

EVALUATION OF THE MOTILITY INDOLE UREASE (MIU) TEST TO DETECT *HELICOBACTER PYLORI* INFECTION

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Abstract. The aim of this study was to evaluate the diagnostic value of the new motility indole urease (MIU) test and Campylobacter Like Organism (CLO) test with culture as the gold standard. This study were done on 225 biopsy samples from gastric antra taken from chronic dyspeptic patients from several hospitals in Jakarta. On the MIU test, the biopsy tissue sample was submerged in the MIU tube agar to a depth of about 2/3 from the surface, and incubated at room temperature. Another piece of biopsy tissue was used for CLO and cultured. The CLO and MIU tests were considered positive if the color changed from yellow to red, and were considered negative if there was no color change within 24 hours. The CLO test compared with culture had 75.9% sensitivity, 78.6% specificity, 76.6% positive predictive value and 78% negative predictive value, whereas the result of the MIU test compared with the culture method showed 96.3% sensitivity, 82.1% specificity, 83.2% positive predictive value, and 96% negative predictive value. The MIU test with its higher sensitivity and specificity may be used as an alternative diagnostic method for *H. pylori* infection.

INTRODUCTION

Helicobacter pylori is a gram-negative microorganism with a U or S form. This microorganism often causes chronic bacterial infection in humans and infects almost half the world's population. The habitat of this bacterium is the human gastrointestinal tract. *H. pylori* infection is the main cause of active chronic gastritis, gastric ulcers, and duodenal ulcers (Graham, 1991; Marshall, 1996). It has been associated with increased risk of gastric cancer (Nomura and Stemmerman, 1993; Goodwin, 1997; Watanabe *et al*, 1998; Uemura *et al*, 2001).

The prevalence of *H. pylori* infection is quite high in both developed countries and in developing countries, like Indonesia. The

prevalence of *H. pylori* infection in developed countries is around 40-50%. In developing countries, where the infection may occur at a young age, the prevalence may be up to 80% (Taylor and Blaser, 1991; Ching, 1995; Asaka, 1996). The high cost of therapy has a negative impact on the individual and the community by decreasing financial resources. A simple, low cost detection method for *H. pylori* infection which has a good sensitivity and specificity is needed.

Generally, people who suffer from *H. pylori* infection do not have any specific symptoms. Therefore, laboratory testing is important in making the diagnosis of *H. pylori* infection. One widely used method is the urease test which is done using the CLO kit. However, it often gives false negative results and is expensive. Another method to test the urease enzyme of *H. pylori* is the MIU technique, which is cheaper and easier.

The aim of this study was to assess the sensitivity, specificity, positive and negative prediction values of the MIU and CLO tests

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compared with the culture method.

MATERIALS AND METHODS

Biopsy samples of the gastric antrum and corpus were taken in 225 patients (110 women, 115 men; mean age 35 years, range 20-70 years old) complaining of chronic dyspepsia, who visited the endoscopy unit and agreed to the study after giving informed consent. The samples were collected from several private and general hospitals in Jakarta. Four antrum biopsy samples from different locations were taken from each patient, 2 samples for the CLO and MIU test, and another 2 for the culture.

Two antrum biopsy samples were aseptically cut into small pieces and cultured in sulfur glycolate broth, on Brucella blood agar, and DENT blood agar. Cultures were incubated microaerobically at 37°C for 3-5 days in an anaerobic jar using a "Campylobacter" BR 56 (Oxoid) kit containing BR 46 catalyst. Small translucent colonies grown on Brucella blood agar were presumed to be *H. pylori* colonies. On DENT blood agar the *H. pylori* colonies had a grey color. Confirmation were made by microscopic observation with Gram stain and biochemical tests. Observation of curved or spiral shaped gram-negative bacteria and biochemical test results for catalase, oxidase, and urease were used to confirm the

bacteria was *H. pylori*. On sulfur glycolate culture, *H. pylori* were seen as spirals or bold tail-like lines coming out of the biopsy tissue. Identification was done by microscopic observation and reculturing the tissue on Brucella blood agar. On semisolid MIU culture, a color change from yellow to red accompanied by positive motility was presumed to be *H. pylori*. Further identification was done by microscopic observation and other biochemical tests.

RESULTS

Comparing the MIU with culture gave true positive results in 104 cases, false positive in 21 cases, false negative in 4 cases and true negative in 96 cases. Based on the above, the MIU diagnostic test had a sensitivity of 96.3% specificity of 82.1%, positive predictive value of 83.2% and negative predictive value of 96%. Comparison of the CLO with culture gave true positive results in 82 cases, false positive in 25 cases, false negative in 26 cases and true negative results of 92 cases with a sensitivity of 75.9%, specificity of 78.6%, positive predictive value of 76.6% and negative predictive value of 78%. The differences between the MIU and CLO tests are shown in Table 1.

DISCUSSION

Comparing the CLO test with culture give

Table 1
Diagnostic value of MIU and CLO tests for *Helicobacter pylori*.

Tests	Culture		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Likelihood ratio (+)	Likelihood ratio (-)
	Positive (n=108)	Negative (n=117)						
MIU								
Positive	104	21	96,3	82.1	83.2	96.0	5.37	0.05
Negative	4	96						
CLO								
Positive	82	25	75,9	78,6	76,6	78.0	3.55	0.31
Negative	26	92						

a sensitivity of 75.9%. This may be because the bacteria in the biopsy tissue was present in only a small amount so the urease enzyme was not enough to react with the urea material contained in the CLO. This meant the pH did not change quickly. Thus, the 24 hour time period for reading the CLO test may have resulted in false negative results (Weiss *et al*, 1996). It is possible that if more biopsy tissue was used this factor could be overcome (Laine *et al*, 1996; Lim *et al*, 2004). Proton pump inhibitor use may also prolong the period of time required for detection due to decreased activity of the urease enzyme and motility in *H. pylori*. This slows the breakdown of urea into ammonia and bicarbonate (Tsutsui *et al*, 2000; Graham *et al*, 2004). Omeprazole, a proton pump inhibitor, selectively inhibits urease enzyme activity (Gowan *et al*, 1994; Tsuchiya *et al*, 1995, Laine *et al*, 1998). Another reason is the consumption of antibiotics, like amoxicillin, suppresses the growth of bacteria but does not eradicate it. As a consequence, the number of bacteria were fewer, but not eliminated. The bacteria may be present in other parts of the stomach, such as the corpus and fundus. Biopsies of just the antrum may give false negative results (Genta and Graham, 1994).

MIU is a semisolid medium. It contains urea, amphotericin B as an antifungal and phenol red as an indicator of pH change. The MIU test at 24 hours had a sensitivity of 96.3%, specificity of 82.1%, positive predictive value of 83.2% and negative predictive value of 96%. These are higher sensitivity and specificity values than the CLO test. This may be because MIU contains more nutrients, the concentration of urea and pH are nearer to microaerobic conditions, which is beneficial for the growth of *H. pylori* (Sjostrom and Larsson, 1996; Kuo *et al*, 2002).

According to several studies conducted in the West, the sensitivity and specificity of the rapid urease test may be influenced by several factors, such as the location of the

biopsy in the antrum, fundus and corpus of the stomach. There is patchy distribution of *H. pylori* in the antrum, fundus, and corpus. The microorganisms may be present in one biopsy specimen and absent in another. This has a significant effect on the results of the urease test (Quintana-Guzman *et al*, 1999; Misra *et al*, 2000; Tang *et al*, 2005).

MIU also contains amphotericin B which can inhibit the growth of contaminant yeast. This can reduce false positive results. Yeast may cause an alkaline environment causing the color to change from yellow to red, giving a false positive results.

In conclusion, the MIU test has advantages over the CLO test in that it can show bacterial motility, is easy to perform, has a better sensitivity and specificity, and a low cost.

REFERENCES

- Asaka M. Epidemiology of *Helicobacter pylori* infection. *Asian Med J* 1996; 39: 171-7.
- Ching CH. *Helicobacter pylori* infection a significant problem in Asia. Singapore: TheAga/Singapore Gastroenterological Meeting 1995: 6-9.
- Genta RM, Graham DY. Comparison of biopsy sites for the histopathologic diagnosis of *Helicobacter pylori* density and distribution. *Gastrointest Endosc* 1994; 40: 342-5.
- Goodwin CS. *Helicobacter pylori* gastritis, peptic ulcer, and gastric cancer: Clinical and molecular aspects. *Clin Infect Dis* 1997; 25: 1017-9.
- Gowan CC, Cover TL, Blaser MJ. The proton pump inhibitor omeprazole inhibits acid survival of *Helicobacter pylori* by a urease independent mechanism. *Gastroenterology* 1994; 107: 738-43.
- Graham DY. *Helicobacter pylori*: its epidemiology and its role in duodenal ulcer disease. *J Gastroenterol Hepatol* 1991; 6: 105-13.
- Graham DY, Opekun AR, Jogi M, *et al*. False negative urea breath tests with H₂-receptor antagonists: interactions between *Helicobacter*

- pylori* density and pH. *Helicobacter* 2004; 9: 17-27.
- Kuo CH, Wu DC, Lu CY, *et al.* The media of rapid urease test influence the diagnosis of *Helicobacter pylori*. *Hepatogastroenterology* 2002; 49: 1191-4.
- Laine L, Chun D, Stein C, El-Beblawi I, Sharma V, Chandrasoma P. The influence of size number of biopsies on rapid urease test results : a prospective evaluation. *Gastrointest Endosc* 1996; 43: 49-53.
- Laine L, Estrada R, Trujillo M, Knigge K, Fennerty MB. Effect of proton pump inhibitor therapy on diagnostic testing for *Helicobacter pylori*. *Ann Intern Med* 1998; 129: 547-50.
- Lee N, Lee TT, Fang KM. Assessment of four rapid urease test system for detection of *Helicobacter pylori* in gastric biopsy specimens. *Diag Microbiol Infect Dis* 1994; 18: 69-74.
- Lim LL, Ho YK, Ho B, Salto-Tellez M. Effect of biopsies on sensitivity and specificity of ultra-rapid urease test for detection of *Helicobacter pylori* infection. *World J Gastroenterol* 2004; 10: 1907-10.
- Marshall BJ. *Helicobacter pylori*, the etiologic agent for peptic ulcer. *JAMA SEA* 1996; 12: 28-30.
- Misra V, Misra S, Dwivedi M, Singh UP, Bhargava V, Gupta SC. A topographic study of *Helicobacter pylori* density distribution and associated gastritis. *J Gastroenterol Hepatol* 2000; 15: 737-43.
- Nomura A, Stemmermann GN. *Helicobacter pylori* and gastric cancer. *J Gastroenterol Hepatol* 1993; 8: 294-303.
- Perez G L, Olivares AZ, Cover T L, Blaser MJ. Characteristics of *Helicobacter pylori* variants selected for urease deficiency. *Infect Immun* 1992; 60: 3658-63.
- Quitana-Guzman EM, Schosinsky-Neuermann K, Arias-Echandi ML, Davidovich-Rose H. Comparative study of urease tests for *Helicobacter pylori* detection in gastric biopsies. *Rev Biomed* 1999; 10: 145-51.
- Segal ED, J. Shon J, Tomplins L S. Characterization of *Helicobacter pylori* urease mutants. *Infect Immun* 1992; 60: 1883-9.
- Sjostrom JE, Larsson H. Factors affecting growth and antibiotic susceptibility of *Helicobacter pylori*: effect of pH and urea on the survival of a wild type strain and a urease-deficient mutant. *J Med Microbiol* 1996; 44: 425-33.
- Tang CA, Wang WM, Wu DC. Comparison of the clinical feasibility of three rapid urease tests in the diagnosis of *Helicobacter pylori*. *Dig Dis Sci* 2005; 50: 449-52.
- Taylor DN, Blaser MJ. The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev* 1991; 13: 42-58.
- Tsutsui N, Taneike I, Ohara T, *et al.* A novel action of the proton pump inhibitor rabeprazole and its thioether derivative against the motility of *Helicobacter pylori*. *Antimicrob Agents Chemother* 2000; 44: 3069-73.
- Tsuchiya M, Imamura L, Park JB, Kobashi K. *Helicobacter pylori* urease inhibition by rabeprazole, a proton pump inhibitor. *Biol Pharm Bull* 1995; 18: 1053-6.
- Uemura N, Okamoto S, Yamamoto S, *et al.* *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001; 345: 784-9.
- Watanabe T, Tada M, Nagal H, *et al.* *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. *Gastroenterology* 1998; 115: 642-8.
- Weiss K, Poirier L, Laverdiere M, Ducic S. A new urea broth test to detect *Helicobacter pylori* presence in upper gastrointestinal biopsies. *Diag Microbiol Infect Dis* 1996; 24: 61-4.