Evaluation of Locally made Rapid Urease Test for Diagnosis of Helicobacter Pylori

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ABSTRACT

The rapid urease test (RUT) is used for diagnosing Helicobacter pylori infection. This is an in-house method, which has not been validated. To validate our practice of reading the RUT immediately after endoscopy at 10 min by comparing this with a reading at 30 minute and 1 hour and with histological analysis.

Giemsa staining methods were used to identify H. pylori. The RUT was read immediately (within 10 minutes of upper endoscopy) and each specimen was re-read at 30 minute, 1 hour. Sensitivity, specificity, positive and negative predictive values and accuracy rate were calculated.

Histological examination revealed H. pylori in 44 (55%) cases and by RUT 39 (48.75 %). Sensitivity, specificity, PPV, NPV, accuracy rate when RUT test read at 10 minutes were (68.2 %(52.3 – 80.9)), (100% (88.0 – 100)), (100% (85.9 – 100)), (72.0% (57.3 – 83.3)) and 82.5%. At 30 min (84.1% (69.3 – 92.8)), (100% (88.0 – 100)), (100% (88.3 – 100)), (83.7% (68.7 – 92.7)) and 91.3%. At 1 hour (88.6% (74.6 – 95.7)), (94.4% (80.0 – 99.0)), (95.1% (82.2 – 99.2)), (87.2 (71.8 – 95.2)) and 91.3%. No false positive RUT at 10 and 30 minutes but 2(5.6%) false positive cases when the test read at 1 hour. Good positive prediction of RUT read at 30 and 60 minutes (Kappa coefficient 0.83).

We concluded that the most appropriate time point at which to read the positive RUT test is at 30 minute when there is maximal agreement in specificity and minimal gain in sensitivity by reading the test beyond this time.

Key Words: H. pylori, RUT

INTRODUCTION

Recognition of the role of Helicobacter pylori gastric infection in human disease has dramatically changed the management of peptic ulceration, and the Nobel honours bestowed on Marshall and Warren in 2005 in acknowledgement of their landmark research has focused public attention on this organism. Furthermore, the neoplastic potential of H. pylori, which is defined as a type 1 carcinogen, is well recognised and has resulted in some advocating the widespread, worldwide eradication of this infection. A diagnosis of H. pylori infection is readily made using non-invasive tests, such as 13C and 14C urea breath testing, stool antigen analysis or H. pylori serology. Alternatively, infection can be detected in gastric biopsies obtained at endoscopy, through histological staining, tissue culture or polymerase chain reaction (PCR). Far simpler, however, is to diagnose the bacterium by detecting the presence of an H. pylori–derived enzyme, urease, within gastric biopsies. The urease-driven production of ammonia, when H. pylori-infected gastric tissue is added to a urea–containing medium, elicits a pH-dependent colour change that forms the essence of this diagnostic test. Many versions of the ‘urease test’ have been developed, with varying reported sensitivities and specificities. The RUT is inexpensive and easy to perform; however, this in-house method has never previously been validated. In addition, while not evidence based, it is common practice in our clinic to use a single biopsy of the gastric antrum for this investigation and to analyse colour change within 5 minutes of RUT commencement and determine the need for eradication therapy based on this result. 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. They are relatively expensive and may therefore not be available to all clinicians, especially in developing countries.

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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 bacterium⁹. 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 confirmed¹⁰.

Sensitivity and specificity of RUT was found to be good, when the test was read within 24 hours of duration. So, this prospective study has been designed to check sensitivity and specificity of RUT when the test is read earlier.

**MATERIALS & METHODS**

The aim of this study was therefore to determine the validity of reading the RUT early after endoscopy (at 10 minutes), by comparing this with a reading at 30 minutes 1 hour and with histological examination.

80 symptomatic patients with endoscopic diagnosis of antral gastritis who underwent endoscopic antral biopsy were included in this study. Patients were subjected to endoscopy using fibre optic endoscope without any premedication. 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 al¹¹. 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 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 at 10 minute, 30 minutes and 1 hour at the endoscopy room. 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 patients who were found RUT positive were prescribed anti H. pylori treatment from the endoscopy room.

| Table 1. RUT results at 10 min, 30 min and 1 hour using with HPE |
|------------------|------------------|------------------|------------------|
| RUT              | RUT at 10 min    | RUT at 30 min    | RUT at 1 hr      |
| True positive    | 30               | 37               | 39               | 44               |
| False positive   | -                | -                | 2                | -                |
| True negative    | 36               | 36               | 34               | 36               |
| False negative   | 14               | 7                | 5                | -                |

**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 2. RUT results at 10 min, 30 min and 1 hour using with HPE of H. pylori as gold standard |
|-----------------------------------------------|------------------|------------------|------------------|
| Predictors                                    | RUT at 10 mins   | RUT at 30 mins   | RUT at 1 hr      |
| Sensitivity (95% CI)                          | 68.2% (52.3 – 80.9) | 84.1% (69.3 – 92.8) | 88.6% (74.6 – 95.7) |
| Specificity (95% CI)                          | 100% (88.0 – 100)    | 100% (88.0 – 100)    | 94.4% (80.0 – 99.0)    |
| Positive predictive value (95% CI)            | 100% (85.9 – 100)    | 100% (88.3 – 100)    | 95.1% (82.2 – 99.2)    |
| Negative predictive value (95% CI)            | 72.0% (57.3 – 83.3)  | 83.7% (68.7 – 92.7)  | 87.2 (71.8 – 95.2)     |
| False positive rate                           | 0                | 0                | 5.6%              |
| False negative rate                           | 31.8%            | 15.9%            | 11.4%             |
| Accuracy rate                                 | 82.5%            | 84.1%            | 91.3%             |
| p value                                       | <0.0001          | <0.0001          | <0.0001           |
| Kappa coefficient (95% CI)                    | 0.66 (0.50 – 0.82) | 0.83 (0.70 – 0.95) | 0.83 (0.70 – 0.95) |
Sensitivity, specificity, PPV, NPV, accuracy rate is increased when RUT positive test read beyond 10 minutes. No false positive RUT at 10 and 30 minutes, 2(5.6%) false positive cases when the test read at 1 hour. Good positive prediction of RUT read at 30 and 60 minutes (Kappa coefficient 0.83).

DISCUSSION

In present study we compared results of RUT of H. pylori when the test was read at 10 min, 30 min and 1 hour. Sensitivity of the test was found to be increased from 68.2% at 10 minute to 84.6% at 30 minute and 88.6% at 1 hour, but the specificity was decreased from 100% at 10 and 30 minutes to 94.4% at 1 hour. No false positive cases were found when the test was read at 10 and 30 minutes, however false positive rate was 5.6% (2 out of 39cases) at 1 hour. The accuracy of the test was increased from 82.5% at 10 minutes to 91.3% at 30 minutes and 1 hour. The development of false positives with delayed reading of the urease tests attributed to other urease-producing bacteria, or to relatively small numbers of H. pylori, which are not identified histologically.

Morio et al in their study showed sensitivity and specificity of RUT were 62.5% and 98.4%at 5 minutes and 84.4% and 98.4% at 30 minutes respectively.

W Y Chiu, W K Chick, K H Kwok compared the results of homemade urease test at 1 hour and 24 hours. They also found increases sensitivity (from 73% to 94%) and decrease specificity (100% to 99%). They reported 1 false positive case at 24 hours. Chomvarin C et al showed the sensitivity and specificity of the iRUT result at 30 minutes, 1 hour and up to 24 hours were 77.1% and 100%, 77.6% and 100%, and 94.1% and 94.2%, respectively. Li Lin Lim, Khek Yu Ho also found similar results when they compared the sensitivity and specificity of the RUT at 5, 30, 60 minutes and 24 hours: 50%, 82.1%, 85.7%, 96.4% and 100%, 100%, 100%, 96% respectively.

Some other studies have reported that increased in sensitivity and reduced in specificity of RUT with longer incubation times. In our study, 5 out of 39 cases (11.9%) were false negative specimens on the RUT. However, the specificity of the RUT was excellent, no false positive results found within 30 min, only 2 out of 39 cases (5.6%) were found false positive at 1 hour. Several factors may affect the results on RUT, such as the amount of urea, the incubation temperature, the indicator used in the medium and the number of biopsies used in a RUT. The number of biopsies used in a RUT affects the results because some researchers have shown that taking two biopsies instead of one has resulted in an earlier positive RUT result (Lim et al, 2004).

In our study, 1-h reading of the test was found to give the optimal sensitivity/specificity combination; this does not automatically ensure that it is the best time to read the test. Despite the fact that the H. pylori infection is a chronic disease, the necessity to treat it soon after endoscopy is desirable to minimize the cost as well as the inconvenience of a second clinic visit. If this is the practice, then the most appropriate time point at which to read the test is 30 min when there is maximal specificity and minimal gain in sensitivity by reading the test beyond this time.

Most importantly, reading the test at 30 min allows the physician to prescribe H. pylori eradication treatment to the patient before discharging him or her from the endoscopy suite. Although we have regarded the reading at 1 hour as the final result, 84.1% of tests were positive after 30 minutes.

CONCLUSION

The most appropriate time point at which to read the positive RUT test is 30 min when there is maximal agreement in specificity and minimal gain in sensitivity by reading the test beyond this time. Most importantly, reading the test at 30 min allows the physician to prescribe H. pylori eradication treatment to the patient before discharging him or her from the endoscopy room.

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