods like smear examination, culture, DNA probe technique, others are indirect methods like urease testing & serology. The plethora of diagnostic aids for presence of *H. pylori* indicates that none of them are 100% accurate. The situation therefore demands a battery of test to be applied for maximum possible positivity. So to conclude it is necessary to use more than one test i.e. usage of rapid urease test, Grams staining and serology Ig G stands better to detect *H. pylori* infection. So, a subject was defined as *H.pylori* positive, if the bacteria was identified by at least two of the above four diagnostic methods used.

CONCLUSION: The present laboratory study was carried out to know the role of *H. pylori* infections among individuals with symptoms of acid peptic diseases under-going endoscopy.

Randomly selected 82 males and 28 females in the age group of 25 to 50 years under went diagnostic endoscopy and full thickness multiple endoscopic biopsy specimens were collected and subjected for tests to detect *H. pylori* infection.

H. pylori could be identified in 56 Grams stained smears of crushed biopsy materials, 64 positive rapid urease tests with biopsy materials, culture isolated from 22 cases & IgG in sera of patients against *H. pylori* were detected in 62 cases.

No single test can be considered sensitive or specific to detect or rule out *H. pylori* infection, so it is necessary to use a combination of tests.

A combination of Grams stained smear examination; rapid urease test & IgG detection appear to be highly sensitive & specific for detection of *H. pylori* infection in patients undergoing endoscopy.

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