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Original Research Article

A Preliminary Evaluation of Stool Antigen Test in *H. pylori* Infections

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Abstract: The discovery of Helicobacter pylorirevolutionised the concept of gastroduodenal pathology& diverted the world wide attention from pH to Hp (*H. pylori*). Surgical management is replaced by antibiotics. Endoscopy guided biopsy is diagnostic. No endoscopic tests obviate need for invasive procedure. Aim: Evaluation of *H. pylori* stool antigen detection test by comparing it with conventional diagnostic tests like antral mucosal biopsy urease test and culture. Material& methods-Study group – 35 patients with upper GI disorders. Control group -10 asymptomatic individuals (total 45). Informed consent and ethics committee clearance obtained. Antral mucosal biopsy sample transported in 20%glucose broth .Stool sample obtained simultaneously from the both groups .Urease test done on 10% Urease agar.Microaerophilic culture on BHI agar with 5% sheep blood and VCNT supplement .Colonies identified by morphology on staining, hanging drop, urease test, oxidase test catalase test.H pylori antigen was detected in the stool sample by EIA (Meridian bioscience Inc.).Results: Urease test was positive in 19 of 35(54%) of symptomatic cases.14of35 (40%) cases in the symptomatic group were positive by culture. 18 out of35 (51.42%) of the cases were found to have Helicobacter pylori antigen in stool.Conclusion:*H. pylori*antigen detection in stool is an easy noninvasive alternative diagnostic procedure in pretreatment screening & monitoring the effectiveness of *H. pylori* treatment.
Keywords: Helicobacter pylori,H.pyloristool antigen test

INTRODUCTION

The discovery of association between *Helicobacter pylori* and peptic ulcer disease by Warren and Marshall in 1982 in Australia was a landmark breakthrough in the understanding of upper gastrointestinal disease. Helicobacter pylori are the most common bacterium causing chronic infection in humans. It is said that Helicobacter colonises half the human population of the world [1]. Presently its role has been established in chronic antral gastritis, duodenal ulcer, chronic gastric ulcer, dyspepsia, gastric cancer and gastric lymphoma. WHO added *Helicobacter pylori* to its list of known carcinogens [2].The diagnosis of *Helicobacter pylori* infection is currently based upon endoscopic biopsy based tests like rapid urease, culture, Grams staining and histopathology. These procedures are invasive, tedious and hence every person complaining of symptoms suggestive of gastroduodenal disease cannot be subjected to these tests [2]. The non-invasive tests obviate the need for endoscopy and comprise of serology, C13& C14 urea breath tests. There are 3 available non-invasive methods- C13 or C14 labelled urea breath tests, serology which detects an immune response in the patients and Helicobacter pylori stool antigen, a micro well based enzyme immunoassay for detection of Helicobacter pylori antigen in stool.

Studies evaluating the accuracy of these tests have highlighted that each has advantages and disadvantages making it more or less appropriate depending on the clinical situation [3]. The endoscopic tests are best for a primary diagnosis of Helicobacter pylori infection because endoscopy allows assessment of treatment indications. Non- endoscopic tests should be preferred in situation where the additional information yielded by endoscopy is not needed [4].

Proper screening, accurate diagnosis and effective treatment of *Helicobacter pylori* infection alleviates the enormous morbidity caused in peptic ulcer disease.Presence study was taken up to know the prevalence of *Helicobacter pylori* in various upper gastro intestinal disorders and to evaluate the efficacy of various invasive and non- invasive methods in diagnosing it.

The discovery of *H.pylori*revolutionized the concept of gastroduodenal pathology & diverted the world wide attention from pH to Hp (*H. pylori)*. Surgical management is replaced by antibiotics.Endoscopy guided biopsy is diagnostic. No endoscopic tests obviate need for invasive procedure

AIM

Evaluation of *H. pylori* stool antigen detection test by comparing it with conventional diagnostic tests like antral mucosal biopsy urease test and culture

MATERIAL&METHODS

Patients attending department of gastroenterology with symptoms related to upper gastro intestinal lesions were enrolled in the study. The patients were interviewed regarding demographic details, past history & family history of gastro duodenal disorders and history of previous abdominal surgery, any current illness and dyspeptic symptoms. In addition history of treatment with NSAIDS, antibiotics, PPI's & H2 receptor antagonists or antacids in the previous month also was taken.

Patients with history of previous gastric surgery, history of ingestion of NSAIDS's, antibiotics, PPI's in the Preceeding month or H2 receptor antagonists in the Preceeding week and those with hematemsis, melena or systemic illness were excluded from the study.Study group comprised of 35 patients with upper GI disorders.10 asymptomatic individuals were included in the study as control group. Informed consent and ethics committee clearance were obtained.

The endoscopistaccertained the diagnosis just before taking the biopsy. From each patient 3 antral mucosal biopsy samples were taken within 5cm of gastric pylorus for urease test, microscopy and culture respectively.

Simultaneously stool samples were obtained from the patients which was collected in a sterile wide mouthed screw capped container for detection of *Helicobacter pylori* antigen in stool.

The specimens were picked from the biopsy forceps with the help of sterile disposable needle. The specimen was directly inoculated on urease agar slope. The second specimen was kept on a sterile slide and by firm pressing of the specimen with another sterile slide, impression smears were made to extrude the organisms. The specimens for culture were transported in a rubber cork tubes of 15ml capacity containing 3ml of 20% sterile glucose broth.

Stool sample obtained simultaneously from the entire cases .Urease test done on 10% Urease agar. The test was read positive when red or pink color developed around the biopsy specimen. The tests were read at room temperature at 2hrs, 3hrs and 24hrs. The medium not turning pink within 24hrs were regarded negative.

Microaerophilic culture was done on BHI agar with 5% sheep blood and VCNT supplement. The plates were examined on 4th day for the characteristic colonies. The plates that were without growth within 7 days were considered negative. The colonies of *Helicobacter pylori* were 1-2 mm in diameter, circular, convex, domed, greyish and translucent with entire edge and weakly β hemolytic on BHI agar with sheep blood.

Colonies were identified by morphology on staining[Gram negative, curved, slender bacilli, sea gull shaped],hanging drop [actively motile] ,urease test, oxidase test catalase test all being positive.

H pylori antigen was detected in the stool sample by enzyme immune assay (Meridian bioscience Inc.) according to manufacturer's instructions.

RESULTS

35 patients weretested by urease,culture&HpSA test.19 of 35 were urease positive.18 of them were found to have HpSA (table-1)

Out of 35 cases tested 14 were positive by culture.18 were positive for HpSA(Table-2)

Table 1: Detection of *H. pylori* by urease&HpSA test in symptomatic group

Endoscopy finding	No of cases tested	Urease positive (%)	HpSApositive (%)
Duodenal ulcer	**1**	**0**	**0**
Gastritis	**21**	**12**	**12**
Duodenitis	**9**	**4**	**4**
Esophagitis	**4**	**3**	**2**
Total	**35**	**19(54%)**	**18(51.42%)**

Table 2: Detection of *H. pylori* by culture&HpSA test in symptomatic group

Endoscopy finding	No of cases tested	Culture positive (%)	HpSA positive (%)
Duodenal ulcer	**1**	**0**	**0**
Gastritis	**21**	**9**	**12**
Duodenitis	**9**	**3**	**4**
Esophagitis	**4**	**2**	**2**
Total	**35**	**14(40%)**	**18(51.42%)**

Table 3: Comparison of urease, culture and HpSA tests in symptomatic& asymptomatic groups

Study group	Urease positive	Culture positive	HpSA positive
Symptomatic group(n=35)	54%	40%	51.42%
Asymptomatic group(n=10)	10%	10%	40%

DISCUSSION

Helicobacter pylori has been established as a causative organism of chronic active gastritis which is associated with non-ulcer dyspepsia, peptic ulcer and gastric carcinoma [5].The invasive tests which include rapid urease test histology and culture are best for a primary diagnosis because endoscopy allow assessment of lesions and their treatment. The non-invasive tests obviate the need for endoscopy and comprise serology, urea breath test and Helicobacter pylori antigen detection in stool. Different tests widely used are urease test, culture,antibody detection and *H. pylori* antigen detection in stool. The role of antibody tests in diagnosing active infection or following therapy is limited [6].In this study urease test was taken as gold standard as it is a conventional test proved to be simple,reliable,cost effective and sensitive test.In the present study an invasive test i.e culture of biopsy sample and non-invasive test i.e *H. pylori* antigen detection in stool have been compared with the urease test.

The study sample comprised of 35 consecutive patients of either sex aged between 20 and 60 yrs complaining of symptoms related to upper gastrointestinal disorders. Ten asymptomatic apparently subjects aged between 20 and 40 yrs were taken as control group.

Urease test was positive in 19 of 35symptomatic cases. Control group showed 1person urease positive out of ten. Other Indian studies showed differing results in urease positivity i.e.44% by Gupta *et al.*[1];71%by Ramesh[3]&72% by Arora *et al.*[2];The lower positivity may be because of the frequent usage of metronidazole for amoebiasis in the place of study to which Helicobacter is sensitive.

Microaerophilic culture for *H. pylori* is sensitive &specific but difficult. In the present study 18 cases in the symptomatic group were positive by culture. All these cases gave a positive urease test. This result co-relates with that of other workers like Ramesh (39.4%)[3], Arora *et al.;* (28%) [2]. .Fewer isolations by culture maybe because of fastidious nature of the organism and in some cases overgrowth of competing microflora. Sampling error, technical factors and patchy distribution of the organism can reduce diagnostic yield from infected patients.

In the control group only one case was culture positive which was urease positive also.Helicobacter pylori antigen detection in stool by enzyme immune assay gives extremely accurate results.It is simple easy to perform &does not require any special equipment. It is cost effective when compared to other non-invasive tests like urea breath test.Visible color change makes the test objective simple. In this study 35 cases were tested for antigen in stool.18 (51.42%) of the cases was found to have Helicobacter pylori antigen in stool. Similarly Pietz *et al.;* [7] got 47% detection of antigen in stool.In the control group of 10, four subjects found to have antigen in stool (40%).This supports the statement that in industrialised nations antibody surveys show that approximately 50% of adults older than age 60 are infected [8].

Table 4: Comparison of urease, culture and HpSA tests in symptomatic& asymptomatic groups

Study group	No.tested	Urease positive	Culture positive (%)	HpSApositive (%)
Symptomatic group	35	19(54%)	14(40%)	18(51%)
Asymptomatic group	10	1(10%)	1(10%)	4(40%)

Table 5: Sensitivity, Specificity of tests

Method	sensitivity	Specificity
Culture	73.68%	100%
HpSA	84.21%	87.5%

The results of antigen detection when compared to culture are not statistically significant (p =0.65) (Table 6), but further studies with more sample size may be needed to conclude. Culture of biopsy specimens is specific cost effective. It has disadvantages like sampling error, low sensitivity and is invasive. HpSA test is sensitive (ELISA) and specific. Moreover, it is non-invasive. It is easy to perform even by untrained personnel according to manufacturer's instructions the result can be obtained within 2 hrs by visible colour change.The only disadvantage is that it is expensive.

CONCLUSION:

*H.pylori*antigen detection in stool can be an easy noninvasive alternative diagnostic procedure in pretreatment screening & monitoring the effectiveness of *H. pylori* treatment.

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