A Comparative Evaluation of Microbiological versus Histopathological Diagnostic Methods for Helicobacter pylori Infection

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ABSTRACT

Helicobacter pylori infection is a factor in the pathogenesis of gastroduodenal mucosal inflammation and gastric carcinoma. Though Histopathology plays a pivotal role in diagnosis of H.pylori infection, it has several limitations. This study was aimed at evaluating an in-house urease test and crush cytology for diagnosis of H. pylori infection. This cross sectional study was done for a period of three months on outpatients undergoing upper gastrointestinal endoscopy for various clinical indications. Antral biopsy specimens were obtained from 120 patients with findings of gastroduodenitis with or without gastric and duodenal ulcer. The samples were subjected to the following tests namely an in-house rapid urease test (RUT), Giemsa staining of crush cytology and histopathology. Kappa statistics was used to evaluate the various tests. RUT was positive in 45% of patients, Giemsa staining of crush cytology in 44.2% and histopathology in 45.8%. Considering histopathology report as the gold standard. Kappa statistics showed almost perfect degree agreement of HPE with Giemsa staining of crush cytology (Kappa-0.96 and 98% agreement) and RUT (Kappa 0.92 and 96% agreement). The sensitivity and specificity of Giemsa stain was 96.4% and 100% respectively and that of RUT was 94.5% and 96.9% respectively. Giemsa stain of crush cytology is a rapid and specific diagnostic test and is comparable to histopathology for the diagnosis of H. pylori infection.

Keywords
Helicobacter pylori, crush cytology, inhouse rapid urease test.

Introduction

Since its discovery in 1982 by Barry J. Marshall and J. Robert Warren from the gastric epithelium, Helicobacter pylori has been detected in every human population studied and is associated with inflammation of gastroduodenal mucosa. H.pylori is a gram negative helical bacterium which can be seen Haematoxylin & Eosin stain (H&E), the sensitivity and specificity of which has been reported as 69-93% and 89-90% respectively. Though HPE has the added advantage byits ability to detect the associated pathology, it has several limitations such as higher cost, longer turnaround time, interobserver variations etc. (Lee et al., 2015).

The aim of the study was to evaluate the Giemsa stained crush cytology and in-house rapid test with histopathological examination.
Materials and Methods

Study design and population: This cross sectional study was done over a period of three monthson new out patients attending the endoscopy clinic with clinical indications for upper gastrointestinal endoscopy such as dyspepsia, upper abdominal pain, vomiting. Patient’s consent and Institutional Ethics Committee clearance were obtained.

Inclusion criteria: Patients with endoscopic findings of gastroduodenitis with or without gastric or duodenal ulcer were included.

Exclusion criteria: Patients with active bleeding ulcer (Lee et al., 2000), postgastrectomy, recent treatment with proton pump inhibitors or bismuth or Helicobacter pylori eradication therapy in the past one month were excluded.

Sample size: 120 patients

Specimen collection and transport

Biopsy specimens were obtained using a flexible gastroduodenoscope which was thoroughly rinsed with water and disinfected with 2% gluteraldehyde for ten minutes after each use. The biopsy forceps was cleaned and rinsed in 70% ethanol between each sample collection. Three biopsy specimens were taken from within 3 cm from the pylorus.

One bit was inoculated into urea broth. The other bits were transported in sterile screw capped vials containing isotonic saline and 10% formalin for crush cytology and histopathological examination respectively.

Rapid Urease Test (Chiu et al., 1999):

Inhouse rapid urease broth was prepared as follows.

Stock solution A (1 % phenol red solution)

1 gm phenol red was dissolved in 32.5 ml of 0.1 mol/L sodium hydroxide and made up to 100 ml with distilled water. The solution was autoclaved for 15 minutes at 121°C.

Stock solution B (10% urease solution)

10 gm of urea was dissolved in 100 ml of sterile distilled water taking aseptic precautions. The pH of the solution was adjusted to 6.8 and dispensed on 0.5 ml aliquots in sterile vials. Two drops of phenol red was added to each vial containing 0.5 ml of 10% urea solution. A change in colour to pink within 30 minutes was interpreted as positive.

Crush cytology and staining (Nijhavan et al., 1993; Soltesz et al., 1992)

One of the two specimens in the transport medium was crushed between sterile frosted glass slides, air dried, fixed with methanol and stained with Giemsa stain for 5 minutes and washed (Qualigenes, India). The smear was examined for deep purple bacilli with typical helical morphology.

Histopathology (Barthel et al., 1990)

The antral samples were sent to the Histopathology laboratory for further processing and Haematoxylin and Eosin staining.

Results and Discussion

The study population consisted of 120 adults with males and females in the ratio 19:5 in the age range of 20-70 years. The endoscopic findings included gastritis/duodenitis in 51 patients (42.5%), gastric ulcer in 31 patients (25.8%) and duodenal ulcer in 38 patients (31.7%).
Table 1 Results of Inhouse RUT and Giemsa stained crush cytology

| Test evaluated | HPE |  |
|----------------|-----|-----|
|                | +   | +   |
| Giemsa stain   | 53  | -   |
|                | -   | 65  |
| Inhouse RUT    | 52  | 2   |
|                | 3   | 63  |

Table 2 Comparative Evaluation of biopsy based tests with HPE

|                      | Giemsa-crush cytology | RUT |
|----------------------|-----------------------|-----|
| sensitivity          | 96.4%                 | 94.5% |
| specificity          | 100%                  | 96.9% |
| PPV                  | 100%                  | 96.3% |
| NPV                  | 97%                   | 95.4% |
| % of false positives | 3.6%                  | 3.1% |
| % of false negatives | nil                   | 5.4% |
| Kappa value          | 0.96                  | 0.92 |
| Interpretation of kappa value | Almost perfect agreement | Almost perfect agreement |

The in-house rapid urease test was positive in 45%(n=54/120), Giemsa staining of crush cytology in 44.2%(n=53/120) and Histopathology in 45.8%(n=55/120). Among the 54 RUT positive samples, 11 (20.4%) turned positive within 1 minute and 36 specimens (66.7%) in 1-5 minutes, 6 specimens (11.1%) in 30 minutes. Thus by 10 minutes 53 of 54 samples (98.1%) had changed colour. One specimen (1.8%) became positive at one hour. The results of rapid urease test, Giemsa stain and RUT were compared to that of histopathology by H&E stain and are shown in Table 1 & 2.

A number of cytological techniques have been used to identify *H. pylori* such as imprint smears, brush cytology and crush cytology. In imprint or touch smears, only the superficial part of the biopsy sample is harvested on the slides while crush smears represent the whole biopsy tissue. The present study found an excellent sensitivity and specificity for Giemsa stained crush cytology with almost perfect agreement with histopathology. Other studies have noted good concordance of 76.5% between crush cytology and histology (Soltesz et al., 1992).

The rapid urease test is advantageous in that the results are available in the endoscopy room itself. This study observed a good sensitivity and specificity for urease test (94.5% & 96.9% respectively). Other studies have also reported a similar sensitivity and specificity of 94% and 99%. A higher concentration of urea and omission of buffer in the in-house test induces a more rapid colour change (Working party of the European Helicobacter Study Group, 1997). In our study, 98.1% of the samples had turned positive by 30 minutes. This is comparable to the study by Thijs in which 94.54% of the positive rapid urease tests changed colour within 30 minutes (Thijs et al., 1996).

In this study, there were two samples positive by RUT but negative by HPE. These samples showed delayed colour change beyond 5 minutes. The delayed colour change could be due to other urease
producing bacteria which produce much less urease than H. pylori. Similarly three samples were negative by RUT but histopathologically positive. Such false negative RUTs could be due to the presence of gastric atrophy where in the organisms are low in numbers or absent, or due to patchy distribution of the organisms (Calam, 1996). False negative results due to sampling error are diminished by testing multiple biopsies, discontinuation of antibiotic for at least 1 month and proton pump inhibitors 2 weeks before endoscopy (Working party of the European Helicobacter Study Group, 1997).

The H. pylori status in the study population based on HPE was 45.8%. Other studies have reported prevalence rates varying from 60% to 100% in duodenal ulcer and 20% to 100% in gastric ulcer (Philip Abraham. Helicobacter pylori, 1997). The prevalence of Helicobacter pylori in gastro duodenal disease during routine upper gastrointestinal endoscopies in South India was reported as 58% in patients with gastroduodenitis (Thayumanavan, 1997).

In conclusion, this study indicates that Giemsa stained crush cytology is a simple, highly sensitive and specific method for rapid detection of Helicobacter pylori infection. As the result can be made available in the endoscopy room itself ,it is more useful for rapid diagnosis of H.pylori in out patients than histopathology.

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