Rapid Urease Test to Diagnose Helicobacter Pylori Infection

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Abstract

Helicobacter pylori, a common cause of peptic ulcer, may also lead to gastric mucosa associated lymphoid tissue lymphoma and gastric adenocarcinoma. A study was done for 17 months from 1st January 2004 to 31st May 2005. Rapid Urease Test (RUT) and microscopy were performed on endoscopic gastric biopsy material obtained from 302 patients suspected to have peptic ulcer. Thirty three (10.93%) specimens were positive by RUT and 25 of those were positive by microscopy. RUT, having high sensitivity (90-95%) and cent per cent specificity, and also being simple cheap, rapid and convenient to perform, can be done in all the microbiology laboratories. Rapid diagnosis of H.Pylori by RUT helps the patients in effective treatment.

Key Words

Helicobacter pylori, Peptic ulcer, Rapid urease test.

Introduction

Helicobacter pylori is a gram negative, curved, microaerophilic and motile organism with multiple polar flagella. It resides in the stomach of man and other primates, lining up the gastric mucus secreting cells. More than 50% of the world population is colonized with H.pylori (1,2). H.Pylori commonly causes peptic ulcer, a chronic inflammatory condition of stomach and duodenum, presenting as recurrent abdominal pain. It is a major cause of morbidity in infected patients as it is associated with 90% of duodenal ulcers and 80% of gastric ulcers (3). The disease has a low mortality. But it results in substantial human suffering and hence loss of manpower. H.Pylori is also associated with gastric mucosa associated lymphoid tissue (MALT) lymphomas and gastric adenocarcinoma. Before the scientists Warren and Marshall, isolated H.Pylori from mucosal specimens of patients with chronic active gastritis and peptic ulcer in 1983, the disease was attributed to stress, dietary factors and injurious effects of digestive secretions such as gastric acid. Humans appear to be the only reservoir of H.pylori infection and therefore human contacts remain the major mode for its transmission. Iatrogenic spread through contaminated gastrointestinal equipment has been documented (2). Water has been shown to be a source for H.pylori infection (4).

Various methods to diagnose H.Pylori infection are grouped as (a) Invasive methods and (b) Non invasive methods. The invasive methods are based on collection of endoscopic gastric biopsy specimens that are subjected to urease test, staining, culture, histology and molecular diagnostic techniques. The non invasive methods comprise urea breath test and serology.

Material and Methods

This study was conducted in the Department of Microbiology, Christian Medical College and Hospital, Ludhiana, Punjab. Many patients visit this hospital from the adjoining states in North India as it serves as a tertiary care centre. The study was conducted over a period of 17 months from 1st January 2004 to 31st May 2005. During this period a total of 302 endoscopic gastric biopsy
specimens were collected from patients with dyspeptic symptoms attending endoscopy suite. Each specimen was subjected to rapid urease test. The medium used for the test was urea broth. It consists of urea, phenol red indicator and distilled water. 10 gm of urea is dissolved in 80ml of distilled water and final volume is made upto 100ml. To it 0.002 gm of phenol red is added. pH is adjusted upto 6.4 to 6.8 using dilute hydrochloric acid. The broth is sterilized by steaming at 100°C for 20 minutes. The medium is distributed in the quantity of 1.5 to 2ml in aliquots.

One biopsy piece from each sample was inoculated immediately after collection into 1.5ml to 2ml of urea broth. It was incubated at 37°C in the incubator for one and a half hour. The change in colour of the broth from pale yellow to deep pink was taken as a positive test.

For microscopy, a piece of biopsy tissue was crushed between two sterilized glass slides. Each preparation was air dried and heat fixed. The smears were overlayed with crystal violet for 30 seconds, followed by washing with distilled water. Then Gram’s iodine solution was poured for one minute followed by decolourisation with pure alcohol for 30 seconds. The counterstaining was done with dilute carbolfuchsin for 30 seconds. After washing with distilled water, smears were air dried and screened for H. Pylori under oil immersion lens of light microscope. Serology by ELISA test was not done because of the cost factor.

Results

Out of 302 patients, 197 (65.23%) were males and 105 (34.77%) were females. Thirty-three (10.93%) yielded positive results by rapid urease test. But only 25 (8.28%) were positive by microscopy. All microscopy positive samples were positive by rapid urease test. Out of 33 positive cases 23 were males and 10 were females. Table 1 indicates the results obtained sex wise. Most of the positive cases i.e. 27 out of 33 (81.82%) belonged to poor families.

Table 1

<table>
<thead>
<tr>
<th>Results obtained by Rapid Urease Test</th>
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<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
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<td>Total</td>
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Discussion

Helicobacter pylori is a spiral urease producing organism that lies in the interface between gastric epithelial cell surface and the overlying mucus gel. A variety of host factors and bacterial factors contribute to the pathogenesis of gastrointestinal diseases resulting from Helicobacter pylori infection. It is intensely antigenic and secretes various factors like urease, catalase, mucinase, lipase, hemolysin and alkaline phosphatase that decrease viscosity of mucus. The production of catalase protects the bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophils from hydrogen peroxide. The multiple polar flagella permit them to penetrate the mucus layer. Adherence of H. pylori to gastric epithelial cells and vacuolating cytotoxin are the virulence factors, as they are associated with degenerative changes in the epithelial cells (3).

Rapid urease test is one of the invasive tests. It is based on the principle that abundant urease enzyme produced by H. pylori hydrolyses urea to ammonia. The consequent rise in the pH of the medium is detected by phenol red indicator.

$$\text{NH}_2\text{CO-NH}_2 + 2\text{H}_2\text{O} + \text{H}^+ \rightarrow 2\text{NH}_4^+ + \text{HCO}_3^-$$

Several modifications of Christensin’s original urea medium have been developed with the aim of obtaining quick results and improving sensitivity and specificity. Various rapid urease tests are available commercially like CLO test, HP test and Pylori-Tek test. These provide comparable results with high sensitivity and specificity. However, simpler and cheaper in-house urease test medium giving similar results can be made in most of the Microbiology Departments as done by us. The sensitivity of test depends on pH of the medium, concentration of urea, indicator used and temperature of incubation.

Microscopy may be false negative if number of organisms is quite low. In that case RUT yields positive result as H. pylori get sufficient time to multiply in the urea broth. This clarifies the reason for getting 33 positives by RUT but only 25 positives by microscopy in our study. RUT is gold standred test for H.pylori infection. It can be used as a rapid diagnostic technique as we get results with in 90 minutes. Thus RUT, a simple and cheap test, is quite beneficial in tracing H.pylori...
infection and thereby helps us in treating the patients in time. Serology by ELISA is quite costly and laborious. A duration of 3 to 5 hours is needed to complete ELISA test. Moreover while performing ELISA, a batch of tests is put up at one time keeping the economic factor in mind as we need to run positive and negative controls simultaneously. Therefore sometimes, waiting for the receipt of samples, diagnosis may take a long time. But RUT is run independently for each biopsy specimen. Moreover sensitivity and specificity of ELISA tests lie between 80-85% and 75-80% respectively. Therefore these may yield false negative and false positive results. As RUT utilizes biopsy material, the specimen once obtained can be used for other studies like histopathology which are again beneficial in achieving an appropriate diagnosis. An inverse relationship exists between the prevalence of the infection and socio-economic factors with higher infection rates in developing countries (5-6). In our study also majority of the patients (27/33) had poor socio economic status.

More than 90% of individuals with gastric mucosa associated lymphoid tissue (MALT) lymphomas are infected with Helicobacter pylori (7). Most patients with low grade lymphoma achieve partial or complete regression after H. pylori infection is eradicated (8). The highest and consistent eradication rates have been achieved by two antibiotics usually clarithromycin and amoxycillin in combination with proton pump inhibitors given for one to two weeks duration. Adenocarcinoma of the stomach is one of the most common malignancies in the world. The relative risk of developing gastric adenocarcinoma is 1.6 to 2 folds greater in association with H. pylori infection versus controls. Therefore H. pylori was categorized is a definite carcinogen by WHO in the year 1994 (7).

Any antibiotic active against H.pylori will cause a reduction in the number of bacteria in the stomach (9). It might be the factor contributing towards the less positivity of the results obtained by microscopy of the stained biopsy material in our study. But RUT remains reliable as the organisms get multiplied in the urea broth during the test. Moreover RUT is extremely valuable because it gives a positive result for H.pylori infection before the patient leaves the endoscopic suite (10). RUT has sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy as 98%, 100%, 100%, 98% and 99% respectively as reported by Said et al (11). These values reinforce its standrdness against screening capability. This study was taken up with an aim to focus on the role of RUT in diagnosing H.pylori caused dyspepsia, peptic ulcer and other associated ailments that are upsurging at present.

References