DETECTION OF HELICOBACTER PYLORI ANTIGEN IN STOOL BY ENZYME-LINKED IMMUNOSORBENT ASSAY AND COMPARISON WITH CONVENTIONAL METHODS

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ABSTRACT

Helicobacter pylori (H. pylori) bacteria are 'slow' bacterial pathogens and are associated with gastritis, peptic ulcers, gastric adenocarcinoma and gastric Mucosa-Associated Lymphoid Type (MALT) B-cell lymphomas. Several methods, both invasive and non-invasive, are available for detection of H. pylori infection. Invasive methods involve endoscopy and examination of gastric biopsies, e.g. by culture, rapid urease test or histology and are not appropriate for large-scale population studies. Non-invasive methods include the urea breath test, serology and stool antigen test. The latter approach is non-invasive, does not require highly specialized equipment and unlike serology is more likely to provide evidence of active rather than past infection. Furthermore, it may be more appropriate for use in paediatric patients, where techniques such as serology are insensitive and invasive methods are undesirable. Additionally, it may be used for treatment follow-up purposes. Pathogen-specific stool antigen tests are a valid alternative to the Urea Breath Test for non-invasive detection of H. pylori.

METHODOLOGY

A total of 120 patients who underwent upper gastrointestinal endoscopy for various gastrointestinal disturbances like dyspepsia were included in the study. Stool samples were obtained from the patient on the day of endoscopy and stored at – 20°C. Three biopsy samples were collected, two from the gastric antrum and one from the corpus. One biopsy sample from the antrum was used for performing Rapid urease test at the Endoscopy room and the other two samples were placed in 10% formalin and sent to the laboratory for histopathological examination.

RESULTS

Sensitivity, specificity, positive and negative predictive values of ELISA was 100%, 77%, 52% and 100% respectively.

CONCLUSION

H. pylori stool antigen (HpSA) is suitable to use particularly in developing countries and for selection of patients for endoscopy. Detection of HpSA shows high sensitivity and specificity and might be useful for non-invasive diagnosis of H. pylori infection in children and adult patients.

KEYWORDS

HpSA, Non-Invasive Test, Antigen Detection.

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INTRODUCTION

Helicobacter pylori (H. pylori) bacteria are 'slow' bacterial pathogens and the name comes from Latin meaning 'spiral rod of the lower part of the stomach.' It was first isolated in 1983 in Australia by Warren and Marshall and was found to be present in patients suffering from type B gastritis.[1] The H. pylori have now been associated with gastritis, peptic ulcers, gastric adenocarcinoma and gastric Mucosa-Associated Lymphoid Type (MALT) B-cell lymphomas.

The mechanism of tissue injury are not clearly established, and both bacterial and host factors may be determinants of outcome.

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H. pylori do not appear to invade tissues, except as an incidental finding. Ammonia, produced by urease and by deaminases, may potentiate neutrophil induced mucosal injury. Urease is a chemoattractant and activator of host phagocytic cells. Both the Cag A and Vac A Proteins are important signalling molecules elaborated by H. pylori and the host mounts antibody response to both.[2,3] Strains from patients with ulcers or stomach cancer more commonly express Cag A compared with controls.

H. pylori bacterium is a small microaerophilic, non-sporing, Gram-negative curved spiral shaped bacterium that is about 3 μm long with a diameter of 0.5 μm with multiple unipolar-sheathed flagella. Urease production is a consistent finding in Helicobacter species of humans that colonise the stomach, but is uncommon in species found in the intestines. Treatment of H. pylori infection is more effective than anti-secretory non-eradicating therapy in preventing recurrent upper gastrointestinal bleeding from peptic ulcer. Consequently, all patients with peptic ulcer bleeding should be tested for H. pylori and eradication therapy should be prescribed to infected patients.[4]

Several methods, both invasive and non-invasive, are available for detection of H. pylori infection. Invasive methods involve endoscopy and examination of gastric biopsies, e.g. by culture, Rapid Urease Test (RUT) or histology, and are not appropriate for large-scale population studies. Non-invasive methods include the Urea Breath Test (UBT), serology and stool antigen test. The latter approach is non-invasive, does not require highly specialized equipment and unlike serology is more likely to provide evidence of active rather than past infection. Furthermore, it may be more appropriate for use in paediatric patients where techniques such as serology are insensitive and invasive methods are undesirable. Additionally, it may be used for treatment follow-up purposes. In the case of upper gastrointestinal bleeding, which is a major cause of morbidity, mortality and medical care costs, peptic ulcer is the most frequent source of bleeding in these patients. Treatment of H. pylori infection is more effective than anti-secretory non-eradicating therapy in preventing recurrent upper gastrointestinal bleeding from peptic ulcer. Consequently, all patients with peptic ulcer bleeding should be tested for H. pylori and eradication therapy should be prescribed to infected patients.[5]

Pathogen-specific stool antigen tests are a valid alternative to the Urea Breath Test (UBT) for non-invasive detection of H. pylori. Much experience has been gained with Premium Platinum HpSA (Meridian Diagnostics, Cincinnati, Ohio), the first Enzyme Immunoassay (EIA) available for the identification of H. pylori antigens in fecal samples. This test uses polyclonal anti-H. pylori antibodies and has revealed good overall performance in diagnosing H. pylori infection or evaluating the success of eradication therapy.[6]

AIMS AND OBJECTIVES

The aim of the study is to detect the presence of Helicobacter pylori antigen in stool by ELISA and compare the results with conventional methods like Rapid Urease Test and Histopathological examination.

MATERIALS AND METHODS

The study was conducted with the approval from the Institutional Ethical Committee of a Tertiary Health Care Centre at Salem, Tamil Nadu. Permission to conduct the study was sought from the respective hospital authorities. Informed consent was obtained from the patients before their enrolment into the study. A total of 120 patients who underwent upper gastrointestinal endoscopy for various gastrointestinal disturbances like dyspepsia were included in the study.

The details of complete history, clinical feature of the patients subjected to endoscopy were obtained. Patients on antibiotics, active gastrointestinal bleeding and those with history of gastric surgery were excluded from the study. Pre-invasive procedure preparation for oesophagogastroduodenoscopy was performed as per norms. Stool sample is obtained from the patient on the day of endoscopy. Three biopsy sample were collected, two from the gastric antrum and one from the corpus. One biopsy sample from the antrum was used for performing Rapid urease test and the other two samples were placed in 10% formalin and sent for histopathological examination. Stool sample was collected in a sterile container on the day of endoscopy and stored at - 20° C.

Rapid Urease Test

The test was done using PYLODRY urease kit. The kit was opened and an antral biopsy sample was placed on the urea strip and one drop of distilled water added followed by closing of the kit. Colour change from yellow to pink at room temperature within 30 minutes was taken as positive.

Histopathology

One specimen from the gastric antrum and one from the corpus were fixed in 10% formalin, paraffin sections were made and stained with Haematoxylin and Eosin and examined for Helicobacter pylori by an experienced pathologist.

Enzyme-Linked Immunosorbent Assay

COPROELISA kit was used for the detection of Helicobacter pylori antigen in the stool samples. The test was performed according to the kit manufacturer's instructions. The absorbance is determined at 450/620 nm and the results recorded. Optical Density (OD) value of less than 0.15 is taken as negative and OD value of more than or equal to 0.15 is taken as positive.

RESULTS

The patient was classified as Gold standard positive when histopathological examination and urease test were both positive and Gold standard negative when both these tests were negative. Out of the total 120 samples, 30 were positive by both ELISA and gold standard tests; 4 were positive by Gold standard but negative by ELISA; 16 were positive by ELISA but negative by gold standard tests; 70 were negative by both ELISA and gold standard tests.

Endoscopy Findings	Total	ELISA Positivity	Percentage
Gastritis	56	32	57.14
Oesophagitis	38	16	42.1
Lax LES	22	8	36.36
Duodenitis & Duodenal ulcer	18	10	55.55
Gastric ulcer	10	4	40
Normal study	20	2	10

Table 1: ELISA Positivity vs Endoscopic Diagnosis

Table 1 shows 57% of gastritis patients and 56% of duodenitis patients are positive for HpSAg by Enzyme-Linked Immunosorbent Assay.

Test Name	Positive No (%)	Negative No. (%)
ELISA	46 (38.3%)	74 (61.7%)
Rapid Urease Test	34 (28.3%)	86 (71.7%)
Histopathology	24 (20%)	96 (80%)

Table 2: Efficacy of Different Laboratory Tests (N = 120)

Sl. No.	Statistical Analysis	Percentage (%)
1.	Sensitivity	100
2.	Specificity	77
3.	Positive predictive value	52
4.	Negative predictive value	100
5.	False positivity	48
6.	False negativity	0

Table 3: Results from ELISA

DISCUSSION

The present work is based on comparative evaluation of invasive and non-invasive methods of detecting Helicobacter pylori infection. Two biopsy based tests namely rapid urease test and histopathological examination and one stool antigen EIA were analysed. The conclusions from the study give fruitful thought about the relative merits and demerits of the methods.

Among the total 120 patients, 74 (61.67%) were males and 46 (38.33%) were females. The maximum number of patients in this study was in the age group 50-59 and is in line with the study conducted by D. Nair et al.[7] The endoscopic examination of the study population revealed that gastritis accounted for 47%, oesophagitis in 32%, lax LES in 18% and duodenal ulcer in 15%. Among the patients with peptic ulcer disease the predominant symptom was epigastric pain in 80% of cases, dyspepsia in 67%, vomiting in 48%, loss of weight in 28%, loss of appetite in 18%, haematemesis in 10% and melena in 4% of the cases. Epigastric pain was the predominant symptom among patients with acid peptic disease.

Out of 120 samples studied by Rapid urease test, 34 (28.3%) were positive. The overall positivity of Rapid Urease Test (RUT) correlated well with reports by Sivaprakash et al (38.7%).[8], while it was lower than that reported by Maimooma et al (65.8%).[9] In the present study, 96% of the cases were positive within the first 20 minutes. This is comparable to 95% reported by Sengupta et al.[10] Marshall et al using an RUT reported that 75% of the positive tests are detected within 20 minutes, 92% at 3 hours and 98% at 24 hours.

Histology has been considered by some to be the gold standard for detection of H. pylori. Unfortunately, histology is an imperfect gold standard as the detection of H. pylori relies upon a number of issues including the site, number and size of gastric biopsies, the method of staining and the level of experience of the examining pathologist. In the present study, Histopathological examination by Haematoxylin and Eosin was positive in 24 (20%) cases.

In the present study, ELISA was positive in 46 (38.3%) cases. The sensitivity, specificity, positive predictive value and negative predictive values were 100%, 77%, 52% and 100% respectively. This correlates with a study by Rani et al[11] in 2000 with a sensitivity of 100% and specificity of 57.9%.

Syam et al[12] evaluated the Helicobacter Pylori Stool Antigen (HpSA) for the detection of H. pylori infection in 63 dyspeptic patients. The sensitivity and specificity of HpSA test were 66.7% and 78.9% respectively. They concluded that HpSA stool test may be useful for the primary diagnosis of H. pylori infection in peptic ulcer.

Arikan et al[13] conducted a prospective study to examine the reliability of the HpSA test. The HpSA test had a sensitivity of 91% and specificity of 83%. HpSA test proved to be as equally reliable as pathological examination for confirming the existence of H. pylori in humans. Thus, the HpSA test was an useful method for detecting H. pylori in patients for whom endoscopy was not indicated. Fanti et al (1999) in his study to evaluate EIA for HpSA, found that this method has a sensitivity of 98.2%, negative prediction value of 96.4%, specificity of 93.1% and a positive prediction value of 96.4%.[14]

Vaira et al (1999).[15], in a multi-centre prospective study, found a sensitivity rate of 94.1% and a specificity rate of 91.8% for HpSA testing.

The HpSA test and the Urea Breath Test (UBT) were conducted 4 weeks after eradication treatment. Sensitivity and specificity of HpSA test was 90% and 95.3% respectively and that of UBT was 90% and 98.9% respectively. Thus, unlike serologic testing that requires several months to achieve significant reduction in antibody titer, the HpSA and UBT with C.[13] can be used to detect prognosis at 4 weeks after the end of the treatment.

In a study conducted in Iran on 54 patients with gastrointestinal problems by Ebrahami et al shows sensitivity and specificity of HpSA before treatment as 78.6% and 92.3% respectively. In 2005 a study was carried out on 100 children with dyspeptic symptoms in Tabriz, Iran. They compared three diagnostic methods (histology, serological test and HpSA). HpSA sensitivity and specificity was 54.8% and 79.4% respectively.[16]

Inelmen et al[17] evaluated the accuracy of HpSA in the diagnosis of H. pylori infection in 85 elderly patients affected by medication. Among 56 patients who were not taking Proton Pump Inhibitors (PPI), the sensitivity and specificity of the Helicobacter pylori stool antigen test were 76% and 93% respectively. Among 29 patients who had received pharmacological therapy with PPIs, the sensitivity and specificity of HpSA tests were 82% and 83% respectively. They concluded that HpSA was a useful test in elderly people. The test was easy, simple to perform and non-invasive.

Forne et al (2000) compared HpSA testing with histological methods, UBT and the rapid urease test for the diagnosis of H. pylori infection and to evaluate the use to determine H. pylori status after treatment. Before treatment, the HpSA test has sensitivity and specificity rates of 89.5% and 77.8% respectively. The specificity is lower than that of UBT, histological evaluation and rapid urease test. Within 24 hours after treatment, the sensitivity for HpSA is 0%. Within 6 weeks after treatment, the sensitivity is 70.4% and 81.6%. Six months after treatment, the sensitivity and specificity is further reduced to 50% and 79.3%.[18]

Thus, they conclude that the HpSA test is beneficial for the primary diagnosis of H. pylori with a similar sensitivity as other standard tests, but with a lower specificity. HpSA testing is not useful for early monitoring to determine the efficacy of treatment. Within 6 weeks and 6 months after treatment for further evaluation of the result of eradication treatment, HpSA testing is not very accurate compared to the UBT.

In this study, we found that HpSA was positive in 57% of gastritis cases and 56% of duodenitis cases. These observations suggest that HpSA is a highly reliable diagnostic method for Acid Peptic disease at primary care level where endoscopy facility is not available.

CONCLUSION

HpSA is suitable to use particularly in developing countries. Detection of H. pylori antigens using enzyme-linked immunosorbent assay shows a high sensitivity and specificity and might be useful for non-invasive diagnosis of H. pylori infection in children and adult patients. HpSA may be useful particularly in selection of the cases requiring endoscopic examination, in monitoring the response to treatment and in epidemiological studies. We recommend using the stool antigen test as a diagnostic test for H. pylori infection.

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