The Detection of *Helicobacter pylori* in Gastric Mucosal Biopsy Specimens by PCR Using Primers Derived from UreC Gene in Patients with Dyspepsia

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ABSTRACT

***Background**: The detection of Helicobacter pylori (H. pylori) in gastric biopsy specimens can be done using CLO (Campylobacter Like Organism) test and histopatological examination, but the sensitivity of both method is influenced by the density of the bacteria in the sample. Beside that, the coccoid form is detected with difficulty by histology and need immunohistochemical stain to confirm. PCR can be used for the detection of both spiral and coccoid form of the bacteria.*

***Objective**: To detect the genome of H. pylori by Polymerase Chain Reaction (PCR) using primers derived from ureC gene of the bacteria in gastric biopsy specimen from patients with dyspepsia.*

***Methods**: Gastric biopsy specimen from 179 patients with dyspepsia in the endoscopic unit Mataram hospital. The biopsy was taken from antrum and corpus and put into sterile saline for the culture of H. pylori and put into 70% ethanol solution for the PCR. The specimen for bacterial culture was carried soon to microbiology laboratory and plated into the appropriate media and grown in microaerophilic condition in CO_2 incubator. The PCR was done using primers derived from ureC.*

***Result**: The H. pylori genome was detected in 79 of 179 biopsy sample (44.13%). The bacterial culture was positive for H. pylori in 22 (12%). The PCR result was positive in 10/35 of patient with normal endoscopy (28.57%). From 22 patients with duodenal ulcer without gastric ulcer the PCR was positive in 15 (68.18%). In patient with gastric ulcer without duodenal ulcer the PCR was positive in 9 patients (42.08%). From 7 patient with combined gastric and duodenal ulcer the PCR was positive in 5 (71.43%), in 3 patient with gastric cancer the PCR was positive in 1 (33.33%).*

***Conclusion**: The study showed that 44.13% of patient with dyspepsia in Mataram hospital was positive for H. pylori by PCR.*

***Keywords**: detection of Helicobacter pylori, gastric mucosal biopsy specimen, polymerase chain reaction, ureC gene*

INTRODUCTION

The diagnosis of *Helicobacter pylori (H. pylori)* infection can be done invasively using endoscopy and mucosal gastric biopsy and non invasively using UBT (Urea Breath Test) or serologic detection of IgG anti-*Helicobacter pylori*. The detection of *H. pylori* in gastric biopsy specimens can be done using CLO (Campylobacter Like Organism) test and histopatological examination, but the sensitivity of both method is influenced by the density of the bacteria in the sample.[1,2] The problem is especially important in patient with long standing dyspepsia that might be treated with acid suppressing drug or antibiotic before endoscopy. In patient not stopping the antibiotic or acid suppressant drug several days before endoscopy the urease producing spiral shaped bacteria might be replaced by coccoid form that does not show urease activity and it is not detectable by CLO. Beside that, the coccoid form is detected with difficulty by histology and may need immunohistochemical stain to confirm. Polymerase Chain Reaction (PCR) can be

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used for the detection of both spiral and coccoid form of the bacteria. Recently it was found that the highest sensitivity and specificity of PCR can be achieved using primers derived from ureC gene.[3]

THE OBJECTIVE OF STUDY

To detect genome of *H. pylori* by PCR using primers derived from ureC gene of the bacteria in gastric biopsy specimen from patients with dyspepsia.

METHODS

Materials of the study were gastric biopsy specimen from 179 patients with dyspepsia in the Endoscopic Unit Mataram hospital. The patients consist of 93 male and 86 female aged between 25 to 88 years. The criteria used for dyspepsia is persistent or recurrent upper abdominal pain or discomfort supposed to be referable to the upper gastrointestinal tract.[4,5] The endoscopy is done without special preparation except fasting for 8 hours before endoscopy. The biopsy was taken from antrum and corpus and put into sterile saline for the culture of *H. pylori* and put into 70% ethanol solution for the PCR. The specimen for bacterial culture was carried soon to microbiology laboratory and picked up using sterile pincet and crushed and then swabbed on the surface of solid agar consisted of Trypticase Soy Agar (TSA) and added with Vitox (Oxoid #SR90A), Dent Supplement (Oxoid #SR 147) and 10% of fresh sheep red blood cell. The culture was incubated in microaerophilic condition (10% CO_2, 85% N_2, and 5% O_2) in CO_2 incubator for 5 days. Small, round, and transparant colony was pick up and subcultured for microscopic examination and biochemical test including urease test, catalase test and sugar test.[6]

The PCR was done using primers derived from gene ureC consisted of

5'AAGCTTTTAGGGGTGTTAGGGGTTT3'

and

5' AAGCTTACTTTCTAACACTAACGC3'.[7]

DNA extraction was done using TRI-zol kit (invitrogen) a modification of Guanidium Isothiocyanate method. First, a piece of gastric tissue was put in a tube containing 300 uL Tri-Zol solution. The tissue was minced using the tip of a pointed pincet then vortexed for 1 minute. The tube was left in room temperature for 5 minutes. Into the tube was added 80 uL chloroform. The organic phase (upper phase) was pipeted into a new tube containing 200 uL absolute ethanol. The tube was shaken and than left in 2-8^{0}C for 1 hour. The DNA was sedimented by centrifuging in 14,000 rpm for 10 minutes and washed twice using 75% ethanol. It was dried by putting in room temperature for ½ hour. DNA was suspended using 50 uL aquabidest. Amplification was done using PCR Core II System (Promega Corp) with reaction volume of 50 uL, consisted of PCR buffer, $MgC1_2$ 25 mM, dNTP mix, Primer, Taq polymerase 0.25 U and 5 uL DNA template. The amplification condition was predenaturation 94^{0}C 10 minutes, denaturation 94^{0}C 1 minute, annealing 45^{0}C 1 minute, extension 72^{0}C 3 minute. The reaction was amplified 35 cycle with the target amplification of 294 bp. Amplification was done using Amplitron I machine (thermolyne).

Analysis of PCR product was done using 2% agarose gel with Tris Borate buffer in 100 V for one hour. The observation of DNA bands was done using ethidium bromide staining and UV light. The DNA bp size was measured using Low DNA Mass Ladder (Invitrogen, USA).

RESULTS

The endoscopic appearance was normal in 35 (19.55%), gastric ulcer in 21 (11.73%), duodenal ulcer in 22 (12.29%). The endoscopic finding can be seen in table 1.

Table 1. Endoscopic finding in patients with dyspepsia

Diagnosis	Number of Patients	%
Normal	35	19.55
Gastric ulcer (without duodenal ulcer)	21	11.73
Duodenal ulcer (without gastric ulcer)	22	12.29
Gastric ulcer combined with duodenal ulcer	7	4.00
Gastric tumor	3	1.68
Erosive gastritis	16	8.94
Anthral gastritis	45	25.12
Pangastritis	2	1.12
Esophagitis	5	2.79
Esophageal varices	5	2.79
Duodenitis	11	6.15
Other findings	5	2.79

The *H. pylori* genome was detected in 79 of 179 biopsy sample (44.13%). It was positive in 46 of male (58.23 %) and in 33 of female (41.77 %). The PCR result was positive in 10/35 of patient with normal endoscopy (28.57%), 20/29 of all patients with duodenal ulcer (68.97%), 14/28 of all patients with gastric ulcer (50%). From 22 patients with duodenal ulcer without gastric ulcer the PCR was positive in 15 (68.18%). In patient with gastric ulcer without duodenal ulcer the PCR was positive in 9 patients (42.86%). From 7 patient with combined gastric and duodenal ulcer the PCR was positive in 5 (71.43%), in 3 patient with gastric cancer the PCR was positive in 1 (33.3%). The gastric cancer consisted of 1 patient with cancer of cardia with negative PCR in 2 cases of prepyloric cancer and 1 of the cases was PCR positive (50%). All 2 patients with pangastritis were

Table 2. The frequency of PCR positive in patients with each endoscopic diagnosis

Diagnosis	Number of Patient	Number of PCR (+)	%
Normal	35	10	28.57
Gastric ulcer (without duodenal ulcer)	21	9	42.86
Duodenal ulcer (without gastric ulcer)	22	15	68.18
Gastric ulcer combined with duodenal ulcer	7	5	71.43
Gastric tumor	3	1	33.33
Erosive gastritis	16	8	50.00
Anthral gastritis	45	17	37.78
Pangastritis	2	2	100.00
Esophagitis	5	2	40.00
Esophageal varices	5	2	40.00
Duodenitis	11	5	45.46
Other findings	5	3	60.00

PCR positive (100%). The frequency of PCR positive in patient with each endoscopic diagnosis can be seen in table 2.

The bacterial culture was positive for *H. pylori* in 22 (12.29%). From 22 patient with duodenal ulcer 5 patient showed positive culture of *H. pylori.*

DISCUSSION

In invasive diagnosis of *H. pylori* infection the frequently used diagnostic were CLO test and detection of *H. pylori* by histology, but it was known that both CLO and histology is affected by the density of *H. pylori* in the gastric tissue. For example, in patient taking acid suppressing drug for long time and the drug was not stopped for several days before endoscopy, the density of spiral bacteria showing urease activity decrased significantly[8] that may caused the false negative CLO test. In the past it was thought that this phenomena was caused by anti-*H. pylori* effect of the acid suppressant. Later the anti-*H. pylori* effect can be explained by the change of spiral bacteria into coccoid form and it was proven that coccoid form of *H. pylori* does not show urease activity despite intact urease genes and production of urease protein.[9] This change is not a permanent one and the coccoid form can change into spiral form again if the acid suppressant is stopped for several days. In patient with *H. pylori* infection where due to administration of acid suppressant or antibiotic the spiral form of the bacteria change to coccoid form, the detection of *H. pylori* by histology can difficult, because in the histologic detection of *H. pylori* the spiral form should be detected. The detection of coccoid form needs immunohistochemistry. Beside that it was also known that detection *H. pylori* by histology is relatively subjective and depund of the degree of expertise of the pathologist.[10,11]

This study showed the significant role of *H. pylori* infection in the development of dyspepsia in Lombok Island. The big role of *H. pylori* can be seen in the duodenal ulcer without concomitant gastric ulcer with the rate of positive PCR of 68.18%. In gastric ulcer without concomitant duodenal ulcer the rate was 42.86% much lower than duodenal ulcer. While in duodenal ulcer combined with gastric ulcer the rate of positive PCR was 71.74%. With the detection *H. pylori* by PCR we can detect spiral and coccoid form, and this is one of the superiority of PCR over CLO test and histology that can not detect the cocoid form.[12] The ureC gene is relatively conserved so that the primer derived from this gene is considered to be better compared with the other urease gene such as ureA and ureB.[3,6,13]

Some studies showed that PCR is more sensitive for the detection of *H. pylori* infection compared with histology especially in cases were the density of the bacteria is low.[14] In patient with long standing dyspepsia that might have been treated by acid suppressing drug PCR is more sensitive compared with histology or Urea Breath Test.

A very interesting fact in this study was the low rate of positive culture of *H. pylori*. The rate of positive culture was much less compared with PCR. It was known that *H. pylori* coccoid form is very difficult to be grown in vitro.

CONCLUSION

The study showed that 44.13% of patient with dyspepsia in Mataram hospital was positive for *H. pylori* by PCR. It suggests that *H. pylori* infection has a significant role in the development of dyspepsia cases in Mataram. In duodenal ulcer alone the positive rate of PCR was 68.18%, in gastric ulcer alone the rate was 42.86%, while in the combined gastric and duodenal ulcer the rate was 71.4%. In patient with functional dyspepsia the rate was 28.57%.

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