Original Article

Detection of Helicobacter Pylori Antigen in Stool

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ABSTRACT

OBJECTIVE: To detect H. Pylori antigen in stool and to determine the diagnostic accuracy of H. pylori stool antigen.
DESIGN: Comparative analytical study.
SETTING: This study was carried out at Pakistan Medical Research Council (PMRC), at Jinnah Postgraduate Medical Centre, Karachi from May 2006 to September 2006.
MATERIAL METHODS: Endoscopic biopsy specimens were collected for rapid urease test and histopathology examination. Stool samples were taken for detection of antigen of Helicobacter pylori. The stool antigen test is a rapid immuno-assay based on "lateral flow chromatography technique" using monoclonal antibodies for qualitative detection of Helicobacter pylori antigen in human stool.
RESULT: Fifty males and 50 females were selected for this study by non-probable purposive sampling technique. Their mean age was 39 years ranging from 15 to 55 years. The sensitivity and the specificity of Helicobacter pylori stool antigen test (HPSA) with rapid urease test was found to be 89.1% and 92.6% respectively and the sensitivity and specificity of HPSA test with histopathology was 92.8% and 89.6% respectively.
CONCLUSION: Helicobacter pylori stool antigen (HPSA) is an accurate easily carried out in routine laboratory and is non-invasive.

KEY WORDS: Helicobacter pylori, sensitivity, specificity, rapid urease test, histopathology.

INTRODUCTION

Helicobacter pylori infection is associated with pathogenesis of chronic gastritis, gastric and duodenal ulcer and gastric cancer in infected individual. Recently it was associated with extra-gastrointestinal disease such as iron deficiency anemia[1]. Helicobacter pylori is spiral gram negative rod. It is approximately 0.5 x 3.0 micrometer in size. The catalase positive organism has 4-6 sheathed flagella attached to one pole which is used for motility[2]. The Helicobacter pylori is found in colonized gastric mucosa or adherent to epithelial lining of stomach [5].

The antrum of stomach is the region where Helicobacter pylori usually prefers to colonize at first[2]. In some individuals Helicobacter pylori also infects the corpus region of stomach. This results in a more widespread inflammation that predisposes, not only to ulcer in the corpus regions, but also to stomach cancer[4]. The infection usually remain in stomach for decades finally leading to atrophy and intestinal metaplasia. In few patients metaplasia may lead to dysplasia and gastric carcinoma[6]. The prevalence of Helicobacter pylori infection is high in infants and children in developing countries particularly communities of low socio-economic status[8]. Helicobacter pylori infection is almost always acquired in early childhood and usually persist throughout life, unless specific treatment is given[9]. Up to 50% of the world population is infected with Helicobacter pylori [6], but it can cause ulcer in 10% to 15%[4]. Actual mode of transmission has not been determined. The ability to culture this bacterium from stool has supporting evidence for an oral-fecal mode of transmission[2]. Acute infection is acquired by ingestion of organism. It is commonly asymptomatic but may be associated with epigastric burning, abdominal distension or bloating, belching, nausea, flatulence and halitosis. Helicobacter pylori gastritis produce no symptoms in 90% of the infected persons[5]. Severe complications include bleeding and perforation. The pathogenic role of Helicobacter pylori in peptic ulcer disease, both duodenal and gastric, is well recognized; up to 95% of patients with duodenal ulcer, and 80% of patients with gastric ulcer are infected with Helicobacter pylori[5].

Diagnosis of Helicobacter pylori is the first key-step in the management of Helicobacter pylori associated with gastroduodenal diseases[7]. Currently there is no single test which can be considered as the gold standard[10]. It is recommended that two or more tests should be performed to improve the diagnostic accuracy in clinical trial of Helicobacter pylori infection[7]. Several invasive and non-invasive diagnostic tests are used for the detection of Helicobacter pylori infection[11]. Non-invasive diagnostic tests of Helicobacter

pylori infection include stool culture, detection of antibody in serum, urea breath test and stool antigen test[6]. Invasive diagnostic test of Helicobacter pylori infection is by biopsy specimens which are obtained at gastro-duodenoscopy for rapid urease test and for histopathology, and microbiology culture[10]. The early diagnosis and treatment of Helicobacter pylori infection is recommended to reduce morbidity and the potential for malignancy[13].

Invasive test requiring endoscopic biopsy includes culture, histopathology and rapid urease test. Non-invasive tests do not require endoscopic biopsy[7]. Histological examination is the most accurate invasive method for diagnosis of H. pylori infection[11]. But it is biopsy based and needs technical assistance. The invasive rapid urease test is in-expensive and provides results within 30-minutes and final reading after 24 hours[6]. It is specific, therefore the additional testings are probably unnecessary. Sampling error can occur because of patchy nature of infection in biopsy[17] and false negative results are common in children because of smaller bacterial load[26]. At present there is increasing interest in non-invasive tests as these tests do not need endoscopy for the diagnosis of H.pylori infection and are not influenced by sampling error[17].

This study was done to detect the H.pylori in symptomatic patients, to compare the results of invasive and non-invasive methods as well as to determine the sensitivity and specificity of HPSA test, compare it with other diagnostic tests.

MATERIAL AND METHODS

One-hundred patients, suspecting gastritis and peptic ulcer were selected from Pakistan Medical Research Council (PMRC), Jinnah Postgraduate Medical Centre, Karachi, from May 2006 to September 2006 and analysed at Department of Microbiology BMSI, JPMC Karachi.

The study was designed (Cross sectional prospective) for the detection of H.Pylori antigen in Stool sample by premier platinum Helicobactor pylori Stool antigen test Patients select who had history of burning abdominal distension or bloating belching, nausea flatulence, epigastric pain and dyspepsia for the last six months and provisionally diagnosed cases of gastritis or peptic ulcer. Only those patients were selected who had not taken any antibiotic, non - steroidal anti-inflammatory drugs, bismuth compound, proton pump inhibitors and oral anticoagulant for last four weeks. Adults were included in this study. Children already diagnosed cases of gastritis or peptic ulcer by endoscopy, carcinoma of stomach, patients with antibiotic or H_2 antogonist, bismuth salts or proton pump inhibiting drugs for less than four weeks, chronic use of corticosteroids or non-steroidal anti-inflammatory drugs, bleeding disorders, bleeding peptic ulcer, metabolic disorders, congenital deformity, Malformation, diarrhoeal or watery stool, recently received blood transfusion, severe concomitant diseases, pregnancy or lactation were excluded from the study.

Informed consent was obtained prior to the inclusion in the study. The motivation and compliance was 100%, nevertheless prior counseling in routine practice is recommended. All patients were instructed about fasting. Stool sample was collected after the endoscopy. At the time of collection of stool and biopsy, the personal data history was taken and recorded on especially designed Performa.

The sample was collected according to WHO (2005) Criteria in small plastic container with lid in polythene bags. All samples were properly labeled with patient's name, age, sex, case no, ward and date of collection. The stool sample was collected in plastic container with a tight fitting lid provided to the patients. The patients were asked to pass stool sample direct into plastic container. The kit was kept at room temperature before performing the test.

The biopsy specimen was removed from the biopsy forceps with the help of sterilized needle on urease media for rapid urease test. Other biopsy specimen was taken in container with 4% formalin for histopathology (Giemsa stain). The results of stool antigen test, rapid urease test and histopathology may be positive or may be negative. The Rapid Urease test was taken as a gold standard in this study. Tiny pieces of tissue (biopsy tissue) were taken with the help of endoscopy forceps, from antrum of stomach & first part of the duodenum and multiple specimens were collected due to patchy nature of infection and handed over to the author for study purpose.

Mucosal biopsy specimen for Rapid urease test

The cap of the urease test tube was lifted. The biopsy specimen was removed from the biopsy forceps with the help of sterilized needle and inserted into the medium by stabbing to the bottom of the tube. The cap was replaced tightly and incubated preferably in an incubator at 37˚C. The color change was observed after 24 hours. Development of dark pink color indicates a positive test.

Helicobacter Pylori Stool antigen test (HPSA)

Specimen preparation: Stool was mixed thoroughly as possible prior to sampling. Watery, diarrheal specimens were not taken for testing. Before performing test the kit and specimens were kept at room temperature for 2 hours. One ml of sample diluent was transferred in a test tube or vial. Sample stool portion of

approximately 5-6 mm size, was added with a swab, a wooden applicator or a bacteriology loop, and shaked gently in order to suspend it into the diluent, vortexed for 15 seconds and centrifuged for one minute. The solid particles settled at the bottom 500 microliters of supernatant was transferred to another test tube, with a pipette. The reaction strip was dipped in the second test tube with the arrow pointing to the bottom. The liquid must not reach the blue area above the arrow-heads. Result was read after exactly 5 minutes in the white area.

Negative test result: Only one blue coloured band (Control line) appeared.

Positive test result: In addition to the Blue band (Control line), a distinguishable pink-red band appears.

Invalid test result: The blue band (Control line) is absent, with or without a visually detectable pink-red band appears

RESULTS

Total 100 patients were included in the study. Their mean age was 39 years and age ranged from 15 to 55 years. The major symptoms were epigastric pain (88%) and dyspepsia (65%). The maximum number of our study patient, belonged to age group 25 to 34 years. The peak duration of illness was found to be 7 to 12 months, which is 25%. On endoscopy 39 had normal findings. Remaining 61 patients had positive findings, 3 patients had esophagitis, 42 patients had gastritis, 10 patients had duodenitis and 6 patients had gastric and duodenal ulcers. Out of total 100 cases 46 were positive RUT and 45 were positive HPSA test. In 61 positive endoscopy cases 36 were RUT positive and 33 were HPSA positive. In 39 negative endoscopy cases 10 were RUT positive and 12 wee HPSA positive. The sensitivity and specificity of HPSA with RUT was 89.1% and 92.6% respectively. The sensitivity and specificity of HPSA with histopathology examination was 92.8% and 89.6% respectively. These values are almost the same. SPSS statistical software was used for analysis.

TABLE I:

ENDOSCOPY VS RAPID UREASE TEST AND STOOL ANTIGEN TEST (n=100)

Endoscopy	Subjects	Rapid Urease Test +ve	Stool Antigen +ve
		Number (%)	Number (%)
Positive	61	36(59.0)	33(54.1)
Negative	39	10(25.6)	12(30.8)

TABLE II:
SCREENING TEST FOR STOOL ANTIGEN

Stool antigen	Subjects	Rapid Urease Test		Histopathology	
Test		+ve	-ve	+ve	-ve
Positive	45	41	04	39	06
Negative	55	05	50	03	52
Total	100	46	54	42	58

	Urea activity	Histopathology
Sensitivity of stool antigen:	41/46X100=89.1%	39/42X100=92.8%
Specificity of stool antigen:	50/54X100=92.6%	52/58X100=89.6%
Positive predictive value:	41/45X100=91.1%	39/45X100=86.7%
Negative predictive value:	50/55X100=90.9%	52/55X100=94.5%

DISCUSSION

The mean age of our study patients was 39±13.9 years which is in accordance with Li et al.[15], Rehman[21] and Syam et al, [12] who found mean ages 43.7 years, 41 years and 39 years respectively in their studies. However our mean age is lesser than that of western countries, mainly due to high incidence of contamination.

The basic non-invasive test includes, fecal culture, serology, urea breath test (UBT) and polymerase chain reaction (PCR)[6]. Fecal culture should be regarded as the most specific test for the presence of H.pylori. The successes rate for the isolation of H.pylori from feces has been rather poor[18]. The serological test is widely used, it takes several months or even years for the antibody to decrease significantly to confirm successful eradication[7]. PCR test have been developed to detect H.pylori in human feces. This test is scarcely suitable to detect the organism[17]. Stool specimen false negative result may be due to presence of PCR inhibitors or due to genetic variability[10].

The UBT is considered to be a highly accurate and reliable diagnostic method before and after eradication therapy[19]. The UBT requires an expensive instrument such as mass spectrometer, which is not always available in routine clinical laboratories[20]. A newly developed H.pylori antigen test in stool specimen (HPSA) detects bacterial material in feces[22]. The stool antigen assay is a convenient pre-treatment diagnostic test. The results can be monitored visually, thus making it very useful for a general practitioner[20]. In

this study the results were compared for detection of H.pylori with Rapid urease test (RUT), histopathology examination and Helicobacter pylori stool antigen test (HPSA). RUT was considered as a gold standard. Forty-one cases out of 46 patients were both positive for RUT and HPSA test. In this study HPSA was 89% sensitive and 92% specific. The different researchers have compared the results of different invasive and non-invasive methods.

Trevisani et al,[17] compared results of gastric biopsies for Rapid urease test (RUT) and recorded reading after 24 hours with stool antigen test. It was 94% sensitive and 90% specific. Li et al,[15] reported that the HPSA test has a diagnostic value comparable to the gold standard in detecting of H.pylori. The sensitivity and specificity of the HPSA for the diagnosis of H.pylori infection were 92.6% and 88.5% respectively. According to Baqai et al,[23] the sensitivity and specificity of the HPSA for the before treated patients was 65% and 81% respectively. Vaira et al,[24] reported the accuracy of HPSA which was assessed before treatment by comparing results with gastric biopsies using special stain as well as culture, rapid urease test (RUT) and urea breath test. They found 94% sensitivity and 92% specificity of HPSA.

Tanaka et al,[7] reported that the HPSA test is useful for the initial diagnosis of H.pylori infection. Tanaka compared the diagnosis based on culture, biopsy and Rapid urease test (RUT). He showed that the HPSA test was highly accurate with a sensitivity of 98.3% and specificity of 95%. Antos et al,[25] performed HPSA test in children to establish the diagnosis of H.pylori infection. The results of the mono colonal stool antigen test were compared with culture, histological (H&E, Giemsa stain) finding for sensitivity of 88% and specificity of 88.1% before treatment was recorded.

The HPSA sensitivity and specificity in this study is consistent with most of the workers. It is lower than the findings of Tanaka al,[7] et Because in his study there were high percentages of endoscopic findings positive patients compared with this study which have less number of endoscopy positive patients. However our findings are more sensitive and specific than Baqai[23], who did his study in Karachi in 2003 on only 34 patients. This difference may be due to the less number of patients. In this study we also performed histopathological examination of the tissue obtained by endoscopy. The sensitivity and specificity of HPSA with histopathological was found to be 92.8% and 89.6% respectively. These results show that the histopathological examination is slightly more sensitive but less specific than RUT test. These values are almost the same that we got for RUT. The RUT was easy to perform, time saving and does not need histopathologist, so we have taken RUT as the gold standard for this study. This study suggests that HPSA is cheap and easy to perform. It is almost as sensitive and as specific as other costly, invasive test which is difficult to perform. In my opinion HPSA test can replace other tests because this study favours the use of HPSA test for the diagnosis of H.pylori.

CONCLUSION

Helicobacter pylori stool antigen test (HPSA) is accurate, relatively inexpensive, easily carried out in routine laboratory and is non-invasive technique.

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