Identification of *Helicobacter pylori* in Gastric Biopsies; Comparison of the R of the Rapid Urease Test with the Histological Giemsa Staining Technique

B Seneviratne[1#], WMDR Fernando[2]
[1]*Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*
[2]*Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahara, Sri Lanka*
[#]bimalka03@yahoo.com

Abstract— Helicobacter pylori (H. pylori) remains a prevalent chronic infection in the developing countries. H.pylori is an important agent associated with peptic ulcer disease, chronic gastritis and gastric malignancies. It can be detected by various invasive (rapid urease test and histology) and non-invasive tests (stool antigen test, urea breath test and serology). The aim of this study was to compare the performance of the Rapid Urease Test (RUT) with histological Giemsa staining technique. Data was collected from histopathology reports of antral biopsies, endoscopy and RUT reports of the patients (n=429) who underwent endoscopic examination at a tertiary care private Hospital from July 2013 to May 2014. The overall positivity for H. pylori was considered when either or both tests were positive. H.pylori were found in 183 (42.65%) patients by the RUT method and in 191(44.55%) patients with the Giemsa staining technique. Only 18 (4.19%) cases showed dis-concordant results between Giemsa staining and the RUT method. With 95% confidence interval, proportion of the dis-concordant result (18/429) of two identification techniques is not statistically significant (p-value=1.00). Sensitivity and specificity of the Giemsa staining method was 98.45% & 100% and the RUT was 88.14%, 94.89% respectively. Even though no statistical significance was found for dis-concordant results by both methods, substitution of the RUT test by the histopathology Giemsa staining technique should be considered with caution.

Keywords— *Helicobacter pylori*, Rapid Urease Test

I. INTRODUCTION

Helicobacter pylori (*H. pylori*), first described by Warren and Marshal remains a prevalent chronic infection in developing countries.*H.pylori* is associated with chronic gastritis, peptic ulcer disease and gastric malignancy(Kuo CHet al, 2002).A study in 2002 has shown that the prevalence of *H. pylori* in a dyspepticSinhalese population was 75.4% (Fernando et al, 2002). Although, theroutes of transmission of *H.pylori* are still poorly understood, in developing countries faecal-oral transmission is thought to be common(Logan RPH et al, 2001). The organism colonizes the gastric mucosa particularly the antrum and cardia. Individuals infected with *H.pylori* have a 10 to 20% lifetime risk of developing peptic ulcers and a 1 to 2% risk of acquiring gastric cancer (Kusters JG et al, 2006). Recently, *H.pylori* has been classified as gastric carcinogen class 1 (Syam et al, 2006). Accurate diagnosis will enable the complete eradication of *H.pylori* infection. Diagnosis of *H.pylori* is routinely carried out in several ways. These include invasive methods and non-invasive methods. Non-invasive methods are carbon urea breath test, blood antibody test and stool antigen test. Non-invasive methods are not appropriate for determining the underlying disease associated with *H.pylori* infection. Invasive methods require endoscopic biopsy of gastric mucosa for rapid urease test (RUT), histopathology, culture and genetic amplification. Culture of biopsy samples is a complicated procedure as special transport media are required. Histological examination of gastric biopsy samples should be mandatory at the initial presentation of the patient because it gives insight on the status of the gastric mucosa, and it has been considered by some to be the gold standard for detection of *H. pylori.* European guidelines indicates that the gold standard needs to be generally represented by at least two different detection methods (Pity et al, 2011). In histology sections, *H.pylori* is recognized as short, curved or spiral bacilli resting on the epithelial surface or in the mucus layer of gastric mucosa (Pity IS et al, 2011).Following treatment, the density of *H.pylori* becomes lower or even absent and the shape of bacteria may be changed into round or vibrio shape. Such modified forms are difficult to be identified by the routine hematoxylin-eosin(H&E) stain. Therefore several modified staining methods are used in routine practice. Generally it is agreed that the modified Giemsa stain is preferable because of its high sensitivity & specificity and low cost (Kheiralla, 2012). In upper gastrointestinal endoscopy, RUT became the initial test of choice, because of its simplicity with widely available commercial kits(Syam AF et al, 2006(2) *H.pylori* is able to neutralize

the acid in its environment by ammonia, produced in the reaction of its urease with urea present in the stomach. This principle is used in the RUT kit, in which the biopsy is incubated in a medium containing urea and a pH-sensitive colour marker. Production of urease by *H. pylori* causes a rise in pH value and a change in the colour of the medium. Positive results are shown within one hour. The aim of this study was to compare the performance of the RUT with the histological Giemsa staining technique.

II. MATERIALS AND METHOD:

An analytical cross sectional study wasconducted in the Endoscopy unit and histopathology Laboratory of a tertiary care private Hospital, Sri Lanka. Data was collected from Histopathology reports of antral biopsies, endoscopy reports and rapid urease test (Pronto dry) reports of the patients who underwent endoscopic examination from July 2013 to May 2014. As instructed by the manufacturer, RUT wasperformed and results were monitored at room temperatureafter 01 hour. A positive result is defined as a colour change on the test from yellow to pink-magenta. Histological assessment of the biopsies was made by the principal investigator. Modified Giemsa stain was performed in all biopsy samples to detect *H. pylori* infection. Data from the records of 429 of patients were included. Dis-concordant results of the two tests were compared by one proportion test using Minitab statistical software. Specificity, sensitivity, negative predictive value (NPV) and positive predictive (PPV) value were calculated by Binary Classification Test using 2‹2 contingency table. Data collection was commenced following theethical clearance by the ethics review committee of Faculty of Medical Sciences, University of Sri Jayewardenepura. Data was encoded and tabulated by the investigators.

III. RESULTS

Patients from 18- 80 years of age were included in the study. The overall positivity (either of the tests) of *H.pylori* was 45.22% (194/429). The RUT was positive in 42.65% (183/429) and Giemsa histological staining was positive in 44.55% (191/429). Only 18 (4.19%) cases showed dis-concordant results between the histopathology technique and the RUT method. With 5% significance level, the proportion of dis-concordant results (18/429) was not statistically significant (p-value=1.00). In a study done in 2010 using 50 patients to compare the RUT and the Giemsa staining technique, *H.pylori* has been positive in 22 cases by histological method and in 19 by the RUT method. A statistically significant correlation between the two tests (Kappa=0.876, p value < 0.01) (Wichai K, 2010) has been found.

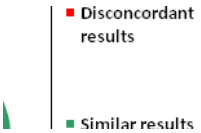

Figure 1: Percentage of dis-concordant results of both methods.

Table 1. Sensitivity, specificity, true positive& true negative values of the RUT and Giemsa Staining technique compared with the “gold standard” in which the two tests are taken together

	Sensitivity	specificity	True Positives	True Negatives	False Positives	False Negatives
RUT	88.14 %	94.89 %	171	223	12	23
Giem-sa test	98.45 %	100%	191	235	00	03

IV. DISCUSSION AND CONCLUSION

According to the findings of this study, the Giemsa staining method revealed a high sensitivity (98.45%) than the RUT (88.14%) and also a high specificity (100%) than the RUT (94.89%). In a study done at the Teaching Hospital Karapitiya, sensitivity and specificity of RUT has been reported as 32% and 68% respectively. For the Giemsa technique sensitivity has been81% and specificity 100% (Waidyaratne EI et al, 2012). According to the findings of our study, identification of infected patients from cases of positive test results (PPV) by the RUT was 93.44% and by the Giemsa staining was 100%. The identification of uninfected persons from cases of negative test results (NPV) by the RUT was 90.65% and by the Giemsa staining was 98.74%. Several studies have found that medications such as antibiotics, bismuth, or proton Pump Inhibitors (PPI) can reduce the density and/or urease activity of *H. pylori,* thereby decrease the sensitivity of the RUT (Fernandez MC et al,2004and Goddard AF et al, 2003). Acute ulcer bleeding at the time of testing may also decrease the sensitivity and negative predictive value of the RUT. As such, the RUT can rarely be used as the sole means of identifying *H. pylori* infection. Simplicity, rapidity of test results and the cost

effectiveness make the RUT a practical means of testing for *H. pylori* in suspected patients. The RUT test also has the advantage of not requiring refrigeration. Histology may be an imperfect gold standard as the detection of *H. pylori* relies upon a number of issues including the site, number, and size of gastric biopsies, method of staining, and the level of experience of the examining pathologist. Observation can be incorrect in patients who had used proton pump inhibitors prior to the endoscopic examination.

The proportion of dis-concordant results between the two methods was not statistically significant. Even though no statistical difference was found to decline any one of the twomethods, substitution of the RUT by Giemsa staining technique should be considered with caution.

REFERENCES

Fernandez MC, D. Muñoz S, Díaz EG, Jurado MVG Alonso CR. 2004. Diagnosis of *Helicobacter pylori* infection using urease rapid test in patients with bleeding duodenal ulcer: influence of endoscopic signs and simultaneous corporal and antral biopsies. *Rev Esp Enferm Dig (madrid*); 96:599-605.

Fernando N, Holton J, Vaira D, DeSilva M, Fernando D. 2002. Prevalence of *Helicobacter pylori*in Sri Lanka as determined by PCR. *Journal of Clinical Microbiology*; 40(7):2675-2676.

Goddard AF, Logan RPH. 2003. Diagnostic methods forHelicobacter pyloridetection and eradication. *J Clinical Pharmacology*; 56 273–283

Kheirallah AK. 2012. H. pylori: Comparison of progressive and regressive Giemsa staining. *The biomedical scientist*; 56(2):76-78

Kuo CH, Wu DC, Lu CY, Su YC, Yu FJ, Wu YL, Lira SR, Liu CS, Jan CM, Wang WM. 2002. The Media of Rapid Urease Test Influence the Diagnosis of Helicobacter pylori. *J of Hepato-Gastroenterology*; 49:1191-1194

Kusters JG, van Vliet AH, Kuipers EJ (July 2006). Pathogenesis ofHelicobacter pylori Infection. *Clin Microbiol Rev* **19** (3): 449–90

Logan RPH, Walker MM. 2001. ABC of the upper gastrointestinal tract Epidemiology and diagnosis of Helicobacter pylori infection. *British Medical Journal*; 323:20

Pity IS, Baizeed AM. 2011. Identification of Helic*obacter pylori* in gastric biopsies of patients with chronic gastritis: histopathological and immunohistochemical study. *Duhok medical journa*l; 5(1):69-77

Syam AF, Abdullah M, Rani AA, Nurdjanah S, Adi P, Djumhana A, Tarigan P, Wibawa IDN. 2006. Evaluation of the use of rapid urease test: Pronto Dry to detect *H. pylori* in patients with dyspepsia in several cities in Indonesia. *World Journal of Gastroenterology*; 12(38): 6216-6218.

Waidyarathne EI, Mudduwa LKB, Lekamwasam JDVC, Lekamwasam S. 2012. *Helicobacter pylori* detection techniques: comparison of sensitivity, specificity and cost. *Galle Medical Journal*; 17(2)

Wichai K. 2010. Comparison of pronto dry and histopathology in identifying of *Helicobacter pylori* in Patients with perforated duodenal ulcer. *KhonKaen Medical Journal*; 34(1)

BIOGRAPHY OF AUTHORS

Dr. Bimalka Seneviratne is a Consultant Pathologist / Senior Lecturer, attached to the departmentof Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura. She was Head ofthe Department of Pathology from 2010 – 2012. She is the current President of the Faculty ofMedical Teachers' Association, USJP. She completed her Postgraduate training in Histopathologyat the Royal Prince Alfred Hospital in Sydney, Australia. Her special interests include theinterpretation of renal allograft biopsies, IgG4- related systemic diseases and breast and thyroid pathology. She has been a member of the Senate and Dean's Advisory Committee from 2010 –2012.