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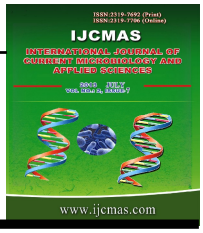

Original Research Article

Comparative study of invasive methods for diagnosis of *Helicobacter pylori* in humans

V.Subbukesavaraja[1]* and K.Balan [2]

[1]Department of Microbiology, MES Medical College, Kerala, India
[2]Department of Microbiology, KIMS, Tamil Nadu, India
**Corresponding author e-mail: arsenio82@gmail.com*

A B S T R A C T

To compare the various invasive tests (Rapid urease, conventional Urease, Gram stain, Geimsa stain) for identification of *Helicobacter pylori.* (*H.pylori*). A total of 81 endoscopic biopsy specimens were collected from gastric antrum of both the sexes in age group 20-70 over a period of 12 months from tertiary care hospital. Of 81 biopsy specimen, 59 were male (72.4%) and 22(27.6%) female, and the maximum number of patients was in the age group 40-49. The endoscopy results revealed that duodenal ulcer accounted for 36%, gastritis in 30%, gastric carcinoma in 16%, and gastric ulcer in 17%. In the present study rapid urease detected 35 positive cases, Giemsa staining 22 cases and Grams stain detects 18 cases. Majority of cases were in the age group of 40-49 years of male preponderance. Rapid urease test was positive in 43.21% of the samples of which 64.15% were positive in the first 10 minutes and Gram stain was positive in 22.2% whereas Giemsa stain was positive in 27.1%. More number of samples was found to be positive for *H.pylori* by Giemsa stain (27.1%) as compared to those prepared by Gram stain (22.2%).

Keywords

Biopsy; urease test; Gram's stain; Giemsa stain; *Helicobacter pylori.*

Introduction

The discovery of *Helicobacter pylori* in 1982 by Marshall and Warren was the starting point of a revolution concerning the concepts and management of gastroduodenal diseases. *H.pylori* is a gram negative curved motile rod found in the deeper portion of the mucous gel coating the gastric mucosa. It is extraordinary among bacteria in its ability to colonize and survive in this environment for decades despite host defenses and gastric acidity.

International Agency for Research on Cancer has declared this pathogen as an independent carcinogen in addition the etiologic association of this infection with an increasing number of disorders including cardiovascular diseases (Franceschi *et al*., 2005) and metabolic syndrome (Gunji *et al*., 2008) are being investigated. Therefore it is of utmost importance to detect the infection and pursue with eradication therapy and follow up. *H.pylori* is a strong producer of urease and its presence is detected by rapid

urease tests. The advantage of these tests is that they can be readily performed in the endoscope suite. Another rapid test is smear evaluation smears stained by Giemsa or Gram stain provide a diagnostic hint to histopathological examination of gastric biopsy specimens.

Culture is probably the most difficult approach to the diagnosis of *H.pylori*. The advantages are that it is gold standard, highly specific and the antibiotic sensitivity can be detected. High rate of false negatives due to the fastidious nature of the organism. Chronic *H.pylori* infection elicits local and systemic immune response that lead to production of antibodies. The presence of IgG antibodies to *H.pylori* can be detected by immunoassays. Serology is sensitive for primary diagnosis but is not useful in assessing post treatment *H.pylori* status (Lerang *et al*., 1998). The urea breath test relies on the urease activity of *H.pylori* to detect the presence of infection. Sensitivity is excellent because the whole stomach is sampled. Unlike serology it is useful for determining the success of the eradication therapy. Even though the test is more accurate than serology its usage is limited due to high cost and lack of facilities for testing.

With the advent of Polymerase chain reaction (PCR), many possibilities have emerged for diagnosing *H.pylori* infection. PCR allows identification of the organism in samples (Rudi *et al*., 1999) with few bacteria and it has been successfully used to detect *H.pylori* CagA and VacA virulence genes in gastric biopsy samples. PCR is being evaluated for its utility in identifying *H.pylori* in samples of dental plaque, saliva and other easily sampled tissues. The potential advantage of PCR includes high specificity, quick results and the ability to identify different strains of bacteria for pathogenic and epidemiologic studies. The major limitation of PCR is that it is costly and relatively few laboratories currently have the capacity to run the assay.

In the present study Rapid urease test, conventional urease test, Gram's stain, Giemsa stain of biopsy specimens were used to detect presence of *H. pylori* in selected samples.

Materials and Methods

The study was conducted after obtaining approval from the institutional ethical committee from tertiary care hospital. Informed consent was obtained from the patients before their enrolment in the study.

This is a prospective cross sectional study done for the period of one year from the Outpatients and Inpatients of both the sexes in age group 20-70, attending surgery department with complaints suggestive of upper gastro intestinal diseases or with gastric ulcer, duodenal ulcer, antral gastritis and gastric carcinoma. Patients with previous gastric surgery and active bleeding were excluded from the study

Specimen collection and transport

Biopsy Samples

Patients fasted overnight before endoscopy. Endoscopy was done using fiber optic endoscope. The endoscope and the biopsy forceps were rinsed thoroughly with water and soaked in 2% gluteraldehyde for 20 minutes (Coudron *et al*., 1989) and were thoroughly rinsed with sterile normal saline just before the collection of specimen.

Two biopsy samples were taken from the antrum (2cm from the pylorus) and body of the stomach and were transported in normal saline for Urease and staining procedures.

Processing of specimen

2 Biopsy tissues were placed in two different sterile glass slides with a drop of normal saline and teased with sterile scalpel so as to make the tissue into smaller fragments. Another sterile glass slide was placed over the teased first tissue and the tissue was crushed between the two glass slides. Then the minsed tissue was used to make smears. The same was repeated to the other biopsy tissue and stained with grams and giemsa stain.

The biopsy specimen is further subjected to rapid urease test (Pyloday, Helix diagnostics) and conventional urease test with positive and negative control .

Result and Discussion

Of 81 biopsy specimen, 59 were male (72.4%) and 22(27.6%) female, and the maximum number of patients in this study group was in the age group 40-49 (Table 1).

The endoscopy results revealed that duodenal ulcer accounted for 36%, gastritis in 30%, gastric carcinoma in 16%, and gastric ulcer in 17 %. (Figure.1)

In the present study rapid urease detected 22 positive cases, Giemsa staining 22 cases, Grams staining 18 cases (Table 2).

The present work is based on using conventional and rapid diagnostic method for detecting *Helicobacter pylori* infection. Four biopsy based tests namely rapid urease test, conventional urease test, gram stain, giemsa stain were done. The conclusions from the study give fruitful thought about the relative merits and demerits of each method.

A total of 81 patients with upper gastrointestinal symptoms were enrolled in the study of which 59 (72.4%) were males and 22(27.6%) were females. (Table.1).

The maximum number of patients in this study was in the age group 40-49, which correlates with study by Nair *et al*.,(1997) were out of the 136 patients, 116 were male and 20 were female in comparable to the present study which also showed males were more affected than females.

The endoscopic examination of the study group revealed that duodenal ulcer accounted for 36%, gastritis in 30%, gastric carcinoma in 16%, and gastric ulcer in 17%. (piediagram 1). Out of the 81 samples studied by rapid urease test35 (43.21%) were positive, the overall positivity of rapid urease test correlates with study by Sivaprakash *et al.,* (1994). (38.7%) at the same time a study by Maimooma *et al.,* (1994) found that positivity was 65.8%.

Conventional urease test were positive for 22(27.16%) cases which was comparatively lesser than rapid urease test which gave 43.31% positivity. Rapid urease test could have given false positivity for 7 (20%) out of 35 cases as all other battery of tests showed negativity (Tee *et al*., 1991). The direct Gram stained smears in our study showed positivity in 18% which is comparable to the study by Arora *et al.,* (2003) ,while Anjana *et al*., (1998) reported 72.3% while other studies report values, ranging between 44-74% (Philip *et al*., 1989).

Table.1 Demographic Profile of Study Population

Age	Male	Female	Total	Percentage (%)
20-29	7	2	9	11.1
30-39	10	5	15	18.5
40-49	18	7	25	30.8
50-59	14	6	20	24.3
>60	10	2	12	14.8
TOTAL	59	22	81	100

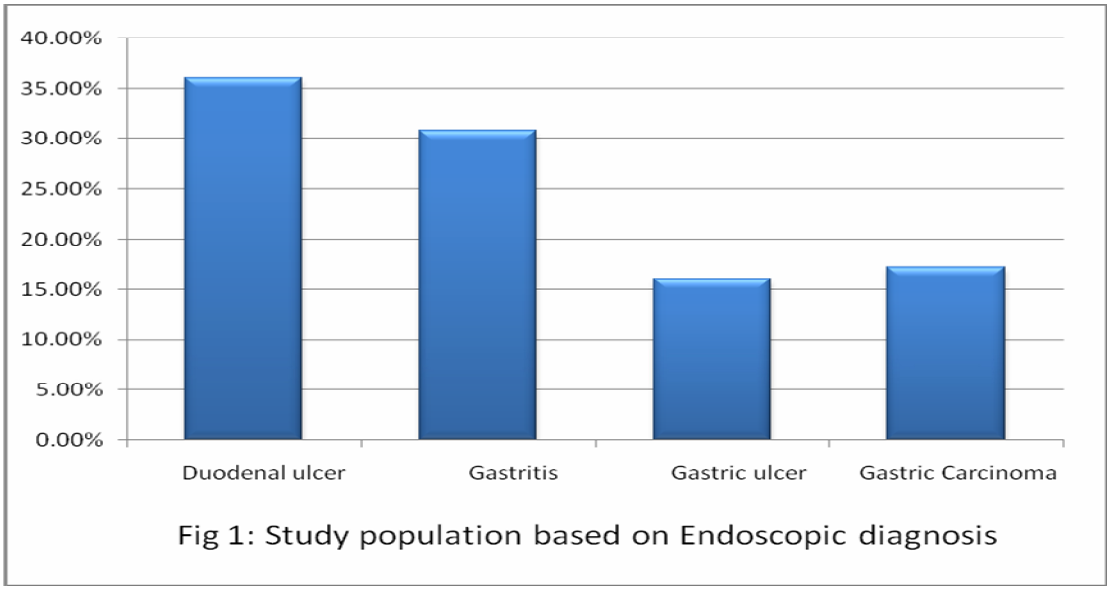

Fig 1: Study population based on Endoscopic diagnosis

Table.2 Comparison between Urease test and staining procedures

Endoscopic diagnosis	Urease	Gram's stain	Giemsa stain
Duodenal ulcer	10	8	9
Gastritis	7	7	8
Gastric ulcer	3	1	2
Gastric Carcinoma	2	2	3
Total	22	18	22

In the present study Giemsa staining of the crushed smear was positive in 22% which correlates with study by Coudron *et al*., 1989 were positivity was 31% (Philip *et al*., 1989). In the present study rapid urease detected 35 positive cases, Conventional urease 22 cases, Giemsa staining 22 cases, Grams staining 18 cases. As there is vast difference in Rapid and conventional method for urease detection it would be wise to compare conventional urease and giemsa stain. A similar picture emerges in the study by Anjana *et al.*, (1998) in which 34 out of 47 cases was urease positive, while Giemsa was positive in 38 out of 47 of the cases.

The majority of cases, out of a study population of 81 patients, were in the age group of 40-49 years of male preponderance and epigastric pain was the most common symptom in both gastric carcinoma and acid peptic disease. Duodenal ulcer was the commonest endoscopic finding observed during the course of the study.

Rapid Urease test was positive in 43.2% of the samples of which 64.1% were positive in first 10 minutes whereas Gram's stain was 22.2% and Giemsa stain was positive in 27.1% of the cases. More number of samples was found to be positive for *H.pylori* by Giemsa stain (27.1%) as compared to those prepared by Gram stain (22.2%).The Seroprevalence of the study population was 35.8%. As this is institutional based limited study further evaluation of the test has to be done with a bigger sample size to arrive at a conclusion for this disparity.

Acknowledgement

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