The Result Discrepancies between Histological and PCR Method for Detecting *Helicobacter pylori* in Patients with Dyspepsia due to Inappropriate Preparation before Endoscopy

Maruni Wiwin Diarti, Haris Widita**, Soewignjo Soemohardjo*, Weny Astuti**, Troef Sumarno***, Yunan Jiwintarum*****, Zainul Mutaqin*, Retno Handayani *****

*Biomedical Research Unit, Mataram General Hospital, Mataram
**Department of Internal Medicine, Mataram General Hospital, Mataram
***Department of Pathology, Faculty of Medicine, University of Airlangga, Surabaya
****Department of Biochemistry, Faculty of Medicine, University of Airlangga, Surabaya
*****Department of Laboratory Technology, Institute of Health Technology, Mataram

ABSTRACT

***Background**: False negative result of Helicobacter pylori (H. pylori) detection in gastric tissue can be due to inappropriate preparation before endoscopy. The objectives of this study is to compare the result of H. pylori detection in gastric biopsy by histological method and ure C polymerase chain reaction (PCR) in patients with dyspepsia who underwent upper gastrointestinal (GI) endoscopy without preparations other than six hours fasting before endoscopy.*

***Method:** We obtained 156 paraffin blocks of gastric endoscopic biopsy samples, taken from antrum and corpus of patients with dyspepsia who underwent endoscopic examination at the Endoscopy Unit of Biomedika hospital, Mataram. All biopsy samples were stained with Hematoxylin and Eosin for tissue diagnosis and Giemsa stain for detecting H. pylori Ure C PCR were done on all blocks. Cag PCR were performed on all Ure C PCR positive samples.*

***Results:** Of 156 paraffin blocks, only 17 blocks (10.9%) were positive for H. pylori by histological examination. All of the 17 samples showed positive results on PCR method. Of 156 paraffin blocks, positive results were found in 73 patients (45.9%) by ure C PCR method. The PCR method has increased the positivity rates of H. pylori more than four times compared to histological method. This study showed that the rate of cag a was 63.0%.*

***Conclusion:** Ure C PCR is superior to histological examination in patients who did not stop consuming acid supressor drug and antibiotic two weeks prior to endoscopy. This phenomenon can be explained by the change of spiral form into coccoid form of H. pylori, which is hardly detected using Giemsa stain.*

***Keywords:** Helicobacter pylori, histology, ureC, Cag a, PCR*

INTRODUCTION

One of the important methods for detecting *Helicobacter pylori* (*H. pylori*) of endoscopic gastric biopsy sample is the histological method. Giemsa is the most popular stain used since it is very simple, easy, and cheap. It has been known for a long time that histological method is sensitive and specific for detecting of *H pylori* in gastric tissue. However, histological detection would be difficult if the bacterial content of the specimen is low, and particularly, it becomes very difficult if the bacteria is in a cocoid form.[1,2] The expertise of the pathologist is a very crucial factor and histological method is also considered to be relatively subjective. In recent years,

Correspondence:
Soewignjo Soemohardjo
Biomedical Research Unit
Mataram General Hospital
Jl. Pejanggik No. 6 Mataram Indonesia
Phone/Fax: +62-370-638329
E-mail: klinikbiomedika@yahoo.co.id

PCR method has become popular for detecting *H. pylori*. PCR using ure C (glmM) gene fragment as primers has been proven to be the most specific and sensitive method compared to PCR using other primers such as *ure A* and *ure B*.[3,4,5,6] The superiority of PCR method over histologal method for detecting *H. pylori* has been reported by Cesar et al.[7]

Although most endoscopist realize the importance of appropriate preparation before endoscopy for detecting of *H. pylori* such as stopping acid suppressor drugs and antibiotics at least two weeks before endoscopy. In order to avoid false negative result, a test based on urease activity such as CLO (rapid urease testing) will be performed. However, in daily practice, it is very difficult to do such preparation since the patient cannot wait for two weeks and they want prompt endoscopic examination and prompt relief of the symptoms. Such situation might also affect the diagnosis of *H. pylori* by using histological method.

The objectives of this study is to compare the result of *H. pylori* detection in gastric biopsy by histological method and ure C polymerase chain reaction (PCR) in patients with dyspepsia who underwent upper GI endoscopy without preparations other than 6 hours fasting before endoscopy.

METHOD

There were 177 upper GI endoscopies in patients with dyspepsia at Endoscopy Unit of Biomedika Hospital Mataram since January to December 2007. Endoscopic gastric biopsy was taken from antrum and corpus of the stomach in all patients. There was no special preparations before endoscopy except six hours fasting before the procedure. The patients were not told to stop any medications including either acid suppression drugs or antibiotics. Of 177 paraffin blocks, only 156 blocks were sufficient for this study.

All of the biopsy samples were stained with hematoxylin and eosin for tissue diagnosis and Giemsa stain for *H. pylori* detection.[1,2] Ure C PCR was performed according to method described by Lu et al.[5] Cag PCR was conducting using primer and method as described by David et al.[6] Histological examination was done by an experienced pathologist. Of originally 177 paraffin blocks, 23 (12.99%) blocks showed positive *H. pylori* and 154 were negative by histological examination. There only 156 blocks available for this study including 17 blocks that were positive and 139 blocks that were negative *H. pylori* by histological examination. Ure C PCR (glmM) were performed on blocks with *H. pylori* positive and negative results. Cag PCR was done on all Ure C PCR positive samples.

RESULTS

The sample consisted of paraffin blocks of 100 male and 56 female patients aged 15 years to 88 years. All of the patients had dyspepsia complaint for more than 6 months. Table 1 showed endoscopic diagnosis of the cases and the result of *H. pylori* diagnosis by histological and PCR method. Figure 1 showed the electrophoresis bands of *H. pylori*-positive by Ure C PCR (glmM).

Of 17 blocks with *H. pylori*-positive by histological method, all were also *H. pylori*-positive by PCR (100%). Of 139 blocks with negative result for *H. pylori* detection by histological method, 56 (40.7%) showed positive results by PCR method. Of 17 blocks with *H. pylori*-positive results by histological and PCR method, 14 were positive for cag a (82.38%); moreover, of 56 samples with positive PCR but negative results of histological method, 32 were positive for cag a (57.14%). Hence, of total 156 patients with dyspepsia, 73 patients were positive for *H. pylori* by histological or PCR (46.8%).

Table 1 shows that regarding the results of histological method, there are only minority of patient with gastric ulcer and duodenal ulcer who also show *H. pylori*-positive result. In contrast, by using PCR method, the positive rate is much higher (around 50%).

Table 1. Result of histological method for *H. pylori*

Endoscopic diagnosis	Total number of patient	Number of patient *H. pylori*-positive by histological method
Gastric ulcer	27	0
Duodenal ulcer	12	1 (8.3%)
Chronic gastritis	2	1 (50.0%)
Antral gastritis	98	13 (13.2%)
Acute gastritis	2	0
Atrophic gastritis	0	0
Pan gastritis	0	0
Gastric malignancy	12	2 (16.7%)
Normal endoscopy	3	0
Total	**156**	**17 (10.9%)**

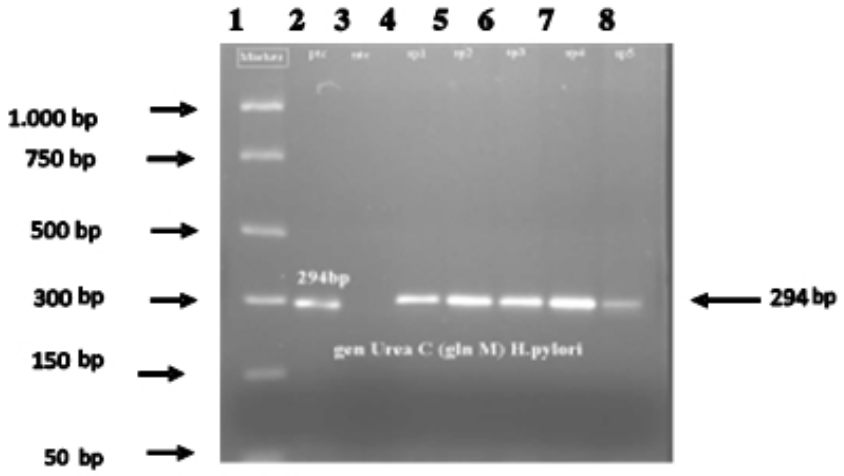

Figure 1. Showed *H. pylori* positive by PCR ure C (glmM)
Note: (1) G316A Marker; (2) Negative control ATCC 53726 *H. pylori* 294 bp; (3) Negative control (aquadest); (4) Sample B88 positive for *H. pylori* (294 bp); (5) Sample B99 positive for *H. pylori* (294 bp); (6) Sample B104 positive for *H. pylori* (294 bp); (7) Sample B112 positive for *H. pylori* (294 bp); (8) Sample B118 positive for *H. pylori* (294 bp)

Table 2. Result of histological and PCR method for *H. pylori* detection

Endoscopic diagnosis	Number of patient (%)		Total
	H. pylori-positive by PCR or histological method	*H. pylori*-negative by PCR and histological method	
Gastric ulcer	13 (48.1%)	14 (51.9%)	27
Duodenal ulcer	6 (50.0%)	6 (50.0%)	12
Chronic gastritis	1 (50.0%)	1 (50.0%)	2
Antral gastritis	45 (45.9%)	53 (54.1%)	98
Acute gastritis	1 (50%)	1 (50.0%)	2
Atrophic gastritis	0	0	0
Pan gastritis	0	0	0
Gastric malignancy	5 (41.7%)	7 (58.3%)	12
Normal endoscopy	2 (66.7%)	1 (33.3%)	3
Total	73 (46.8%)	83 (53.2%)	156

Table 3. Parafin blocks available for the study obtained from patients with dyspepsia

Sex	Number of patient (%)		Total
	H. pylori-positive by histological method or PCR	*H. pylori*-negative by histological method and PCR	
Male	53 (53.0%)	47 (47.0%)	100
Female	20 (35.7%)	36 (64.3%)	56
Total	73 (46.8%)	83 (53.2%)	156

Table 4. Parafin blocks w*ith H. pylori*-positive by histological method and PCR

Sex	Number of patient (%)		Total
	H. pylori-positive by histology	*H. pylori*-negative by histological method, but positive by PCR	
Male	14 (26.4%)	39 (73.6%)	53
Female	3 (15.0%)	17 (85.0%)	20
Total	17 (23.3%)	56 (76.7%)	73

Using Chi-square analysis with Fisher's test with 95% confidence interval, we found that the *H. pylori*-positive rate of male is significantly higher compared to female patients ($p < 0.05$).

Table 4 indicated that the rate of cag a in blocks with Ure C PCR positive was 63.01%. The difference of cag a positivity between the group with *H. pylori*-positive by histological method and the group with

Tabel 5. The presence of cag a in patients with *H. pylori* infection

H. pylori status	Number of patient (%)		
	cag a - positive (PCR)	cag a - negative (PCR)	Total
Histological method positive; PCR positive	14 (82.4%)	3 (17.6%)	17 (100%)
Histological method negative; PCR positive	32 (57.1%)	24 (42.9%)	56 (100%)
Total	46 (63.0%)	27 (37.0%)	73 (100%)

H. pylori-negative by histological method but positive by PCR were analyzed by using T-paired test samples with 95% confidence interval and p = 0.05. According to the test, the difference of cag a positivity between the both groups was markedly significant (p = 0.000).

This study shows that *H. pylori* in the biopsy specimens with positive results by histological method has a higher rate of cag-a-positive compared to the specimens of *H. pylori*-positive by PCR, but negative by histological method. The reasons for this phenomenom has not been known, but it might be due to the higher *H. pylori* DNA content in the specimens with positive results in histological method.

DISCUSSION

The result of this study, i.e. 45.9% of patients with dyspepsia was *H. pylori*-positive by Ure C PCR is almost similar to the results of the study by Soemohardjo et al.[8] Soemohardjo et al reported that 41.13% of patients with dyspepsia in Mataram General hospital showed *H. pylori*-positive results by using PCR ureC. Arinton in his dessertation reported that only 2% out of 104 patients with dyspepsia showed positive *H. pylori* infection by Ure C PCR; in contrast to the infection rate of 36.5% by Ure C PCR.[9] The positive rate of *H. pylori* infection done by a PCR method was as high as 45.9%; while there was only 10.9% by histological method. It is really astonishing fact and there must be a reason for this large discrepancy.

Several studies showed that histological diagnosis of *H.pylori* infection is accurate with high specificity and sensitivity. But it should be remembered that the histological detection of *H. pylori* depends on the appearance of spiral bacteria which is commonly seen near the area of gastric inflammation.[1,2] The spiral bacteria can be seen abundantly for *H. pylori* infection in patients who didn't take antibiotics or acid suppressant for a long time.[10,11] It is recommended that the patient should not take antibiotic and acid suppressant drugs at least for two weeks period before endoscopy to avoid false negative result. However, it becomes difficult since it has been known that there are only several patients with dyspepsia who can stop acids suppressant drugs for two weeks.

The histological diagnosis for *H. pylori* infection is difficult if the bacterial content of the sample is low and the spiral bacteria can be seen only after a time-consuming search and sometimes, it cannot be detected. The procedure become very difficult since under some stress condition, the bacteria will change it appearance from spiral form into curved form or cocoid form. In that case, the immunohistochemical staining is needed. However, such staining is costly and complicated. Reports showed that in patients with *H. pylori* infections who took proton pump inhibitors (PPI) for a long time, the diagnosis which is based on the presence of urease enzymatic activity such as urea breath test (UBT) or CLO test will show false negative results. The urease enzymatic activity reappears several days after PPI consumption has been stopped.[10] The reason of this phenomenon has not been known. Jekti et al reported a study that demonstrated spiral form of *H. pylori*, which was followed up prospectively under condition of several stress - such as prolonged incubation without new nutrient supplementation, aerobiosis, and with the addition of amoxicillin. The study clearly showed that the spiral form change to coccoid form with the stress. In all stages of coccoid development, the urease enzymatic activity was negative.[12] The change of *H. pylori* into coccoid form may explain the negative results of *H. pylori* by histological method and also the false negative result of UBT. A very interesting study was reported by Renz et al in 2000.[11] They reported 35 patients with chronic gastritis who underwent endoscopic examination. The diagnosis of *H. pylori* infection was determined by CLO test, culture, antibody, and Ure C PCR, and histological method. Fifteen patients were positive for CLO test and culture. All patients showed numerous helical forms of *H. pylori*. Nine subjects indicated CLO-negative, culture-negative, and positive-PCR results. Histological method in these subjects showed cocoid forms, or mixed cocoid form with scant helical form, or mixed cocoid form with helical form in the equal proportion.

This study shows that in the diagnosis of *H. pylori* infection by using Ure C PCR is superior to histological diagnostic; especially, in patients without good preparation before endoscopy. In the future, PCR may be used routinely for detecting *H. pylori* in endoscopic

biopsy sample since it can routinely detect all form of *H. pylori* including the coccoid form.

The accuracy of histological method is less effective compared to PCR method due to high false negative result in patients without appropriate preporation prior to endoscopy.

CONCLUSION

We conclude that there is marked discrepancy between the results of *H. pylori* detection by histological method and PCR in patients with dyspepsia without appropriate preparation before endoscopy. Only minority of patients show positive result when we use histological method; however, while using PCR, the positive rate is much higher. It means that histological method cannot be used in this situation when appropriate preparation before endoscopy is not done, and it should be replaced by PCR. The reason of this finding is that majority of patients with dyspepsia might be still taking acid suppressor drug or even antibiotics before endoscopy. This situation may cause the change of spiral form into coccoid form that decrease the possibility of histological detection of *H. pylori*, while PCR can detect the genome of both spiral and coccoid form of *H. pylori.*

REFERENCES

1. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of *Helicobacter pylori*: comparison of staining methods. J Clin Pathol 2000;53:756-59.
2. Logan RPH, Walker MM. Epidemiology and diagnosis of *Helicobacter pylori* infection [Clinical Review]. BMJ 2000;323:920-22.
3. De Reuse H, Labigne A, Mengin-Lecreulx D. The *Helicobacter pylori* ureC gene codes for a phosphoglucosamine mutase. J Bacteriol 1997;179:11:3488-93.
4. Bickley J, Owen RJ, Fraser AG, Pounder E. Evaluation of the polymerase chain reaction for detection for detecting the urease C gene of *Helicobacter pylori* in gastric biopsy samples and dental plague. J Med Microbiol 1993;39:338-44.
5. Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q, Chong SKF, Lee CH. Comparison of five PCR methods for detection of *Helicobacter pylori* DNA in gastric tissue. J Clin Microbiol 1999;37:772-4.
6. David E, Canavez FC, Carvalho CM, Miteldorf CATZ, Lopes LHC, Leite KMR. *Helicobacter pylori* and cag a gene detected by polymerase chain reaction in gastric biopsi: correlation with histological findings, proliferation, and apoptosis. Sao Paulo Med J 2005;123:113-8.
7. Cesar ACB, Cury PM, Payao SLM, Liberatore PR, Silva AE. Comparison of histological and molecular diagnosis of *Helicobacter pylori* in benign lessions and gastric adeno-carcinoma. Brazilian J Microbiol 2005;36:12-6.
8. Soemohardjo S, Palgunadi I, Gunawan S, Muttaqin Z, Widita H, Achwan W. The detection of *Helicobacter pylori* in gastric mucosal biopsy specimen used PCR ureC in dyspepsia patient. Indones J Gastroenterol Hepatol Dig Endosc 2007;8:40-3.
9. Arinton IG. Level of serum gastrin and ratio pepsinogen I to pepsinogen II as a biomarker of chronic gastritis due to *Helicobacter pylori* [Dissertation]. University of Diponegoro Semarang 2008.
10. Gatta L, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, Miglioli M, Vaira D. Effect of proton pump inhibitors and antacid therapy on ^{13}C urea breath tests and stool test for *Helicobacter pylori* infection. Am J Gastroenterol 2004;99:823-9.
11. Ren Z, Pang G, Batey R, Routiey D, Russeli A, Musicka M, Dunkley M, Beagley K, Ciancy R. Non–urease producing *Helicobacter pylori* in chronic gastritis. Aust NZJ Med 2000;30:578-84.
12. Jekti D, Soemohardjo S, Muttaqin Z. The Absence of urease enzymatic activity of *Helicobacter pylori* coccoid form. Indones J Gastroenterol Hepatol Dig Endos 2008;9:35-41.