DETECTION OF HELICOBACTER PYLORI BY INVASIVE METHODS AND EVALUATION OF RAPID UREASE TEST IN A DYSPEPTIC PATIENT’S IN AND AROUND HOSAKOTE, BANGALORE RURAL, SOUTH INDIA

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ABSTRACT

Helicobacter pylori is one of the most common bacterial pathogens in human and it seems to be the only bacterium that can survive in the harsh condition in the stomach. Approximately 50% of the world population is infected with this organism. Documenting the presence of H pylori in a gastric biopsy is essential for appropriate patient care. Aims and objective of the study was to know the prevalence of H. pylori in rural population of Hosakote and to compare the diagnostic accuracy of rapid urease test, culture and histopathology. This was a prospective study carried out in the department of Microbiology in collaboration with department of Surgery from September 2012 to August 2013 at tertiary care hospital, south India. A total of 100 patients with dyspeptic symptoms referred for upper gastro endoscopy were studied. Biopsies were taken from antrum which was used for Rapid Urease Test, culture and histopathological examination. Data were analyzed using Microsoft excel software. Of the 100 patients 59 were male and 41 were female with ages ranging from 20yrs to 85 yrs. In the present study a total numbers of infected cases were 61, 37 uninfected & 42 were indeterminate. Of the 100 patients, 32(52.45%) were culture positive, 59(96.72%) were RUT and 56(91.08%) were histology positive for H.pylori. A positive prevalence of 100% was recorded with culture, and 93.33% with histology. Culture gave a NPP of 82.35% whilst histology gave a NPP of 95%.The RUT had a PPV of 98.3 and NPV of 92.5. RUT is a sensitive and highly specific which enables the rapid diagnosis of H.pylori infection before the patients leaves endoscopy room. Also it is helpful in the management of patients with gastritis especially in the rural area like Hosakote where the prevalence is high.

Keywords: Helicobacter pylori, Rapid Urease Test

INTRODUCTION

Helicobacter pylori is a spiral Gram negative, microaerophilic, motile with multiple polar flagella, slowly growing, pathogenic, urease producing organisms that lies in the interface between the gastric epithelial cells and surface & overlying mucus gel (Foroutan et al., 2010; Syam et al., 2006). H. pylori infection is associated with gastritis, gastric ulcer, gastric adenocarcinoma and mucosal associated lymphoid tissue lymphoma (Xiaohong et al., 2010).

It is found in half the population of the world. Its prevalence is highly variable in relation to geography, ethnicity, and age and socio economic factors (Hunt et al., 2010). Approximately 50% of the world population is infected with this organism. In developing countries, its prevalence is 80% to 90% and in developed countries, the prevalence of infection is <40% (Islam et al., 2010) Inadequate sanitation, low socioeconomic status and over crowding seem to be related to a higher prevalence of H. pylori infection (Foroutan et al., 2010).

With the increasing recognition of the role of H.pylori in gastrointestinal disease, there is a need for a reliable, efficient and yet inexpensive test (Goh et al., 1994). In the present study we are evaluating diagnostic efficiency of RUT in comparison with culture and histology in gastric biopsy sample.
MATERIALS AND METHODS

The study was carried out in the department of Microbiology in collaboration with department of Surgery at tertiary care hospital from September 2012 to August 2013. A total of 100 patients with dyspeptic symptoms referred for upper gastro endoscopy were studied.

Excluded patients from the study were
(i) Patients who had partial/complete gastrectomy/gastoejejunostomy
(ii) Patients who had ever received H. pylori eradication therapy
(iii) Patients who had taken any antibiotic, colloidal bismuth compound, proton pump inhibitor in last one month.
(iv) Patients with bleeding peptic ulcer
(v) Chronic use of corticosteroids or immunosuppressant drugs (Sarkar et al., 2004).

The endoscopic examination was performed using an Olympus CV150 fiber optic video endoscope. Three biopsy specimens from antrum (Hypermic patches) were taken from each patients were used for RUT, Culture & Histopathological examination. An endoscopic diagnosis was recorded and the endoscope & biopsy forceps were disinfected with gluteraldehyde.

Culture Examination: The biopsy specimen was ground with 0.3ml of a 20% glucose solution using either a glass tissue grinder or a porcelain pestle and mortar. The homogenate was plated on 5% ox blood agar, chocolate agar and a Brain Heart Infusion agar plates containing 7% sheep blood, 0.4% IsovitaleX and H.pylori selective supplement. The culture plates were then incubated at 37°C under microaerophilic conditions using anaerobic gas pack in anaerobic jar. The cultures were examined after four and seven days. Positive cultures were identified by colony morphology, Gram stain morphology, positive catalase test, positive oxidase test, and strong urease activity (Islam et al., 2010; Sehnell and Schubert, 1989).

Rapid Urease Test: RUT reagent was prepared as described by Thillainayagam et al. An antral biopsy was placed in an eppendorf tube containing 0.5ml of a freshly prepared 10% urea in unbuffered deionised water at a pH of 6.8 to which had been added two drops of 1% phenol red as a pH indicator (Thillainayagam et al., 1991). Change of color from yellow to pink within 30 min was considered as positive rapid urease test.

Histopathological examination: Another biopsy from antrum was fixed in formalin, processed and paraffin sections stained with H&E.

The research protocol was approved by institutional Review Board and the ethical committee. Data were then analyzed using Microsoft excel software.

RESULTS

A total of 100 endoscopies performed were entered into the study. Of the 100 patients 59 were male and 41 were female with ages ranging from 20yrs to 85 yrs.

Table 1: Endoscopic findings in dyspeptic patients

<table>
<thead>
<tr>
<th>Endoscopic findings</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>32</td>
<td>32%</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>06</td>
<td>06%</td>
</tr>
<tr>
<td>Minimal order of gastritis</td>
<td>48</td>
<td>48%</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>03</td>
<td>03%</td>
</tr>
</tbody>
</table>

Normal endoscopic findings seen in 32 dyspeptic patients. Gastric ulcer in 11, duodenal ulcer in 06, and minimal order of gastritis in 48 cases were observed in endoscopic examination. In addition 03 cases of gastric carcinomas was identified (Table 1).

Definition of H.pylori infection:
Patients with positive culture results were considered as infected. In case of negative culture, patients positive by both biopsy urease test & histology were considered as infected. Patients negative by culture positive by either biopsy urease test/ histology were considered as indeterminate (Leodolter et al., 2003).
According to gold standard definition, in our study total numbers of infected cases were 61, 37 uninfected & 42 were indeterminate. Of the 100 patients, 32(52.45%) were culture positive, 59(96.72%) were RUT and 56(91.08%) were histology positive for H.pylori.

<table>
<thead>
<tr>
<th>Test</th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>Likelihood Positive ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>32</td>
<td>0</td>
<td>56</td>
<td>12</td>
<td>72.72</td>
<td>100</td>
<td>100</td>
<td>82.35</td>
<td>88</td>
<td>0.7345</td>
</tr>
<tr>
<td>RUT</td>
<td>59</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>95.16</td>
<td>97.36</td>
<td>98.33</td>
<td>92.5</td>
<td>96</td>
<td>0.9875</td>
</tr>
<tr>
<td>Histology</td>
<td>56</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>96.55</td>
<td>90.47</td>
<td>93.33</td>
<td>95</td>
<td>94</td>
<td>1.079</td>
</tr>
</tbody>
</table>

Histological examination had the highest sensitivity (96.55%) and culture, the lowest (72.72%). While specificity of 100% was observed with culture. The RUT gave highly comparable results with a sensitivity of 95.16% and specificity of 97.36%. A positive prevalence of 100% was recorded with culture, and 93.33% with histology. Culture gave a NPP of 82.35% whilst histology gave a NPP of 95%. The RUT had a PPV of 98.3 and NPV of 92.5.

Diagnostic accuracy of Culture, histology and RUT were 88, 96, and 94 respectively. Likelihood positive ratio in histology was 1.079. Likelihood positive ratio was 0.9875 in RUT and 0.7345 in culture.

DISCUSSION

H. pylori detection is not an easy task due to the difficulty in accessing its ecological niche and the fragile nature of the bacterium (Francis and Philippe, 2007). Invasive methods are based on collection of endoscopic gastric biopsy specimens that are subjected urease test, culture, histology and molecular technique. The noninvasive method comprises Urea Breath test and serology (Vandana and Vidya, 2006). Serology test is quite costly. Serological assay have been hindered by a relatively high number of false negative and false positives and still its role not defined.

Diagnosis of H. pylori in gastric biopsy is now well established. Culture is gold standard but false negative tests are inevitable because of patchy distribution of the bacteria and consequent sampling error. Histology is highly sensitive compared to culture when detecting H. pylori and has a high degree of specificity. False positives are more due to the presence of other spiral bacteria such as *Gastrospirillum homini* which may be mistakenly diagnosed as *H pylori*. Both culture and histology has several drawbacks. Firstly, delay in availability of result. Culture needs 5 to 7 days and histology needs two weeks or more to be available. Secondly, both requires a specific environment, manpower and always be costly.

Rapid urease test is simple, easy to perform in endoscopy room. Various rapid urease tests are available commercially like CLO test, HP test and Pylori-Tek test. But they are relatively expensive and may therefore not available to all clinicians, up in developing countries.RUT is based on the principal that abundant urease enzyme produced by *H. pylori* hydrolyses urea to ammonia. The consequent rise in pH of the medium is detected by phenol red indicator. Limit to the urease test is false negative may occur, if bacterial load is less than 10^5 or if patients is on antibiotics or on proton pump inhibitor or may be presence of nonurease producing H. pylori. RUT is as highly sensitive as histology and as highly specific as culture in detecting *H. pylori*. Results are available before the patient’s leaves endoscopic room which enables clinician to start prompt treatment. Above all cost of RUT is much cheaper compared to histology and culture.

To conclude RUT is a simple, rapid, highly sensitive, highly specific, cost effective test compared to histology and culture for the diagnosis of *H. pylori* in a gastric biopsy specimen.

**Conclusion**

RUT is a sensitive and highly specific which enables the rapid diagnosis of *H.pylori* infection before the patients leaves endoscopy room. Also it is helpful in the management of patients with gastritis especially in the rural area like Hosakote where the prevalence is high.

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REFERENCES


