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DIAGNOSIS OF HELICOBACTER PYLORI INFECTION WITH A NON INVASIVE ANTIGEN BASED ASSAY

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ABSTRACT

Helicobacter pylori (H.pylori is highly adapted pathogen. This bacterium is associated with formation of inflammatory gastroduodenal diseases gastritis, peptic ulcer, lymphoma and gastric cancer in human.

Stool specimen and biopsy specimens were collected from 100 patients which were received by the endoscopy examination in PMRC, JPMC, Karachi. Immunochromatography Helicobacter pylori stool antigen was used to detect H.pylori stool antigen.

Sensitivity, specificity, positive predictive value and negative predictive value of Immunochromatography HPSA for the diagnosis of H.pylori were 89.1%, 92.6%, 91.1% and 9.90% respectively.

The stool antigen test is a simple noninvasive relatively inexpensive, easily carried out in routine laboratory.

KEYWORDS: Helicobacter pylori (H.pylori), HPSA, Stool antigen.

INTRODUCTION

Helicobacter pylori (H.pylori) is highly adapted pathogen (Salma et al., 2000)[1]. This bacterium is associated with formation of inflammatory gastrodudenal diseases gastritis, peptic ulcer, lymphoma and gastric cancer in human (Agnes, 2005)[2]. H.pylori is a helix shaped (hence named helicobacter) (Wikipedia, 2006)[3]. It is gram negative spiral micro-organism (Lui, 2003)[4]

The association between H.pylori gastritis and ulceration was discovered by Robin Warren in 1979 (Kidd, 1999)[5]. Fortunately Robin Warren and Barry Marshall in Royal Perth Hospital, Western Australia. The head of Microbiology department agreed, without research money, his staff culture 100 specimens. After 34 without success, a public holiday weekened resulted in longer incubation and the first culture became positive on April 1982 (Goodwin, 1997)[6].

Both these Australian scientists won the 2005 Nobel Price of medicine for this association. These two scientists insisted that stomach ulcers are caused by H.pylori and not by stress. To prove this theory Dr. Marshal swallowed this bacterium and become very ill thanks to their work, stomach and intestinal ulcer are often no longer a long term frequently disabiling problem. They can now be cured with short term course of drugs and antibiotic (Press Release) (Marshall and Warren, 2005)[7]. H.pylori is among the most prevalent and persistent bacterial infection in human world wide (Aspholm, 2004)[8].

The prevalence is high in African, American, Hispanic, Asian, and Native American populations. Infection rates are similar in males and females (Triantafyllopoulou, 2006)[9]. H.pylori infection is typically contracted in early childhood (BBC News, 2005)11 and usually persist throughout life, unless specific treatment is given (Koletzko, 2003)[10].

In developed countries less than 10% of children younger than 12 years are infected, however, there is seropositive increase with age at a rate of 0.3-1% per year. Studies of seropositive in adult in developed countries revealed prevalence of 30-50%. In USA the estimated prevalence is 20% for people younger than 30 years, and 50% for those who are older than 60 years (Triantafyllopoulou, 2006)[9]. Doudenal ulcer is more common in males than females, which may be related to higher acid out put in male (Goodwin et al., 1997)[6]. It is estimated that 10% and 1% of infected individuals develop peptic ulcer disease and gastric cancer (Adenocarcinoma and MALT lymphoma) (Agnes et al., 2005)[2]. Stomach ulcer is linked with 90% of stomach cancer (Beddingfield, 2004)[11].

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Table 1
Age and Gender Distribution
(n=100)

Age (in years)	Overall (M&F)
15-24	15
25-34	25
35-44	24
45-54	22
55 & above	14
Total	100

Table 2
Sign and Symptoms
(n=100)

Signs & Symptions	Overall (M&F)
Epigastric pain	88
Dyspepsia	65
Flatulence	54
Nausea	64
Vomiting	38
Anaemia	18

Table 3
Duration of Disease
(In months) (n=100)

Duration of disease	Overall (M&F)
Upto 6 months	17
7-12 months	25
13-24 months	24
25-36 months	06
37-48 months	07
49-60 moths	07
> 60 months	14

The bacteria have been isolated from feces, saliva and dental plaque of infected patients, which suggests gastro-oral or fec-oral possible transmission routes (Wikipedia, 2006)[12]. Previously studies suggested that H.pylori is present in ground water, surface water and other drinking water (Degnan, 2003)[13].

To cause disease a pathogen needs virulence factors. The virulence factor of H.pylori aid in colonization provides adhesion and enable H.pylori to cause cellular damage (Cutlan et al., 2006)[14].

The H.pylori is found in colonized gastric mucosa (Meurer and Bower, 2002)[18]. The antrum of stomach is the region where H.pylori is usually prefere to colonize at first (Holston, 1997)[15]. The catalase positive organism has 4-6 sheathed of flagella attached to one pole which is used for motility (Holston, 1997)[15]. Some virulence factors such as urease (enzyme) and flagella are present in all strains and are necessary for pathogenesis and colonization (Vandenplas, 2000)16. Several positive H.pylori virulence factors have been identified that includes urease; the vaculating cytotoxin, (vacA) and a cytotoxin associated antigen (cagA), however mutation in the genes encoding these factors do not have a measurable effect on virulence of H.pylori (Segal et al., 1997)[17].

H.pylori cause three different types of immune reactions, acute, chronic active, and the atrophic stages of gastritis. There are all stages of inflammation caused

	Sensitivity	Specificity
Present study	89.1%	92.6%
Isakov et a., (2000)	91.8%	94.4%
Trevisani (1999)	94%	90%
Li et al. (2003)	92.6%	88.85%
Manes et al. (2000)	93.8%	90%
Baqai (2003)	65%	81%
Vaira et al. (1999)	94%	92%
Tanaka (2003)	98.3%	95%
Antos (2005)	88%	88.1%

Table 4
ENDOSCOPY FININGS
9n=100)

Endoscopy Finings	Ovall (M&F)
Normal	39
Esophagitis	03
Gastritis	42
Duodenitis	10
Gastric ulcer	02
Duodenal ulcer	04

Table 5
Urease Activity and Stool Antigen Test (n=100)

Tests	Overall
Positive	46
Negative	54
Total	100
Stool antigen test	
Positive	45
Negative	55
Total	100

Table 6
Endoscopy vs Urease Activity and Stool Antigen Test
(n=100)

Endoscopy	Subjects	Urea Activity+ve		Stool Antigen+ve	
		Number	%	Number	%
Positive	61	36	59.0	33	54.1
Negative	39	10	25.6	12	30.8

Table 7
Sensitivity and Specificity of HPSA Test in Comparison To Rapid Urease Test (RUT)

Test HPSA	H.pylori True positive HPSA test	H.pylori False positive HPSA test	Total
Positive	41	04	41+4-45
Negative	FN-ve	TN-ve	FN+TN
	05	50	05+50=55
Total	TP+FN	TN+FP	TP+FP+TN+FN
	41+5=46	50+4=54	41+5+50+40=100

Sensitivity: 89.1%, Specificity: 92.6%

by immune system (Cutlan, 2006)[14]. The international agencies of research on cancer (IARC) a part of world health organization classified H.pylori as a group 1 carcinogen (Tanaka et al., 2003)[18]. A variety of highly sensitive and specific test available to diagnosis of H.pylori infection. However, testing should be performed only if treatment is intended (Knigge, 2001)[19]. Several invasive and non-invasive diagnostic tests are used for the detection H.pylori infection (Hino et al., 2004)[20]. Invasive diagnostic test of H.pylori infection is by biopsy speciment which are obtained at gastro duodenoscopy for histopathology, Rapid urease test and Microbiology culture (Goodwin, 1997)[6].

The use of non invasive tet is becoming more and more important as the diagnosis of H.pylori (Testerman et al., 2001)[21]. Non invasive diagnosis test of H.pylori including stool culture, detection of antibodies in serum, urea brath test and stool antigen test (Goodwin et al., 1997)[6]. Early diagnosis and treatment of H.pylori infection is recommended to reduce morbidity and potential for malignancy (Elitsur et al., 2004)[21].

MATERIAL AND METHODS

A total of 100 adult patients, 50 females and 50 males suspecting gastritis and peptic ulcer were selected from Pakistan Medical Research Council (PMRC), Jinnah Postgraduate Medical Centre, Karachi Children, patients with carcinoma of stomach, bleeding disorders, metabolic disorders, congenital disorders, patients treated with antibodies, bismuth, or proton pump inhibiting with-in 4 weeks preceding the study were excluded.

Invasive test using mucosal biopsy for Rapid urease test, were used to establish the gold standard, in order to evaluate the accuracy of stool antigen test.

The biopsy specimen was removed with the biopsy forceps with the help of sterilized needle and inserted into urea medium by stabbing to the bottom of the tube, and incubated preferably in an incubator at 37°C. The colour was observed after 24 hours development of dark pink colour indicates a positive urease test (Rehman, 1995)[22].

Stool sample were collected after the endoscopy, the sample were collected according to WHO (2005)[23] small plastic container with a tight fitting lid provided to the patients. The patients were asked to pass stool sample direct into plastic container. One ml of sample with diluent were transferred in a test tube, sample portion of approximately 5-6 nm. Stool transferred in test tube, centrifuged for one minute, than transferred 0.5ml of supernatant to the other tube, reaction strip dipped, wait for 5 minutes, negative result only blue band appear, positive result blue and pink band appears, invalid test result, is the blue band (control line) is absent, with or without visually detectable pink red band.

RESULTS

One hundred patients were included in the study, belonging to 15 to 55 years or above. The maximum number of our study patients 25 out of 100 belonging to age group 25 to 34 years, the major symptom were with epigastric pain (88%), the peak duration of illness were found to be 7 to 12 months, which is 25%, on endoscopy finding out of 100 patients. 39 gave normal finding. Remaining 61 patients gave positive finding. 3 patients developed esophagitis, 42 patients developed gastritis, 10 patients developed duodenitis and 6 patients developed peptic ulcer. Out of total 100 cases 46 gave positive RUT and 45 gave positive HPSA test. In 61 positive dndoscopy cases 36 gave RUT positive and 33 gave HPSA positive. In 39 negative endoscopy cases 10 gave RUT positive and 12 gave HPSA positive. The sensitivity and specificity of HPSA test were 89.1% and 92.6%.

DISCUSSION

H.pylori is a world wide infection (Mikaeli). The prevalence of H.pylori infection is hgh in developing countries particularly communities of low socio economic status (Shin et al., 2005)[25].

The study was done to detect the H.pylori in symptomatic patients, to determine the sensitivity and specificity of HPSA test.

The mean age of our study patients were 39+13.9 years which is in accordance with Li et al. (2004)[3]. Rehman (1995)[24] and Syam et al. (2005)[27] who found mean ages 43.7 years, 41 years and 39 years respectively in their studies. However our mean age is higher than Ibrahim (1999)[26] who found the mean age in his study were 34 years. 39 cases were found to be normal on upper GI endoscopy. Out of 100 cases 61 patients who have positive endoscopy findings, 3 (5.0%) patients showed esophagitis, 42 (70%) patients showed gastritis, 6 (9.8%) patients showed peptic ulcer and 10 (16.3%) patients with duodenitis.

These finding were comparable with Syam et al. (2005)[27]. They include 63 patients with positive endoscopy finding out of which 4 (6.3%) patients showed esophagitis, 49 (77.9%) patients showed gastritis, 8 (12.7%) patients showed peptic ulcer, and 3 (4.8%) patients showed duodenitis. So duodenitis is slightly more common finding in our study as compared with Syam et al. (2005)[27]. This may be due to high intake of oily and spicy food, self medication such as high use of non steroidal anti inflamatory drugs.

Detection of H.pylori infection has become a key step in the management of patients referred to the gastroenterologist (Goodwin et al., 1997)[6]. Invasive test requiring endosopic biopsy includes culture, histopathology and rapid urease test. Non invasive test do not require endoscopic biopsy (Tanaka et al., 2003)[18].

A newly developed H.pylori antigen test in stool specimen (HPSA) detects bacterial material in feaces (Branden, 2000)[19]. 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 (Kibir, 2001) 320.

In this study the RUT was considered as a gold standard. 41 cases out of 46 patients were both positive for RUT and HPSA test, HPSA test was sensitive and specific. In this study HPSA ws 89% sensitive and 92% specific. The different researchers have compared the results of different invasive and non invasive methods.

CONCLUSION

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

This study suggests that HPSA is cheap and easy to perform. It is almost as sensitive and specific and others are 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.

It can be used for the surveillance of patients with GIT symptoms for identification and control of disease and prevent the development of gastric carcinoma.

STATISTICAL ANALYSIS

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the Kit were calculated according to the gold standard (Li et al., 2004)[3].

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