STUDY OF DIAGNOSTIC TESTS FOR HELICOBACTER PYLORI INFECTION

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ABSTRACT

Helicobacter pylori is the causative agent of most cases of gastritis and peptic ulcer. The diagnosis of H. pylori is an essential element in the management of many common gastrointestinal pathologies.

AIMS

1. Comparison of invasive and non-invasive tests to choose the appropriate test for the diagnosis of H. pylori infection. 2. Validation of the comparison of the different diagnostic tests.

METHOD

Blood and antral biopsy specimens from 100 acid peptic disease patients and blood samples from 10 control subjects were collected. Biopsies were used for Rapid Urease Test (RUT), culture and Gram's stain by conventional method. Serology using Euroimmun Anti Helicobacter pylori IgG ELISA was done. The efficacy of these tests was determined by calculating the sensitivity, specificity, positive predictive value, negative predictive value and accuracy using culture as gold standard.

RESULTS

Of the 100 cases 14% were culture positive, 18% Gram stain positive, 36% Rapid urease test positive and 42% were positive for Serology IgG antibodies for H. pylori. Maximum percentage of positivity was in peptic ulcer cases (52.9%) followed by Gastritis cases (23.6%) and Dyspepsia cases (14.2%). Among the 100 cases of study group, 42(42%) were positive by serological test IgG ELISA for H. pylori, whereas 3(30%) were positive out of 10 in control group. RUT, IgG Serology showed 100% sensitivity and negative predictive value and Gram stain showed highest specificity (90.1%).

CONCLUSION

RUT+Gram's stain+IgG Serology showed highest Sensitivity, Specificity, Positive predictive value, Negative predictive value and Accuracy. IgG Serology indicates a marker for infection. It can be used as a primary diagnostic procedure.

KEYWORDS

Helicobacter pylori, Peptic ulcer, Gastritis, Rapid urease test.

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INTRODUCTION

The discovery of the association between Helicobacter pylori (H. Pylori) and peptic ulcer disease by Warren and Marshall in 1982 in Australia was a landmark breakthrough in the understanding of upper gastrointestinal disease. H. pylori is now recognized as one of the most common bacterial infections in humans.[1] In the industrialized world, the prevalence of H. pylori infection in the general population rises steadily with age. In many parts of the underdeveloped world, this infection is much more common and is often acquired in childhood.[2]

It is considered to play a major role in the pathogenesis of several gastrointestinal diseases including gastric ulcer, duodenal ulcer, gastric Mucous Associated Lymphoid Tissue (MALT) lymphoma and distal gastric cancer.[3] In 1982, Warren and Marshall 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] Experiments demonstrated that these bacteria can colonize the human stomach, thereby inducing inflammation of the gastric mucosa.[5]

The present study was conducted to compare the different diagnostic methods like Gram staining, Culture, Rapid Urease Test and Serology in the local population to select the most sensitive, specific, rapid, reliable and cost effective test for the diagnosis of H. pylori.

MATERIALS AND METHODS

The study was conducted after obtaining approval from the Institutional Ethical Committee. Informed consent was obtained from the patients before their enrolment in the study. The present study has been carried out on 100 patients of different ages and sex, who were referred for endoscopy. Three tissue biopsy samples were taken from each patient from antrum (within 2 cm of pylorus) and two biopsy samples were placed immediately in a screw capped bottle of thioglycolate broth for culture and Gram's staining and was transported to the laboratory within two hours of collection.

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Direct impression smears were prepared from the biopsy tissues on a clean glass slide and were stained with Gram's stain. Gram's staining shows Gram negative, spiral or S shaped or Seagull shaped organisms with bluntly rounded ends of 0.5-1µm wide and 2.5-5µm long (Fig 1).

For culture, biopsy sample was inoculated onto freshly prepared Brain-Heart Infusion Agar supplemented with 5% sheep blood and Columbia Agar with Skirrow's supplement and incubated at 37°C for 7 days in McIntosh Fildes jar under microaerophilic conditions by gas pack (5% O_2, 10% CO_2 and 85% N_2) devoid of any catalyst. Plates were examined on the 3rd, 5th and 7th day.

Colonies of H. pylori are less than 2mm in diameter, greyish, circular, low convex, translucent and weakly haemolytic on Sheep blood agar**.** On Skirrow's Campylobacter medium colonies are slightly bigger, circular, low convex, grey and translucent. H. pylori are actively motile, catalase positive, oxidase positive and strongly urease positive.

Other biopsy sample was inoculated in the endoscopy room, in Christensen's urease broth for Rapid Urease Test (RUT) and incubated at 37^0 C, the tube was examined after 15 mins, 30 mins, 60 mins, 4hrs, 8hrs, upto 24hrs and a color change from yellow to red or magenta was read as positive (Fig 2). Blood samples were collected from the same patients and was used for the detection of IgG antibody by Anti-Helicobacter pylori IgG ELISA kit.

The efficacy of the tests was determined by calculating the sensitivity, specificity, positive predictive value, negative predictive value and accuracy taking culture as gold standard.

1. Sensitivity was defined as the proportion of number of Helicobacter pylori infected who had a positive test and was calculated as:

Sensitivity=	No. of True Positive / No. of True positive + No. of False negative	X100

2. Specificity was defined as the proportion of individual free of Helicobacter pylori that had a negative test and was calculated as:

Specificity =	No. of True negative / No. of False positive + No. of True negative	X100

3. Positive predictive value was defined as:

PPV=	No. of True positive / No. of True positive + No. of False positive	X100

4. Negative predictive value was defined as:

PPV=	No. of True negative / No. of True negative + No. of False negative	X100

5. Accuracy was defined as:

PPV=	No. of True positive + True negative / No. of True positive + True negative + False positive + False negative	X100

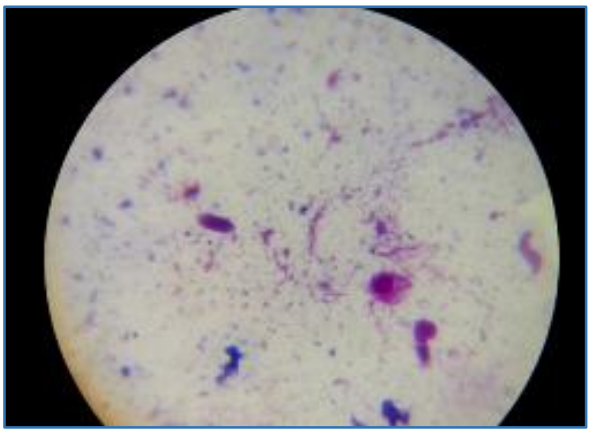

Fig. 1: Curved S-Shaped Gram Negative Helicobacter pylori in grams staining

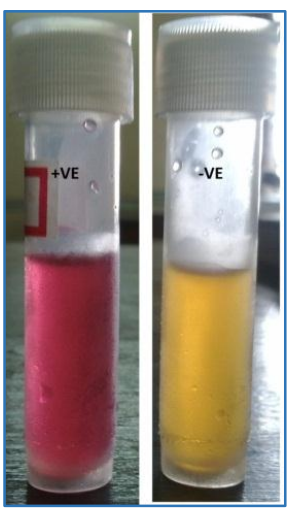

Fig. 2: Urease Broth

RESULTS

Out of 100 cases, 14 were culture positive, 18 Gram stain positive, 36 Rapid urease test positive and 42 were positive for Serology IgG antibodies for H. pylori (Table-1).

Among different combination of tests, maximum positivity was shown by RUT+Serology (28) followed by RUT+Gram stain (17) and RUT+Gram stain+Serology (15) (Table-2).

H. pylori showed maximum percentage of positivity in peptic ulcer cases (52.9%) followed by gastritis cases (23.6%) and dyspepsia cases (14.2%).

Among positive cases for H. pylori, 20-29 years age group showed highest percentage (35.7%) of positive cases and males (57.1%) showed a higher percentage of positivity compared to females (42.8%).

Among the 100 cases of study group, 42(42%) were positive by serological test IgG ELISA for H. pylori, whereas 3(30%) were positive out of 10 in control group (Table-3).

Among various diagnostic tests RUT, IgG serology showed 100% sensitivity and negative predictive value. Gram stain showed highest specificity (90.1%). Gram stain showed a higher accuracy (89.2%) than RUT + Serology (85.5%). The three test criteria (RUT + Gram stain + Serology IgG) showed highest sensitivity (100%), specificity (92.4%), positive predictive value (78.9%), negative predictive value (100%) and accuracy (94.1%) (Table-4).

Diagnostic Test	Positive	Negative
Rapid Urease Test	36	64
Gram stain	18	82
Culture	14	86
IgG Serology	42	58

Table 1: Positivity for H. pylori by various diagnostic tests.

Tests	No. of Positive Patients
Culture	14
RUT+Gram's stain	17
RUT+Serology	28
RUT+Gram's stain+Serology	15

Table 2: Positivity for H. pylori with different combination of tests.

	Total No. Examined	No. of Positive Cases	Percentage
Study group	100	42	42
Control group	10	3	30

Table 3: Serological test (IgG ELISA for H. pylori) in study and control groups.

Tests	Sensitivity	Specificity	PPV	NPV	Accuracy
RUT	100%	73.5%	61.0%	100%	81.3%
Gram stain	85.7%	90.1%	66.6%	96.4%	89.2%
IgG Serology	100%	66.6%	59.1%	100%	77.5%
RUT+ Serology	100%	79.03%	68.2%	100%	85.5%
RUT + Gram stain + Serology	100%	92.4%	78.9%	100%	94.1%

Table 4: Statistical analysis of various diagnostic tests for H. Pylori with culture as gold standard.

DISCUSSION

Culture is the gold standard test for the diagnosis of H. pylori. In the present study, 14% cases were positive for H. pylori by culture. All these cases showed urease test positive. This study was nearer to Navinchandra Motiram Kaore et al.[6] (2012), which showed 8.6% positive by culture.

H. Pylori isolations were few in India compared with other works because it is a fastidious organism, which requires microaerophilic condition and frequent use of anti-secretory agents as well as Metronidazole for protozoan infestations, decrease the positive outcome.[7,8] Other factors like patchy distribution of the organism in gastric mucosa, time from collection to culture, inadequate mincing of the biopsy material and loss of viability of the specimen during transportation may result in low sensitivity.[9]

In the present study, 36% cases were urease positive. This study correlates with Nassir E. Mohsun et al.[10] (35.6%) and is near to V. Subbukesavaraja et al.[11] (27.1%). Rapid urease test may be recommended as the first choice test as result is obtained within one hour (Suerbaum and Michetti, 2002). Sensitivity and Specificity of Rapid urease test was 100% and 73.5% respectively. Sensitivity of this study correlates with Ruba S. Abu-Sbeih et al.[12] (100%) and Specificity was nearer to Saffari Mahmood et al.[13] (80%).

Gram staining is one of the rapid screening procedures for the diagnosis of Helicobacter pylori. In the present study, 18% cases were positive by Gram staining. This study correlates with V. Subbukesavaraja et al.[11] (22.2%). Sensitivity of Gram stain was 85.7% and Specificity 90.1%. Specificity of this study was close to Madhu Sharma et al. (80%). Direct Gram's stained smear is an invasive, highly specific, rapid screening test and if resources are limited may obviate the need for additional testing of positive specimens.

In the present study, 42% cases were positive by serologic test using ELISA to detect IgG antibodies and was nearer to Hoda A. Reffaay et al.[14] (50%). In the present study, Sensitivity of IgG serology is 100% correlating with Nassir E. Mohsun, et al.[10] (100%) and Ruba S. Abu-Sbeih et al.[12] (100%). Specificity of IgG Serology in present study is 66.6% nearer to Madhu Sharma et al. (63.3%). IgG serology serve as an easy, sensitive, cheap and non-invasive method for screening patients before endoscopy. It is useful in management by virtue of early detection of patients with an increased risk of chronic peptic ulceration.[15]

CONCLUSION

Among all tests, the three test criterias (RUT + Gram's stain + IgG Serology) showed highest Sensitivity, Specificity, Positive predictive value, Negative predictive value and Accuracy. So it was the best combination.

If conditions are not favorable for RUT + Gram's stain + IgG Serology, which includes invasive tests also, then IgG serology which is a non-invasive test and was the best because it showed 100% Sensitivity, 100% negative predictive value and also it was a non-invasive test. As it showed 100% negative predictive value, IgG serology indicates a marker for infection. It can be used as a primary diagnostic procedure.

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