Comparative Evaluation of Different Diagnostic Techniques Available for Diagnosis of Helicobacter Pylori

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Abstract- Introduction: Multiple tests are available to determine whether a patient is infected with *Helicobacter pylori*. Some of the methods for identification of this organism include: direct methods like smear examination, culture, histological sections and DNA probe technique; others are indirect like urea breath test and serology. The plethora of diagnostic tests available for the presence of *Helicobacter pylori* indicate that none of them is 100% accurate.

Material and Methods: During a period of one year of this study, blood and antral biopsy specimens from 70 dyspeptic patients and 10 control subjects were collected. All the specimens were processed within 2-3 hours. Rapid urease test, Primary smear, Culture was done on freshly prepared brian-heart infusion agar supplemented with 7% horse blood, vancomycin (6 mg/L) and amphotericin B (2 mg/L). Plates were incubated at 37°C for 7 days in mcIntosh fildes jar under microaerophilic conditions. The prescence of *H.pylori* was confirmed by colony morphology, secondary smear stained with Gram's stain, Oxidase test, Catalase test and Urease test. Histology was done using Heamatoxylin & Eosin staining and Giminez staining. Serology was also performed using a commercial kit - Novum *Helicobacter pylori* IgG and IgA ELISA manufactured in Germany.

Results: Rapid urease test was positive in 51.4% with a sensitivity and specificity of 60% each. Gram's stain was positive for *H.pylori* in 44.2% with a sensitivity ans specificity of 62.5% and 80% respectively. Culture was positive in 4.2% cases only and had a sensitivity and specificity of 7.5% and 100% respectively. The H.pylori positivity was 68.5% by IgA serology with a sensitivity and specificity of 75% and 36.7% respectively. In the control group 30% were positive by IgG and 40% by IgA serology.

Conclusions: The concordance of results of various diagnostic tests is the best available option for determining a patient's true *H.pylori* status.

Index Terms- Diagnostic techniques, H.pylori, Culture, histology, Serology

I. INTRODUCTION

Multiple tests are available to determine whether a patient is infected with *Helicobacter pylori*. Each one of them are as yet not totally fool-proof. Various authors have stressed the importance of each test but the same could not be reproduced [1]. The tests available for diagnosis of *Helicobacter pylori* include invasive and non-invasive techniques. At present, there is no universally accepted 'gold-standard' for diagnosis of *Helicobacter pylori* infection. Culture and histological analysis of antral biopsy specimen have been considered gold standard to diagnose *Helicobacter pylori*, but these techniques are invasive and time consuming. The non-invasive techniques include serodiagnosis and urea breath test. Various studies have demonstrated the clinical usefulness of serological assays for antibody against *Helicobacter pylori* in infected patients[1].

In clinical practice, a rapid and efficient diagnosis of *Helicobacter pylori* would be of great help in the appropriate treatment. Therefore, there is need to evaluate the diagnostic test for their relative usefulness.

II. AIMS AND OBJECTIVES

To evaluate the different diagnostic tests available for diagnosis of *Helicobacter pylori* infection.

III. MATERIAL AND METHODS

The present study was conducted during a period of one year in the department of Microbiology, T.N. Medical College and BYL charitable hospital, Mumbai.

Selection of cases: Patients were selected from those referred for upper gastrointestinal endoscopy and included both outdoor and indoor patients.

Patients-70 patients with dyspeptic symptoms were selected. Patients on antibiotics and NSAIDs for the past 15 days prior to endoscopy, chronic liver diseases, severe cardio-pulmonary or renal diseases were excluded from this study.

Controls- 10 patients who were also attending gastroenterology clinic with lower gastrointestinal problems were included in this study.

Collection of specimens:

The specimens collected for the study were blood and three gastric biopsies from each patient.

Blood :- Prior to endoscopy 5-6 mL of blood was collected aseptically from the antecubital vein of each patient using 10 ml disposable syringe and needle. Blood was transferred to sterile glass test-tubes and allowed to clot. Then the serum was separated and was centrifuged at 2000 rpm for 5-10 minutes to remove any cellular debris from the serum. The clear supernatant serum was aseptically transferred to sterile plastic vials and stored at -20° C until tested.

sBiopsy :- Three biopsies received from each patient from antrum (within 2 cm of pylorum) for microbiological examination in brucella broth and christensen's urea agar for rapid urease test (RUT) and another for histopathological examination.

Processing of Sample :

All the specimens were processed within 2-3 hours of collection. Following test were performed-

i) Rapid urease test [2]
ii) Primary smear- The biopsy specimen in brucella broth was ground in a ground- glass grinder. A loopful of ground biopsy material was compressed between two sterile glass slides, air dried, heat fixed and stained with Gram's stain [3].
iii) Culture- ground biopsy material was inoculated on freshly prepared brain-heart infusion agar supplemented with 7% horse blood, vancomycin (6 mg/L) and amphotericin B (2 mg/L). Plates were incubated at 37°C for 7 days in McIntosh fildes jar under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) devoid of any catalyst[4]. Plates were examined on the 3rd, 5th and 7th day. Colonies of *H.pylori* from primary culture usually took 3 to 5 days to appear and were less than 2 mm in diameter, greyish, circular, low convex, translucent and weakly hemolytic.

Confirmation of *H.pylori*

Suspected colonies of *Helicobacter pylori* were identified by colony morphology[5] - colonies on brain heart infusion agar were circular, convex, translucent, 0.5-1 mm in diameter and weakly hemolytic, secondary smear stained with Gram's stain[5] , oxidase test[6] , catalase test[7] , urease test[5]

Histology –

The formalin fixed specimens were sectioned and stained with heamatoxylin & eosin (H&E) and Gimenez stain[8]. Light microscopic examination was carried out specifically looking for *H.pylori* and inflammatory cell infilterate in the antral mucosa. Gastritis was graded as nil, mild, moderate and severe.

Serology -

Serum samples were tested for the prescence of IgG and IgA antibodies to *Helicobacter pylori*. Commercial kits used were- Novum *Helicobacter pylori* IgG and IgA ELISA manufactured in Germany. The procedure was followed as per the instruction manual of the manufacturer.

Evaluation of parameters –

A subject was defined as *H. Pylori* positive if the bacteria were identified by Histology (Gold- standard). The results of individual diagnostic methods were compared with the histology and evaluated for its sensitivity, specificity, positive predictive value and negative predictive value. For comparison, concordance of four, five and six parameter was also examined.

Statistical Analysis–

Data was statistically analysed by using spss/pc + (statistical package for social sciences). Chi-square test (χ^2) and chi- square with yate's correction were applied whenever possible.

IV. RESULTS

There were 54.16% males and 63.63% females among the total *H.pylori* patients (57.14%). [p> 0.05, not significant]

H.Pylori was positive in 36 patients by rapid urease test, 31 by Gram's stain, 3 by culture, 41 by IgG serology and 48 by IgA serology. All the control patients were negative by urease test, Gram's stain, culture and histology. There were 3 control subjects positive by IgG serology and 4 by IgA serology.(Table - 1)

For the statistical analysis of different tests, histology was taken as gold standard and sensitivity, specificity, negative and positive predictive values of five diagnostic assays were calculated. IgG and IgA serology had highest sensitivity of 75% each, followed by Gram stain 62.5%. Culture had highest specificity of 100% followed by Gram's stain 80% and 63.3% by IgG serology. Validity (combination of sensitivity and specificity) and Youden's *J* value was highest of Gram's stain 71.25% and 0.43 respectively, followed by IgG serology 69.15% and 0.39 respectively. Lowest was with culture 53.75% and 0.08 respectively. (Table- 2)

V. DISCUSSION

In our study, the specificity of RUT was 60% (Table-2). Schnell and Schubert[9] reported 91% specificity, Coudron and Kirby[10] 89% and Maimooma et al[1] 81%. The low specificity in our study was due to 40% false positive cases which can be due to urease producing bacteria other than *H. pylori* in gastric biopsy specimens. This fact limits the diagnostic usefulness of urease tests as a definitive method for detection of *H.pylori*. In a study by Destura et al[11], *H. pylori* culture showed a sensitivity of 45%, positive predictive value was 97% and the negative predictive value was 55% which matches with our study in which sensitivity, positive predictive value & negative predictive value of *H. pylori* culture was 7.5%, 100%, 44.8% respectively. (Table-2)

In our study, direct Gram's stained smear showed overall positivity in 31 patients i.e. 44.2% (Table-2). A positivity rate , in direct Gram's stained smear of biopsy specimens , however varies from 40-90% as reported by various workers[3,12,13]. The presence of *H.pylori* can rapidly, accurately, easily and inexpensively detected with a simple Gram's stain of direct biopsy smear. But sensitivity of this test greatly depends upon the skill of the observer besides other parameters like number of organisms with typical morphology in the specimen.

The sensitivity of Gram's stain in our study was 62.5% (Table-2), which is in concordance with Nichols et al[14], (69%) where as, high sensitivity is reported by Montogomery and Co-worker[3] (92%), Maimooma et al[1] (80.8%) and Tobin et al[15] (91%) the specificity of Gram's stain in our study was 80% (Table-2) and correlated well with Tobin et al[15] (82%). Gram's stain had low false positivity of 20% in our study and same has been reported by many workers[12,13].

In the present study, *H.pylori* was isolated in 3 out of 70 patients (4.2%). (Table-2) Similar isolation rate has been reported by Gaval et al[16]. (2%) Higher culture positivity rate > 90 has been reported by various workers[17,18]. But studies from India have shown low rates of isolation. Ayyagari et al[19] reported 23.9% and Nanivadekar et al[20] reported 24% isolation rate. 8/92 (8.7%) samples were positive by culture with a sensitivity of 8.69% as reported by Kaore et al[21]. In contrast, the Indian studies reported a sensitivity which ranged from 1.09% (as reported by A. Ayyagari)[19] to 63% (as reported by Akbar and Eltahawy)[22]. The low rate of isolation may be because of the fastidious nature of H. pylori and because of a number of other factors like the patchy distribution of the organism, inadequate mincing of the biopsy material, the presence of oropharyngeal flora, the loss of viability of the specimen during transportation, etc. These factors are difficult to control. All these factors together, result in low sensitivity and a low negative predictive value[21].

In our study, the positivity of IgG serology in the patient group was 58.5% as compared to 30% in control group (Table-1). Several authors have observed increase in levels of specific antibody in patients as compared to controls[23,24]. However, a positivity of 30% to 40% in healthy individuals has been reported by various authors which is in general agreement with our findings[25]. This is possibly due to the biopsies failing to detect *H.pylori* in these subjects or the subjects might have cleared the bacterium spontaneously, while antibody response persisted.

The sensitivity and specificity of IgG serology was found to be 75% and 63.3% respectively (Table-2). This was comparable to study by Nair et al[26] (82.8% sensitivity and 50% specificity) and Sharma et al[26] (80% and 53% respectively) but were lower than figures quoted by western studies[27].

In the present study, IgA antibodies were found in 68.5% in patient group and 40% in control group (Table-1). The sensitivity and specificity of IgA serology was found to be 75% and 36.75 respectively (Table-2) The sensitivity of IgA serology was in concurrance to Talley et al[27] 70%. The specificity was lower than the western studies[28.]

The low specificity of ELISA observed in our study may be due to the increased carriage of campylobacter jejuni by persons in developing countries and presence of spiral bacteria like Gastrospirillium which interfere with the results[29]. It is possible that some false negative results by ELISA may occur for patients who are in acute phase of infection. When ELISA test gives false positive results, it should be taken into account that there might be some false negative diagnosis for the gastric *H.pylori* status, arising from sampling errors. Some of the false positive results by ELISA might indicate the previous infection only[30]. The best for the determination of *H.pylori* status of a patient is that which has greatest validity (combination of sensitivity and specificity) and the largest *J* value was highest with Gram's stain, 71.25% and 0.43 respectively, followed by serum IgG serology, 69.15% and 0.39 respectively. They were lowest in culture 53.75% and 0.08 respectively.

Distribution of *H.pylori* may be a patchy phenomenon and histopathology/culture assess only a small area of the stomach. Serology in essence, assays the entire stomach. Therefore, serological test may be better in already established (past) infections where the organism may not be detected bacteriologically and histologically.

VI. CONCLUSIONS

Culture is single best method without any false positive results but the false negativity is highest with it. Direct Gram's stained smear is an invasive, highly specific, rapid screening test and may obviate the need for additional testing of positive specimens, if resources are limited. The diagnostic usefulness of urease test as a definitive method for detection of *H.pylori* is limited because of high false positive results. The non-invasive, IgG serology is an easy, sensitive and accurate method in predicting *H.pylori* status of patient and can be used as a screening test before endoscopy. The concordance of results of various diagnostic tests is the best available option for determining a patient's true *H.pylori* status.

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LIST OF TABLES

Table -1: Various diagnostic tests for *H.pylori*

S.NO.	DIAGNOSTIC TEST	PATIENT GROUP (n=70)			CONTROL GROUP (n=10)		
		POSITIVE (n,%)	NEGATIVE	TOTAL	POSITIVE	NEGATIVE	TOTAL
1.	RAPID UREASE TEST	36 (51.4%)	34	70	---	10	10
2.	GRAM'S STAIN	31 (44.2%)	39	70	--	10	10
3.	CULTURE	3 (4.2%)	67	70	--	10	10
4.	HISTOLOGY	40 (57.1%)	30	70	--	10	10
5.	IgG SEROLOGY	41 (58.5%)	29	70	3 (30%)	7	10
6.	IgA SEROLOGY	48 (68.5%)	22	70	4 (40%)	6	10

TABLE-2: Statistical analysis of various diagnostic tests for *H.pylori*

S. NO		RUT	GRAM'S STAIN	CULTURE	HISTOLOGY	IgG SEROLOGY	IgA SEROLOGY
1.	POSITIVE	51.4%	44.2%	4.2%	57.1%	58.5%	68.5%
2.	NEGATIVE	48.57%	55.71%	95.71%	42.85%	41.42%	31.42%
3.	FALSE POSITIVE	40%	20%	0	_	36.7%	63.3%
4.	FALSE NEGATIVE	40%	37.5%	92.5%	_	25%	25%
5.	SENSITIVITY	60%	62.5%	7.5%	_	75%	75%
6.	SPECIFICITY	60%	80%	100%	_	63.3%	36.7%
7.	POSITIVE PREDICTIVE VALUE	66.7%	80.6%	100%	_	73.2%	61.2%
8.	NEGATIVE PREDICTIVE VALUE	52.9%	61.5%	44.8%	_	65.5%	52.4%
9.	VALIDITY	60%	71.25%	53.75%	_	69.15%	55.85%
10.	YOUDEN'S *J* VALUE	0.2	0.43	0.08	_	0.39	0.12