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Helicobacter. Pylori Infection in Gastro Duodenal Disease Cases. Tertiary Care Centre Study With Invasive & Noninvasive Tests

KEYWORDS

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ABSTRACT *Presence of Helicobacter pylori in cases of acid peptic disease (APD) changes treatment options. The assessment of burden of H.pylori is challenging as it requires invasive test like endoscopy. We conducted a study at tertiary care hospital in Mumbai, among 92 patients suspected of having gastro duodenal disease. Premier Platinum HpSA test (Meridian Diagnostic Cincinnati,OH,U.S.A) was used as noninvasive test. The test is a polyclonal antibody based enzyme immunoassay for stool samples, to detect the H.pylori antigen along with histopathology & Gram stain, Rapid urease test. We found substantial occurrence of H.pylori infection in investigated cases, out of 92, 65 cases were being infected with H.pylori. As this was the first study in Mumbai tertiary care hospital using Noninvasive assay for stool antigen detection of H.pylori, we couldn't compare with any local studies using same noninvasive techniques, but it reinforces the need of noninvasive techniques in routine screening of APD cases.*

INTRODUCTION:

Nobel Prize for physiology or Medicine in 2005 was awarded to Dr.Barry J Marshall & Dr J.Robin Warren for Serendipitous discovery of Helicobacter pylori & its role in gastro duodenal disease, in 1982(1,8,9). It remains one of the most common infections in human being worldwide (1,2,3,). Indian prevalence is about 33-70 %(4,5) . NIH consensus conference & the International agency for cancer research ,an arm of World Health Organization, in 1994, declared it to be associated with gastro duodenal disease, & class I carcinogen respectively(10,6),changing the medical paradigm from "No acid no ulcer" to "No bacterium No ulcer"(7,11). Infection persists lifelong if not treated & eradicated (4,5). Infection can be eradicated with definite diagnosis & antimicrobial therapy, combine with proton pump inhibitor(13). So the study was conducted to estimate the associated H.pylori infection in gastro duodenal cases.

MATERIALS & METHODS:

The study was conducted in a tertiary care hospital in Mumbai for one year period, with Hospital Ethics committee approval.

Selection of cases: The 92 patients with symptoms suggestive of acid peptic disease (APD) from indoor & out patients departments, referred for endoscopy were enrolled in study. Patients on antibiotics, Nonsteroidal anti-inflammatory drugs for past 15 days prior to investigation, pregnant women, lactating mothers & patients with chronic liver disease, severe renal or cardiopulmonary disease were excluded from the study. Enrolled patients underwent endoscopy after obtaining written consent .Depending on the endoscopic findings patients were divided into various groups

Table- 1

ENDOSCOPIC FINDING	NUMBER OF PATIENTS
NORMAL	23
GASTRITIS	55
DUODENAL ULCER	9
GASTRIC ULCER	5

Endoscopic findings in enrolled patients.

Biopsy &stool samples were collected from all enrolled patients. For Stool samples, the morning stool sample was collected in clean airtight container & preserve at -20^{0}c, until tested. Antrum biopsy samples were obtained from each patient. One biopsy sample was subjected to rapid urease test, immediately after collection. Rapid urease test was done using in house 10% urea solution in deionised water with phenol red as indicator. pH of the solution was adjusted to 6.8. After adding biopsy sample rapid urease test was read after 1 minute. Depending upon the color change at room temperature test was interpreted as follows (12).

Orange colour- To pink colour - Positive test

Orange colour- No colour change - Negative test

The biopsy Samples were subjected to gram stain & Histopathology examination.

HpSA Premier Platinum HpSA, enzyme immunoassay procedure followed as per the instruction manual of manufacturer.

Stool sample approximately 5-6 mm diameter was taken on wooden applicator stick,& mixed into sample diluent and vortexed for 15 seconds.15 micro liter of diluted stool sample was added by pipette to appropriate well. Positive & negative controls provided with kits were added. After addition of enzyme conjugate, plate was shaken then sealed with sealer followed by incubation at 22-27 degree Celsius. Manual washing was done after removing the plate sealer. Wash buffer was added to thoroughly remove any debris. Second incubation was done for 10 minutes at 22-27 ^{0}C after adding substrate solution. Results were read visually as well as spectrophotometrically within 15 minutes after addition of stop solution. The results were interpreted according to cut off provided with kit. Spectrophotometric determination was done by ELISA reader, using dual wavelength,450/620 nm. Result was considered Positive if O.D 450/620 nm Was >0.120 & Negative, if OD 450/620 nm was <0.100.

Histopathology-The formalin fixed specimens were processed & stained by Haematoxylene eosin stain,Giminez stain.The slides were observed for H. pylori &inflammatory cell infiltrate in antral mucosa .Gastritis was graded as Nil, Mild, moderate, severe.

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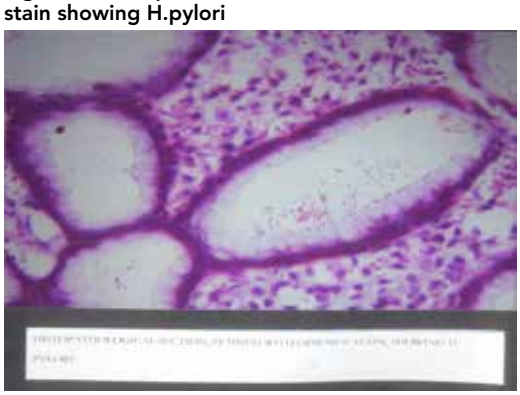
Figure 3 Histopatholoical section stained with Giminez stain showing H.pylori



RESULTS:
Patients were treated as Helicobacter pylori infected when Rapid urease test & Histopathology were positive & Negative if both of these were Negative.

Out of 92 patients, 65 patients were positive by Rapid urease test, 65 by histopathological examination, 5 patients were positive by gram stain. Histopathology shown highest sensitivity of 100% followed by HpSA 95%, rapid urease test 93%, Gram stain sensitivity was lowest, to 83%.

TABLE-2,

ENDSCOPIC FINDING	TOTAL PATIENTS	H.PYLORI POSITIVE	PERCENTAGE
GASTRITIS	55	51	92.7%
DUODENAL ULCER	9	9	100%
GASTRIC ULCER	5	5	100%
TOTAL	69	65	-

Positivity of H.Pylori in APD cases.

TABLE-3

DIAGNOSTIC TEST	H.PYLORI POSITIVE	H.PYLORI NEGATIVE	TOTAL
RAPID UREASE TEST	65(70.6%)	27(29.34%)	92
GRAM STAIN	5(5.43%)	87(94.36%)	92
HISTOLOGY	65(70.6%)	27(29.34%)	92
HPSA	63(68.4%)	29(31.52%)	92

Positivity % of H.pylori by different tests.

TABLE-4,

DIAGNOSTIC TEST	RAPID UREAS TEST	HISTOPATHOLGY	HPSA	GRAM STAIN
TOTAL POSITIVE	65	65	63	5
TRUE NEGATIVE	21	27	24	26
FALSE POSITIVE	1	0	2	60
FALSE NEGATIVE	5	0	3	1
TOTAL	92	92	92	92
SENSITIVITY	93%	100%	95%	83%
SPECIFICITY	95%	100%	92%	30%
PPV	98%	100%	97%	8%
NPV	81%	100%	89%	96%

Sensitivity & Specificity of different tests in APD cases.PPV-Positive predictive value,NPV-Negative predictive value.

DISCUSSION:
Out of 92 samples H.pylori was detected in 65 patients, giving total positivity of 70.6 % .Study of Prevalence &H.pylori association in dyspeptic patients in Southwest Nigeria revealed positivity of 77.5%(16).The detection rate varied with tests used. The Dino vaira etal& Oderda have reported 96% positivity by using HpSA (15,14). In one prospective large multicentre European study out of 501 patients 256 were positive by HpSA with positivity of 51, sensitivity of of HpSA was 95%(17).In our study HpSA detected 63 H.Pylori infection by antigen detection. Gram stain detected lowest positive cases.

Our study depicted significant association of H.Pylori infection with duodenal ulcer & gastric ulcer as was seen in Southwest Nigeria cases(16).Pathogenesis is related with inflammation. Antral inflammation stimulates increased acid production from uninflammed corpus & predisposes to duodenal ulceration(21,23).If inflammation is localized at corpus then it leads to hypochlorhydria & initiates gastric ulceration & adenocarcinoma(21,23).Not all but around 15% colonized develop disease .Strain virulence, host genetic susceptibility attributes to pathogenesis with many virulence factors of H.pylori like CAG pathogenesity island, Cytotoxin A(21).

The annual incidence of H.Pylori infection in developing country is 6-14% & 0.3-0.7% in developed countries (4). In India Prevalence ranges from 33-70 %(4,5).Different modes of transmission are Oral oral route, Faeco-oral route, Iatrogenic route.(18,3)

The factors which induces gastric inflammation are numerous as follows,Interleukin8,Neutrophil adherence to endothelial factor, Lipopolysaccharide, Urease, Lipase, Protease, VacA, Induced contact epithelium gene, Haemolysin, Acetaldehyde. (6,18,19,20,21,22,23,).

CONCLUSION-The association of Helicobacter pylori infection with gastro duodenal disease is substantial. Patients need to be screened for presence of infection for effective management of cases &eradication of infection. Screening can be done with noninvasive test .In our study HpSA stool assay was used as noninvasive test. The assay was having comparable sensitivity &specificity with gold standard invasive test like histopathology. As ours was first study to use it, one need to evaluate this further in population as claim is made of geographical variation. But it can use in adjunct with other method in primary diagnosis of infection. Currently monoclonal antibody assay are available, which are more specific, which allows highly specific binding of H.pylori antigen.

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