A comparative study between Rapid biopsy urease test and histopathological examination of Helicobacter pylori in antral gastritis.”

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ABSTRACT

This prospective study was aimed to assess rapid urease test as an alternative approach for the diagnosis of Helicobacter pylori infection as compared to histopathology in patients with antral gastritis. The study was conducted at Birendra Army hospital from 2063 Bhadra to 2064 Ashad. Total of 80 symptomatic patients with endoscopically antral gastritis were taken as a study group. Rapid biopsy urease test and histological examination of H. pylori were done on all patients. Among 80 cases, H. pylori were positive in 44 (55%) cases of histopathological examination while rapid urease test was positive in 39 (48.75%) cases. In 37 (46.56%) of 80 cases, H. pylori were seen in both rapid urease test and histology. The sensitivity, specificity, PPV, NPV, accuracy of RUT of H. Pylori were 84.1%, 94.4%, 94.9%, 82.9%, 88.8%. Significantly higher no of RUT positive cases were found in histological H. pylori positive cases (p <0.0001*). Agreement between the RUT and HPE of H. pylori positive cases was good (Kappa coefficient from kappa test = 0.78, 95% CI (0.64 – 0.91).

In conclusion, Home made Rapid urease test is quick, simple, reliable and inexpensive test with good sensitivity and specificity for detection of H. pylori infection.

INTRODUCTION

Helicobacter pylorus 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.1

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.2

Although HP can be detected with histology or culture of gastric tissue, simple tests for the presence of urease enable more rapid and convenient diagnosis. Tests for gastric urease are specific for HP because mammalian cells do not produce urease and, except for HP, the stomach is usually sterile. Urease allows the utilization of urea as a nitrogen source and also produces ammonia, which enables HP to tolerate a low pH. Rapid urease test is widely used as standard procedure for the detection of this bacterium, because it is a simple, reliable and inexpensive test, and provides quick results. The commercially available CLO test, which detects presence of urease, is convenient and gives result with in 24 hours, with sensitivity and specificity of 98.0% and 97.0% respectively, but is expensive. Same principle can be applied to a home made rapid urease test, which is very cheap and can be prepared easily. A simpler and cheaper in-house urease test medium giving similar results can be prepared in most of the Microbiology Departments.

As the Rapid urease test is easy to perform and can be finished within a few minutes without the mud

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of sophisticated equipment. By using this test, the physician can determine H. pylori infection of patient at first consultation. The clinician can therefore plan the appropriate therapy almost immediately and the patient is spared another visit to the hospital to receive treatment. Rapid urease test is cost effective, easy, and quick invasive method for detection of H.pylori.  

In Nepal only few studies were done on the RUT of detection of H. pylori. So, this prospective study has been designed to check sensitivity and specificity of RUT. It is hoped that the outcome of this research would be useful for early and easy detection of H. pylori by Rapid urease test.

**METHODOLOGY**

80 symptomatic patients with endoscopic diagnosis of antral gastritis who underwent endoscopic antral biopsy were included in this study irrespective of age and sex. Endoscopically diagnosed case of mild antral gastritis, Patients with gastro duodenal ulcer and cases of gastro duodenal reflux were excluded.

Before the commencement of the study, permission was obtained from the department with an approval of the protocol of the study. All enrolled patients were informed about the nature of the study and their rights to refuse. Their written consent was taken before including them in the study. Patients were subjected to endoscopy using fibre optic endoscope without any premedication. Antral gastric mucosa was carefully studied. Only the symptomatic patients with antral gastritis noted during endoscopy were subjected for gastric biopsy. In all patients trained endoscopist at Birendra Army Hospital carried out an upper gastrointestinal endoscopy. Four gastric mucosal biopsies were taken from the suspicious part of the antrum from each patient for the histological and RUT.RUT reagent was prepared as described by Thillainayagam et al. Unbuffered solutions of urea in deionized water (10 gm of urea in 100ml deionized water) at a pH of 6-8 and 1% phenol red solution (1gm of phenol red in 100 ml deionized water) were prepared and stored at 2 – 8 degree centigrade separately. Each two specimens were placed in the RUT reagent and two sent for histology. Two biopsy tissues were placed immediately into a tube containing 0.5 ml of a freshly prepared test reagent (solution of 10% urea in deionized water, to which had been added two drops of 1% phenol red as a pH indicator). The original color of the solution was yellowish. In each time along with the test sample, a positive control (addition of few drops of NaOH to the solution) with pink color and negative control (reagent tube without tissue and NaOH) was examined for up to 24 hours. The reagent was yellowish when the pH was neutral but it was pink when the reagent was alkaline. The specimens were placed fully in the RUT reagent and the test was interpreted upto 24 hours. Positive RUT results were noted immediately. The change in color of the media from yellow to deep pink was taken as a positive test. The RUT reagent was found to be stable for 2 months. In every 2 months fresh reagents were prepared discarding the old reagents.

For histological examination, Giemsa stain was done in all biopsy specimens.

Expert pathologists from Birendra Army Hospital did the histopathological examination of H. pylori. The histological reports were reported within 1 week.

**STATISTICAL ANALYSIS**

Data were analyzed by using SPSS 11.0 for windows.

The statistical significance for RUT as compared to histological examination was analyzed by chi – square test and Kappa coefficient. Sensitivity, specificity, PPV, NPV, percentage of false positive test and percentage of false negative test of RUT were calculated. HPE of H. pylori was taken as gold standard.

**RESULTS**

A total of 80 cases were taken for this study. Out of 80 cases histologically 44 (55%) cases and by RUT 39 (48.75 %) cases were positive for H. pylori. Both RUT and histologically 37 cases (46.25 %) were positive for H. pylori.

<table>
<thead>
<tr>
<th>Table No.1. Rapid biopsy urease test finding</th>
</tr>
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<tbody>
<tr>
<td><strong>RUT</strong></td>
</tr>
<tr>
<td>H.Pylori(+)+ve</td>
</tr>
<tr>
<td>H.Pylori(-)-ve</td>
</tr>
</tbody>
</table>

Out of 80 cases 39(48.75%) cases were H. pylori positive
Table No.2. Significance of RUT as compared to histopathology

<table>
<thead>
<tr>
<th>RUT</th>
<th>Histopathology positive(%)</th>
<th>Histopathology negative(%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>37 (84.1%)</td>
<td>2 (5.6%)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Negative</td>
<td>7 (15.9%)</td>
<td>34 (94.4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44 (100%)</td>
<td>36 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 84.1%, 95% CI (69.3 – 92.8)
Specificity = 94.4%, 95% CI (80.0 – 99.1)
Positive predictive value = 94.9%, 95% CI (81.4 – 99.1)
Negative predictive value = 82.9%, 95% CI (67.4 – 92.3)
False positive rate = 5.6%
False negative rate = 15.9%
Accuracy rate = 88.8%
\[ \text{LR}^+ = 15.14, \text{95% CI (3.91 – 58.56)} \]
\[ \text{LR}^- = 0.17, \text{95% CI (0.09 – 0.33)} \]
Kappa coefficient from kappa test = 0.78, 95% CI (0.64 – 0.91) i.e. RUT were good accuracy in prediction. Significance of RUT as compared with histopathology was found statistically significant (P value <0.0001)
Correlation between RUT and HPE is good (Kappa coefficient = 0.78)
Kappa value a < 0.0, poor; 0.00 - 0.20, slight; 0.21 -0.40, fair; 0.41 - 0.60, moderate; 0.61 - 0.80, substantial; 0.81 - 1.00, almost perfect.

Bar Diagram No. 2
Significance of Rapid urease test compared to histopathology

DISCUSSION

Although H. pylori can be detected by histological examination of gastric biopsy, a simple and inexpensive RUT enables quick and convenient diagnosis. A positive urease test is strong evidence of H. pylori infection. This is widely used as standard procedure for detection of this bacterium. To date; the rapid urease test is still needed to detect the presence of H. pylori. This test is relatively easy and rapid and thus appropriate therapy is possible if diagnosis of H. pylori is confirmed.

In present study 80 cases were taken. Correlative studies of 80 cases were done including age, sex, and blood group, smoking of H. pylori infection. Our study was centered on rapid biopsy urease diagnosis of H. pylori. At the same time histopathological diagnosis of H. pylori have been evaluated. The sensitivity, specificity, PPV, NPV, accuracy of RUT of H. Pylori were 84.1%, 95% CI (69.3 – 92.8), 94.4%, 95% CI (80.0 – 99.1), 94.9%, 95% CI (81.4 – 99.1), 82.9%, 95% CI (67.4 – 92.3) 88.8%.

Morais M et al did a comparaive study between invasive tests for the diagnosis of helicobacter infections. The sensitivity, specificity, costs and applicability of three invasive diagnostic tests (culture, urease rapid test, and histology) were compared. The sensitivity of Rapid urease test was found to be 86% and specificity of 100%.

Kent-Man Chu and colleagues had done a prospective comparison study of locally made rapid urease test and histology for the diagnosis of helicobacter pylori infection. Three antral biopsy specimens were taken. Results of histological examinations were taken as standards for comparison. 41.1% were found to be positive for H. pylori on histological examination. The sensitivity, specificity, PPV and NNV of the LRUT were 91.6%, 98.3%, 97.4%, and 94.4% respectively.

In Nepal Makaju et al had done a study where they evaluated sensitivity, specificity PPV, NPV of Homemade RUT: 96.4%, 95.2%, 91.55, and 98.0% respectively.
In another study done by W Y Chiu, W K Chick, K H Kwok, the sensitivity, specificity, positive and negative predictive value and accuracy of RUT were found to be 94%, 99%, 99%, 95% and 96% respectively.\(^\text{10}\)

Sensitivity and specificity of RUT in this study was similar to the study done by Morias M et al but lower than the other three similar studies. \(^\text{8,9}\) In the study done by Makaju et al, they used urea in buffer solution for RUT which is different from the reagent used in our study and they used the reagent only for two weeks. In present study, we used the reagent for two months, but every time we checked the reagent before its use. In other two studies\(^\text{8,9}\), they used the reagent (unbuffered urea), which was similar to the reagent used in present study. Low sensitivity of RUT in our study may be because we read the positive test within 1 hour, however in those studies it was read with in 24 hours. The accuracy and positive prediction of RUT were found good in present study.

We compared the results of RUT with HPE of H. pylori and found significantly higher no of positive RUT cases among histological H. pylori positive cases (p <0.0001\(^*\)). We found the agreement between the RUT and HPE of H. pylori positive cases was good (Kappa coefficient from kappa test = 0.78, 95% CI (0.64 – 0.91)).

Similar reports were reported in some other studies. Bermejo F et al showed proportion of positive agreement” of 0.78 between the RUT and HPE of H. pylori positive cases for the antrum, with 0.46 kappa statistic (P < 0.0001).\(^\text{11}\)

Aguilar-Soto et al compared the sensitivity and specificity of rapid urease test to the histological study of H. pylori. They studied 88 patients, 50 women and 38 men. Sensitivity and specificity were as follows: rapid urease test 84.8% and 78.5%, and imprint 75.8% and 83.6% respectively.\(^\text{12}\)

Makaju et al showed significant no of RUT positive (p<0.0001) in histological positive cases. Similar results were reported in other studies\(^\text{9}\).

**CONCLUSION**

The present study verifies the value of rapid urease test in clinical practice. Owing to its high accuracy, albeit with few false-negative results in patients with low-density H. pylori, one can still request for histological examination. As false negative results in RUT is possible if H. pylori density is minimal in biopsy specimen. Alternatively, the biopsy specimens could be taken for histological examination if the urease test is negative to reduce the cost of investigation. Although H. pylori can be detected by histological examination of gastric biopsy, a simple and inexpensive RUT enables quick and convenient diagnosis. In our study we used home made reagent for biopsy urease test and evaluated its utility. We found very good agreement between the RUT and HPE of H. pylori positive cases.

As locally prepared urease solution is very effective, it can be recommended for routine use in gastric biopsy specimen for H. pylori detection.

**REFERENCE**