

Research Article

Comparative Study of Noninvasive Methods for Diagnosis of *Helicobacter Pylori* in Humans

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Abstract: The objective of the study is to compare the noninvasive tests (antigen and antibody detection) for identification of *Helicobacter pylori*. (*H.pylori*). A total of 81 serum and stool specimens were collected from out patients and in patients presenting with upper gastrointestinal symptoms of both the sexes in age group of 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 stool specimens and serum samples were subjected to examination for detection of Antigen and Antibody respectively. Antibody was detected in 29 out of the 81 samples tested (35.8%) whereas Stool Antigen was positive in 23 (28.40%) out of 81 samples tested. Majority of cases were in the age group of 40-49 years of male preponderance. The present study reveals that serology showed a slightly greater number of positive cases.

Keywords: Stool, Antigen, Serum, Antibody, *H.pylori*

INTRODUCTION

The discovery of *Helicobacter pylori* in 1982 by Marshall & Warren [1] 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 [2], and metabolic syndrome [3] is 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 is 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 [4]. 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 [5] 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.

H. pylori culture from stools is not used as a routine diagnostic method. The first report of successful detection of *H. pylori* antigens in stools was made in 1997 by Kozak *et al.* who reported an enzyme-linked

immunosorbent assay (ELISA) performed on stools this test was named *H. pylori* stool antigen test (HpSA).

H. pylori infection is a chronic condition and immunoglobulin G (IgG) (subclasses 1 and 4) is the predominant immunoglobulin class, even in children IgG are present at the mucosal level and detected in virtually all blood samples. IgM are rarely observed, merely because acute *H. pylori* infections are seldom available for study.

In the experimental infection carried out by Morris *et al.*, an initial IgM response was observed. IgA are also elevated in the majority of infected cases but not in all. Therefore, as the relevance of IgM and IgA is limited, commercial kits are primarily designed to detect IgG.

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. Stool specimens and Serum samples were subjected to examination for detection of Antigen and Antibody respectively.

Specimen Collection and Transport

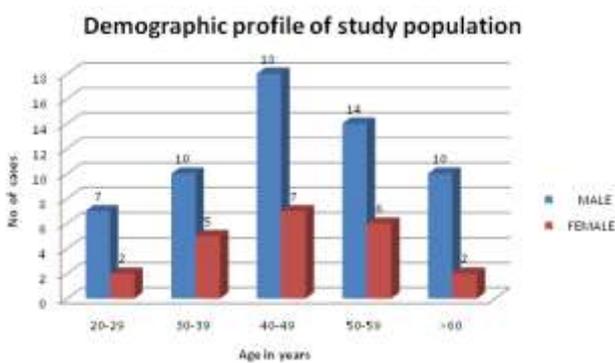


Chart-1: The endoscopic examination of the study population revealed that duodenal ulcer accounted for 36%, gastritis in 30%, gastric carcinoma in 16%, and gastric ulcer in 17%.

Stool Specimen

Patients were asked to collect early morning stool in sterile container provided to them after the day of the performance of endoscopy. A sterile swab was rubbed in stool samples provided in the sterile container. The swab was then inserted into the sample collection tube containing assay diluents. The swab was swirled for atleast 10 minutes in the diluents until the sample was dissolved. The swab was discarded after squeezing it against the walls of the collection tube. After the collection tube was capped with dropper and it was allowed to stay for in flat surface for 5-10 minutes. About 100 ul of the processed specimen was added into the sample well (Rapid diagnostic kit method by Bioline SD diagnostics) of the testing device. The kit was left undisturbed for 15 minutes and the results were interpreted within 10-15minutes. The test results were interrupted as negative, positive and invalid.

Blood specimen

3ml of venous blood was collected under aseptic conditions; serum was separated and stored in 4 °C for further processing. Added 50 µl of plasma or serum in the kit (*H pylori* kit, Tulip Diagnostics) and timer was started. Test results were interpret after 10 min as test results at 10 minutes. The result should not be interpreted after 10 minutes as positive, negative and invalid.

RESULTS

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(Chart: 1)

Table 1: Categorization of the study population based on Endoscopic Diagnosis and histopathology confirmation

Endoscopic Diagnosis	Total	Percentage
Duodenal ulcer	29	35.8
Gastritis	25	30.8
Gastric ulcer	13	16.0
Gastric Carcinoma	14	17.2
Total	81	100

Table 2: Comparison between antibody and antigen detection

Endoscopic diagnosis	Antibody positive	Percentage (%)	Antigen positive	Percentage (%)
Duodenal ulcer	12	41.3	9	39.3
Gastritis	9	31	8	34.7
Gastric ulcer	5	17.2	3	13
Gastric Carcinoma	3	10.3	3	13
Total	29	35.8	23	28.3

DISCUSSION

A total of 81 patients with upper gastrointestinal symptoms were enrolled in the study. Among them 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. In the study conducted by Nair *et al.* [7] 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 present study reveals that serology was positive in 29 out of the 81 samples tested (35.8%) which correlates with the study by Nair *et al* [7] and Sivaprakash *et al* [8] who reported a positive result in 64.9%. The reduced percentage for detection by serology can be due to limited value of certain kits as summoned in a study by Chen Ts *et al* [9] and Goodwin *et al* [10]. The distribution of positive serology results as against endoscopic findings in the study population is as follows- duodenal ulcer 12 cases, gastric ulcer 9 cases, gastritis 5 cases and gastric carcinoma 3 cases. Hence there exists a positive correlation between duodenal ulcer and *H. pylori*, confirming previous reports (Table 1).

Stool Antigen detection by rapid kit method was positive in 23 (28.4%) out of 81 samples tested. This is comparable to study by Mahir Gulcan *et al* [11] who reported positive result in 37 out of 80 children (Table 2) which was comparatively greater than this study.

Serology showed a slightly greater number of positive cases than the conventional tests which may be due to past infection. This is comparable to the study by Arora *et al.*, who reported greater case detection by serology than by conventional tests. The patchy distribution of organism in the gastric mucosa may have resulted in a lower value for biopsy based test. Another factor could be the presence of gastric atrophy and intestinal metaplasia that are hostile to *H. pylori* [12].

CONCLUSION

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. The present study reveals that antibody was positive in 29 (35.8%) whereas antigen was 23 (28.3%) out of the 81 samples tested. 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.

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