ISSN: 2091-2749 (Print)
2091-2757 (Online)

Correspondence
Dr. Yuba Raj Sharma
Associate professor, Department of Internal Medicine
Patan Academy of Health Sciences, Lalitpur, Nepal
Email:dryubarajsharma@yahoo.com

Peer reviewed by
Dr. Reetu Baral
Patan academy of health sciences

Mr. Shital Bhandary
Patan academy of health sciences

Comparison of home made and commercial rapid urease tests for detection of helicobacter pylori in patients with gastroduodenitis and peptic ulcer

[1]Yuba Raj Sharma, [2] Amrita Wagley, [3] Sunil Singh

[1]Associate Professor
Department Of Internal Medicine, Patan Academy of Health Sciences, Laliptur, Nepal

[2]Research Fellow
University of South Korea,

[3]Professor
Department Of Pathology, Army Medical College, Kathmandu, Nepal

ABSTRACT

Introductions: *Helicobacter pylori* is one of the common and medically prominent infections worldwide and an established etiological factor for peptic ulcer disease. This study was conducted to compare the results of two types of Rapid Urease Tests (RUT) for H. pylori infection.

Methods: This study was conducted in patients with gastro duodenal diseases visiting Kantipur Hospital from June to August 2010. Antral biopsies were collected from sixty patients visiting endoscopy unit. The diagnosis was of H. pylori infection carried out using two types of rapid urease tests (commercial and homemade) as well as Histopathology.

Results*: H. pylori* infection was detected in 34 (56.67%) of 60 by histological test, 24 (40%) by homemade kit method and 28 (46.67%) by commercial RUT method. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for RUT (commercial kit) considering histology as gold standard were 76.74%, 92.31%, 92.85% and 75% respectively. The sensitivity, specificity, PPV and NPV for RUT (homemade kit) were 58.82%, 84.62%, 83.3% and 61.11% respectively.

Conclusions: Homemade rapid urease test was sensitive and specific for detection of *H. pylori* infection than commercial test.

Keywords: helicobacter pylori, peptic ulcer, rapid urease test

Plain Language Summary

This study was conducted to compare between home made and commercial rapid urease test kit. Histopathological examination was taken as gold standard. This study concluded that homemade kits were not better than commercial kits.

INTRODUCTIONS

Helicobacter pylori (H pylori) is a well-recognized etiologic agent for gastritis, duodenitis and peptic ulcer disease (PUD).[1] In Nepal, association of H. pylori has been found significantly higher in duodenal ulcer, gastric ulcer and gastritis by various studies.[2]

Urease produced by H pylori is clinically important because it forms the basis for several invasive and noninvasive tests to diagnose infection.[3] The presence of this infection can be diagnosed by non-invasive methods like urea breath test (UBT), serology using ELISA or stool antigen test.

The current study was performed with the objective to find out prevalence of H pylori by comparing the two rapid urease tests, RUT homemade as well as commercial with histopathological test. The findings will be helpful in timely diagnosis and treatment of H. pylori infection.

METHODS

This was a cross sectional study conducted from June to August 2010 at Kantipur hospital, Kathmandu, Nepal. Sixty patients who underwent upper gastrointestinal endoscopy for dyspepsia or upper abdominal pain with burning sensation were included. Biopsy specimens were collected from antral and corpus in case of gastritis, duodenitis and duodenal ulcer. In the case of gastric ulcer, the biopsy specimens were taken from mucosa of adjacent margin of the ulcer. Four biopsies were taken. The first was directly inoculated into the homemade RUT tubes and the second was inoculated directly into the commercial RUT kits. The remaining two were taken to laboratory and placed into a sterile container containing 10% formalin for histopathology.

Homemade RUT reagent was prepared by adding one gram of urea in 9ml of sterile buffer solution (pH 6.8) where, one ml of this solution was filled in a sterile test tube and a drop of phenol red solution was added and stored at 2-8 ^{o}C. The original color of the solution was yellowish. The change in color to red or pink within 24 hours were regarded as RUT positive. The commercial RUT kit 'helikochek',- was used. Histopathological examination was carried out at Kantipur Hospital by a single histopathologist.

In this study, homemade and commercial RUT were used for detection of H. pylori in the biopsy samples and compared with histopathology considered as "gold standard".

RESULTS

Out of the total 60 cases, 24 (40%) were found to be RUT positive by homemade method, 28 (46.67%) by commercial kit, whereas 34 (56.67%) by histopathological test. The age of patients ranged from 15 to 68 years. Among them highest number were from the age group 40 – 60 years. The prevalence of H. pylori infection was also found to be highest in this age group.

Out of 60 cases, 36 were male and 24 were female. Among the 34 histology positive cases, 23 were male and 11 were female, whereas the male and female patients the RUT (commercial kit) positive cases, 19 were male and 9 female; and in RUT (homemade test), 17 were male and 7 female. Male were affected more than females.

Table 1. Comparison of RUT (commercial kit) with reference to histology

	Tests Results	Histology	
		Positive	Negative
RUT	Positive	26	2
	Negative	8	24

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for RUT (commercial kit) considering histology as gold standard were 76.74%, 92.31%, 92.85% and 75% respectively.

Table 2. Comparison of RUT (homemade kit) with reference to histology

	Tests Results	Histology	
		Positive	Negative
RUT	Positive	20	4
	Negative	14	22

The sensitivity, specificity, PPV and NPV for RUT (homemade kit) were 58.82%, 84.62%, 83.3% and 61.11% respectively.

DISCUSSIONS

The H. pylori detection by histological examination is considered gold standard but a simple and inexpensive rapid urease test (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 the detection of this bacterium.[4] The false positive results may be explained by the presence of other urease producing bacteria in the gastric specimen. The false negative urease test may be due to the complete absence of H. pylori or the patchiness of organism.[5]

Out of total 60 patients, the H. pylori were detected in 34 (56.67%) by histology. In Nepal, H. pylori prevalence were found to be 56.8% by Kawasaki et al.[6] using serology, 39.25% by Subedi et al[7] using hitopathology and/or culture, 33.9% by Makaju et al [2] using histopathology, 29.5% by Rai et al [8] using RUT, culture and PCR, 25.5% by Adhikari et al [9] using RUT and/or culture. In studies by Shah et al [10] the prevalence of H. pylori infection was 86.66% by CLO (urease test) and Larsson et al [11] found the prevalence of 70-80% using ELISA test for IgG serum antibodies. In other studies it was 80% using RUT by Shakya et al[2], and 36% using RUT, culture and histology by Dangol et al [12], 67.50% by Marasini et al [13] and 46.56% using RUT and histology by Karki et al.[14]

The prevalence of H. pylori infection varies between regions of the same country or parts of the world. Many variations including studied population, bacterial strains, geographic locations, the efficacy of diagnostic methods, environmental and socio-economic factors may contribute, which makes it difficult to interpret data.[15] Guzman et al.[5] stated that false negative and false positive results in the different methods tested may be due to patchy distribution of H. pylori present in the gastric mucosa. Taking several pieces of tissues from different representative areas minimize this situation.[2]

If the patient uses PPIs, the sensitivity of the RUT may fall below 50%.[16] Bleeding from peptic ulcer disease, presence of blood in the stomach from any source is considered to decrease the sensitivity of the RUT to 60-70%.[17] Longstanding infection can lead to atrophic change especially in the gastric body. Subsequently, an increase in gastric pH in the body reduces the density of H. pylori below the detection level of RUT.[18] On the other side the non acidic environment may harbor other bacteria with urease activity, giving false positive results.[19] Chronic renal failure in which the prevalence of H. pylori infection is lower may also decrease the sensitivity of the RUT.[17] After a failed eradication therapy, H. pylori may require over four weeks to reach the level detected by RUT.[20] It is claimed that the commercially available CLO (urease) test, which detects presence of urease is convenient and gives result within 24 hours with sensitivity and specificity of 98% and 97% respectively, but is relatively expensive. Homemade rapid urease test is cheap and can be prepared easily.[14]

A fundamental distinction among test for H. pylori is whether they provide direct evidence that infection is currently present (active test) or indirect evidence by detecting the presence of antibodies to H. pylori (passive test).[21] Therefore, the available serological test at present in detection of antibody against H. pylori detects IgG only is not useful test for diagnosis of active infection. Simple and non invasive active test such as urea breath test and stool antigen test are expensive and not easily available. The invasive technique such as culture takes long time and has low sensitivity than histology.[22]

Histopathological examination of biopsy specimen detects active H. pylori infection, and has high sensitivity and specificity, is expensive and take days. Hence, RUT is considered to be a better choice for patients undergoing endoscopic examination because of the rapid result that can be obtained within few hours. The most rapid modern test gives positive results in minutes, enabling endoscopist to begin eradication therapy immediately after endoscopy.[23]

Among the homemade and commercial kits available for rapid urease test, homemade RUT is considered to be advantageous in terms of low price, easy reagent preparation and result interpretation and if it has high sensitivity and specificity. However, histological examination should be performed where found essential to detect level of activity and any mitotic changes. The main disadvantage of the RUT is due to the low stability of the reagent. Usually the reagent may give false positive result after two weeks. This could be due to change in pH and contamination of the reagent by urease producing organism. This problem can be overcome by preparing the fresh reagent frequently in required amount. Further study is required for modification of reagent preparation to increase the stability.[2]

In this study, the sensitivity and specificity of RUT Kit either home-made or commercial was not found to be as expected but further studies regarding these tests should be performed for correct assessment. Other tests should be done to confirm diagnosis but RUT could be made more reliable after further study so that it can be used where histopathology is not possible.

CONCLUSIONS

H pylori are commonest cause of gastroduodenitis Histopathology is gold standard but takes several days and is expensive than home made rapid urease test. It is as sensitive and specific as commercials.

ACKNOWLEDGEMENTS

I would like to have special thanks to Kantipur hospital administration for providing me full support to conduct this study. I would like to appreciate Ms. Sita Devi Thapa who provided immense support during endoscopic procedure and collection of samples.

REFERENCES

1. Makaju RK, Tamamd MD, Sharma Y, Sharma N, Koju R and Ashraf M. Prevalence of Helicobacter pylori in Dhulikhel hospital, Kathmandu University Teaching Hospital: A retrospective histopathological study. Kathmandu University Medical Journal 2005; 3(4): 355-359.
2. Shakya S, Dhungel S, Chokani R, Sharma S, Chaudhary CK, Shrestha DR. Association of Helicobacter pylori with duodenal ulcer, gastric ulcer and gastritis in suburban patients attending NMCTH-a preliminary study of dyspeptic patients. Nepal Med Collage J 2001; 3:1-4.
3. Goodwin CS, Armstrong JA, Chilvers T, Peters M, Colins MD, Sly L, McConnell W and Harper WE, et al. Transfer of Campylobacter pylori and Campylobacter mustelae to Helicobacter gen. nov. as helicobacter pylori comb. Nov. and Helicobacter mustelae comb. nov. respectively. Int. J. Syst. Bacteriol 1989; 39:397-405.
4. Marshall BJ, Barrett L, Prakash C. Urease Products Campylobacter pylori from The Bactericidal Effects of Acid. Gastroenterol 1990; 697-702.
5. Gunzaman EM, Nevermann KS, Maria L,Echandi A. Comparative study of urease tests for Helicobacter pylori detection in gastric biopsies. Rev Biomed 1999;10:145-51.
6. Kawasaki M, Kawasaki T and Ogaki T. Seroprevalence of Helicobacter pylori infection in relation to the level of education in Hilly and Suburban Villages in Nepal, Journal of health science 1998; 20,127-132,1998-03-16.
7. Subedi S. Study of Prevalence of Helicobacter pylori in Gastro duodenal Diseases and Evaluation of Antibiotic Sensitivity Pattern of The Isolates (NMTCH and TUTH) Kathmandu. A M.Sc. dissertation submitted to Central Department of Microbiology, Tribhuvan University, 2001.
8. Rai SK, Shah RD, Bhattachan CL, Rai CK, Ishiyama S, Kurokawa M, et al. Helicobacter pylori associated gastroduodenal problem among the Nepalese. Nepal Medical Colledge journal 2006; 8(1):9-13.
9. Adhikari S. Prevalence of Helicobacter pylori among dyspeptic patients attending Bir Hospital, Nepal. A M.Sc. dissertation submitted to Central Department of Microbiology, Tribhuvan University, 2008.
10. Shah RD, Sherchan S, Chaudhary JK. Prevalence of Campylobacter pylori Infection In Peptic Ulcer Disease. J Nep Med Assoc 1990; 28 :208-11.
11. Larson S, Sherchon JB, Shrestha. MP. Preliminary studies on the presence of serum antibodies to Helicobacter pylori in different groups of patients in Nepal- J inst Med (Nepal) 1992;14:36-44.
12. Dangol A. Determination of Prevalence of Helicobacter pylori Infection Among Patients Attending Endoscopy Unit of Tribhuvan University Teaching Hospital and Antibiotic Susceptibility of The Isolates to Contemporary Antibiotics. A M.Sc. dissertation submitted to Central Department of Microbiology, Tribhuvan University, 2011.
13. Marasini DN. Comparative Study of Rapid Urease Test and Serum Antibody Test for The Laboratory Diagnosis of Helicobacter pylori in dyspeptic patients. A M.Sc. dissertation submitted to Kathmandu College of Science and Technology, Tribhuvan University 2010.
14. Karki BB, Pandey PR, Basnet BK, Devkota A. A Comparative Study between Rapid Biopsy Urease Test and Histopathological Examination of Helicobacter pylori in Antral Gastritis. PMJN, 2009; vol 9; no.1.
15. Nawapon M, Siripermpool P, Jatwattanakul T and Chaunthongkum S. The prevalence of Helicobacter pylori infection in patients with gastrointestinal symptoms in Chon Buri, Thailand, South-east Asian J Trop Med Public Health 2005; 36(2):341-346.
16. Yakoob J, Jafri W, Abid S, Jafri N, Abbas Z . Role of rapid urease test and histopathology in the diagnosis of Helicobacter pylori infection in a developing country. BMC Gastroenterol . 2005; l5:38.
17. Gisbert JP and Abraira V. Accuracy of Helicobacter pylori diagnostic tests in patients with bleeding peptic ulcer: a systematic review and meta-analysis. Am J Gastroenterol 2006; 101(4):848-63.
18. Tucci A, Tucci P, Bisceglia M, Marchegiani A, Papadopoli G. Real time detection of Helicobacter pylori infection and atrophic gastritis: comparision between conventional methods and a novel device for gastric juice analysis during endoscopy. Endoscopy 2005; 37(10):966-76.
19. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than Helicobacter pylori in human gastric juice and mucosa. Am J Gastroenterol 2006; 101(8):1756-61.
20. Laine L, Suchower L, Johnson E, Ronca P, and Neil G. Accuracy of CLO test after helicobacter therapy. Gastrointest Endosc 1998; 47(3):250-3.
21. Vakil M, Mark A. How to test Helicobacter pylori. Cleveland Clin J of Med (Suppl) 2005; 72:8-13.
22. Chakraborty P. A textbook of Microbiology 2nd Edition. Kolkata India: New central book agency (P) Ltd.; 2003; 369-71.
23. Goh KL, Cheah PL, Navaratnam P, Chin SC, Xiao SD. Rapid urease test: a new ultra-rapid biopsy urease test for the diagnosis of Helicobacter pylori infection. J Dig Dis. 2007Aug; 8(3):139-42.