Motility Indole Urease as An Alternative Diagnostic Method for Identifying *Helicobacter pylory* Infection

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ABSTRACT

Background: The prevalence of Helicobacter pylori (H. pylori) infection in the world is quite high, especially in developing countries. Usually the patient shows no specific symptoms and chronic gastritis therefore becomes chronically infected. The complication of the infection is the development of peptic ulcer, which is a predisposing factor for gastric carcinoma. Early diagnosis is an important step to avoid these complications by providing immediate accurate therapy.

Methods: In this study, the CLO, MIU (Motility Indole Urease) tests and culture were conducted on 131 biopsy samples of the stomach antrum mucous tissue taken from chronic dyspepsia patients from several hospitals in Jakarta. In the CLO test, biopsy tissue was put in a small well agar to be incubated at room temperature. In the MIU test the biopsy tissue sample was submerged in the small MIU tube agar with a depth of approximately 2/3 rds from the surface, and then incubated at room temperature. Another piece of biopsy tissue was cultured micro-aerophylically. The CLO and MIU tests are considered positive if the color changes from yellow to red, and are considered negative if there is no color change within 24 hours.

Results: Compared to culture, the CLO test demonstrated 38% sensitivity, 96% specificity, 94% positive predictive value and 52% negative predictive value, whereas the results of the MIU test against culture method showed 76% sensitivity, 89% specificity, 88% positive predictive value, and 78% negative predictive value.

Conclusion: The MIU test that showed high sensitivity and specificity, and thus could be further developed as an alternative diagnostic method for H. pylori infection.

***Key Words**: MIU,CLO, H. pylori infection*

INTRODUCTION

Helicobacter pylori (*H.pylori*) is a microorganism that often causes chronic bacterial infection in humans and infects almost half of the world population. The habitat of this bacteria is the human stomach, and therefore the *H. pylori* infection is the main cause of active chronic gastritis, gastric ulcer, and duodenal ulcer[1,2,3,4.]. Recently, it has been proven to be the highest risk factor in stomach cancer[5,6,7,8].

The prevalence of *H. pylori* infection is quite high, in developed countries as well as in developing countries, such as Indonesia. The prevalence of *H. pylori* infection in developed countries is around 40-50%, whereas in developing countries, where the infection occurs from a very young age, the prevalence can reach up to 80%[9,10]. Several complications that can be caused by this infection are: duodenal ulcer, gastric ulcer and gastric carcinoma. The negative impact of this infection on the individual and the community is a deterioration of the quality of human resources and high cost of therapy. For this reason, there is a need for a simple detection method to identify *H. pylori* infection, which can provide high specificity and sensitivity at low cost.

Generally, people who suffer from *H. pylori* infection do not show any specific symptoms. They are sometimes asymptomatic, leading to a chronic disease. Therefore, laboratory procedures are essential to establish the diagnosis of *H. pylori* infection. A widely used method

is the urease test, which is performed using the Campylobacter-Like Organism (CLO) kit. However, it often results in a false negative, and is quite expensive. Another method is by testing the urease enzyme of the *H. pylori*, otherwise known as the Motility Indol Urease (MIU) technique, which was also used in this study.

The aim of this study is to assess the sensitivity, specificity, positive and negative prediction values of the MIU and CLO tests against the culture method as the gold standard.

MATERIAL AND METHODS

Biopsy samples of the gastric antrum were taken from 131 patients with chronic dispepsia who visited the endoscopy department and informedly consented. Samples were collected from several private and general hospitals in Jakarta. Four antrum biopsy tissues of different locations were taken from each patient: 2 tissues for the CLO test and another 2 for culture and the MIU test.

Isolation and Identification

Each antrum biopsy tissue were aseptically cut into 4 pieces. Each piece of tissue was grown in Thioglikolate Broth, MIU, Brucella Blood Agar and DENT Blood Agar. Cultures were incubated micro-aerophylically at 37^{o}C for 3-7 days in an anaerobic jar with a "Campylobacter" BR 56 (Oxoid) kit containing a BR 46 catalyst.

Small translucent colonies grown on Brucella Blood Agar were presumed as the *H. pylori* colonies. On the DENT Blood Agar, the color of the colony was gray. Identification was confirmed through microscopic observation using Gram stain and biochemical tests. Identification of a curved or spiral negative Gram bacteria under the microscope and positive catalase, oxidase and urease biochemical tests confirmed that the bacteria were *H. pylori*.

In sulfur glycolate culture, *H. pylori* are seen as spiral or bold line tail-like shapes coming out of the the biopsy tissue. Identification was performed using the microscope and reculturization of the tissue on a Brucella blood agar. In a semisolid MIU culture, a color change from yellow to red accompanied by positive motility were presumably caused by *H. pylori*. Further identification was conducted using a microscopic and other biochemical tests.

RESULTS

Distribution of *H. pylori* Based on Clinical Report

Out of 131 dyspepsia patients, 107 cases were non-ulcer dyspepsia, 61 of which positive for *H. pylori* from the culture, 18 cases were gastric ulcer, 17 of which were positive for *H. pylori* from the culture; 6 cases were duodenal ulcer, all of which were positive for *H. pylori* from the culture (Table 1).

Table 1. Distribution of *H. pylori* based on clinical report

Clinical Report	*H. pylori* Positive	*H. pylori* Negative	Total
Non-ulcer dyspepsia	61 (57%)	46 (43%)	107 (100%)
Gastric ulcer	17 (94%)	1 (6%)	18 (100%)
Duodenal ulcer	6 (100%)	0 (0%)	6 (100%)

Comparison Between CLO Test and Culture

Compared to the tissue culture, the CLO test demonstrated 38% sensitivity, 96% specificity, 94% positive prediction value and 52% negative prediction value. Marginal Chi-square was 41.405, with a df of 1, and $P < 0.05$. The result can be found in Table 2.

Table 2. Comparison between CLO test and culture

		Culture		Total
		+	-	
CLO	+	29	2	31
	-	48	52	100
Total		77	54	131

$P < 0,05$

Sensitivity: 38 % Positive prediction value: 94 %
Specificity: 96 % Negative prediction value: 52 %

MIU Test Result Against Culture

Comparison of the MIU test result against culture as "Gold Standard" provided 76% sensitivity, 89% specificity, 88% positive prediction value and 78% negative prediction value (Table 3). Marginal Chi-square was 41.405, with a df of 1, and $P < 0.05$.

Table 3. Comparison between MIU test and culture

		Culture		Total
		+	-	
MIU	+	51	7	58
	-	16	57	73
Total		67	64	131

$p > 0,05$

Sensitivity: 76 % Positive prediction value: 88 %
Specificity: 89 % Negative prediction value: 78 %

DISCUSSION

CLO Test Result Compared to Culture

Compared to culture, the sensitivity of the CLO test result was a very low 38%, although the specificity was a relatively high 96%. The low sensitivity of the CLO test could be due to an inadequate number of bacteria in the biopsy tissue. Thus, the urease enzyme yield was insufficient to react with urea within a short time. The urease enzyme requires a longer time to be able to react with the urea, and therefore evaluation within 24 hours using the CLO test often results in a false negative.[11] Another possible reason may be the effect of chemical or long term medication, which reduce urease activity in *H. pylori*, inhibiting breakdown of urea into ammonia and bicarbonate.[12,13] A medicine that can influence urease activity and motility of *H. pylori* is the "proton pump inhibitor", which is usually used to suppress the growth of *H. pylori*.[14] According to Gowan, Omeprazol, a medicine of "Proton pump inhibitor" group, selectively works to inhibit urease activity.[15,16]

Other possible cause of the low sensitivity of the CLO test was the patient's mistake of taking inappropriate doses of antimicrobial drug, causing suppression, but not eradication of bacterial growth. Thus, the drug only reduces the number of bacteria, but allows it to move to other parts of the stomach, such as the corpus area. It seems therefore that collection of biopsy tissue sample from the antrum may only result in a false negative.[17]

Comparation of the MIU Test Result and Culture

Urease test using MIU culture within 24 hours resulted in 76% sensitivity, 89% specificity, 88% positive prediction value and 78% negative prediction value. Compared to the CLO test, the result of MIU showed higher sensitivity and specificity. This might be due to the nutrient contained in the MIU medium and the growth environment, such as the pH and micro-aerophylic condition that were more suitable to promote *H. pylori* growth. Several previous studies in Western countries showed that sensitivity and specificity of the urease rapid test could be influenced by several factors, such as whether the incision area of the biopsy tissue was at the antrum, fundus, and mucosal corpus of the stomach. This is because the number of bacteria found at the antrum, fundus, and corpus could create a positive urease test. Furthermore, urea concentration, nutrient availability and pH of the medium used for the test would also influence the sensitivity and specificity of the urea test[17,18,19] .

An advantage of the MIU test is that it can show bacteria motility for a relatively low cost.

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