DETECTION OF THE *HELICOBACTER PYLORI* CagA GENE IN GASTRIC BIOPSIES FROM DYSPEPTIC PATIENTS USING PCR METHOD IN MATARAM GENERAL HOSPITAL, INDONESIA

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ABSTRACT

A polymerase chain reaction assay (PCR) for the diagnosis of Helicobacter pylori in human gastric biopsies from 44 dyspeptic patients was developed. From endoscopic diagnosis, the sample consisted of 4 patients with gastric cancer, 4 patients with duodenal ulcer, 3 patients with ventricular ulcer, 3 patients with both ventricular ulcer and duodenal ulcer and 31 patients with chronic gastritis. The H. pylori infection was diagnosed using nested PCR, which consisted of two primers of the urease gene fragment. From 44 specimens with urease positive, 39 specimens were positive for cagA strain (88,6%). This reflects a very high frequency of the cagA gene in the Helycobacter pylori infection. The meaning of this is still unclear. There was no significant difference in the cagA status of ulcer dyspeptic patient and non-ulcer dyspeptic patients.

***Key words**: CagA gene, Helicobacter pylori, PCR*

INTRODUCTION

Helicobacter pylori infection has broad clinical manifestations from being asymptomatic up to dyspepsia with active chronic gastritis or peptic ulcer. We also find severe symptoms such gastric cancer. Previous studies showed that most of *H. pylori* infection appear without any symptoms, although biopsies of the gastric mucosa revealed active chronic gastritis. Until now, it is still unclear why some patients with *H. pylori* infection have no symptoms. It may be due to certain host factors or from the *H. pylori* itself. [1,2]

In the long term, the *H. pylori* strain which yields vacuolating cytotoxin invitro has been known to be able to cause vacuolization in epithelial cells of intestine mucosa. Some experts conclude that this cytotoxin was one of the most important factors in this disorder. The product of the vacuolating cytotoxin was encoded by the VacA gene. The vacuolating cytotoxin is always found together with the cytotoxin associated antigen (Cag A) encoded by cagA gene.

At present, the *H. pylori* is divided into two kind of strain with or without cagA production (cagA positive (+), cag A negative (-)). From previous studies, cagA+ has been shown to be more virulent and it is strongly associated with peptic ulcer, gastric cancer or severe active chronic gastritis, while *H. pylori* strain II (without cagA) is less virulent and causes minimal disorder.[3] But from Asian reports such as from Japan, there was no significant difference in the presence of cagA in patients with gastric cancer compared to those without gastric cancer. This data creates a doubt on the association between cagA and severe gastric disorders as mentioned from Western country reports. [2]

AIM OF STUDY

The aim of this study was to know more about the presence of Cytotoxin Associated Antigen by detection of the cagA gene from *H. pylori* strains, isolated from dyspeptic patients in Mataram General Hospital.

MATERIALS AND METHODS

H. pylori was recovered from the antral biopsy of each dyspeptic patients who had undergone diagnostic upper gastrointestinal endoscopy in Mataram General Hospital.[4,5] Detection of *H. pylori* infection was done using nested PCR with two primers used for the detection of the urease A gene. [6,7,8] The specimens which contain the urease gene was checked again to detect cagA gene using the two primers.

DNA was extracted from each biopsy using a phenol choloroform method. The primer set used for the

detection of *H. pylori* infection was 5'GATAATAGGTAAGCTUTGAGG3' and 5'CTGCAAAAGATTGTTTGGCAGA3', while the primer set used for detection of cagA gene was 5'GCGATCAAAATCCTACC3' and 5'AATTCGGTAACGCTGATC3'. [9] All primers were made by BRL, England. All PCR reactions were amplified using the thermocycler Amplitron I from Thermolyne. After PCR, the amplified strands were detected by DNA electrophoresis using 2% agarose gel. The amplified bands were then consecutively stained by ethidium bromide and photographed with Polaroid film. The result was positive if DNA was found in a 144-bp band position. And then, the patients were divided in two groups with or without cagA gene.

Table 1. Cag A status and the endoscopic diagnosis of the patients

Endoscopic diagnosis	Total	CagA+	(%)
Gastric carcinoma	4	4	100
Duodenal ulcer	4	3	75
Ventricular ulcer	3	3	100
Ventricular and duodenar ulcer	3	3	100
Gastritis	31	27	87,1
Total cases	44	39	88,6

RESULTS

Of the 177-biopsy specimens, there were 44 specimens, which showed the urease gene by the PCR method. From these specimens, we found 39 specimens (88.6%) with positive cagA (table 1). Four of them were endoscopically diagnosed as having ventricular ulcer, three had duodenal ulcer, three had both of duodenal and ventricular ulcer, twenty-seven had gastritis and four cases had gastric cancer. From 5 cases without cagA gene, was found one had duodenal ulcer and 4 had gastritis.

From table 1, we can see that most cases with peptic ulcer and gastric cancer was caused by the cag A strain. But it was same as gastritis cases. In this study there were 10 cases with ulcer and 31 cases just non-ulcer dyspeptic cases.

Statistically, there was no significant difference in the cagA status of ulcer patients and non-ulcer patients ($p > 0.05$).

DISCUSSIONS

In this study, it was shown that most of *H. pylori* infections (88.6%) in Mataram General Hospital was caused by the cagA positive strain. This findings is different to the previous study such as European study which found that cagA+ strain in 60 % cases. [2] Because most cases in this study was caused by cagA positive strain, we could use this marker (cag A status) to make a difference between severe and mild manifestation.

Table 2. Cag A status of ulcer and non ulcer dyspeptic patients.

Endoscopic diagnosis	Total	CagA positive	%
non-ulcer	31	27	87.1
ulcer	10	9	90

In Indonesia, there were some studies regarding detection of the cag A gene. The first study was reported by Suata et al in 1997. They found 45% cagA positive strain from *H. pylori* infection using imunobloting method. This method only detected the higher anti cagA.[10] The second study was reported by Mulyadi et al. He found 30% of biopsy specimens with cagA positive strain using nested PCR.[11]

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