

Correlation of CagA-Positive Strains of *Helicobacter pylori* with Topographic Distribution and Chronic Gastritis Grading

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ABSTRACT

Background: *CagA* gene is a marker for the presence of *Cag* pathogenicity island. *CagA*-positive strains of *Helicobacter pylori* can identify individuals who have higher risk of developing gastrointestinal diseases.

Aim: To discover the correlation of *CagA* status of *Helicobacter pylori* with topographic localization of *Helicobacter pylori* and chronic gastritis grading.

Methods: Gastric biopsy specimens were taken from 104 patients. The specimens were obtained from gastric antrum, corpus and incisures for histological and polymerase chain reaction (PCR) studies. The histological chronic gastritis was assessed semi-quantitatively (grades 0-3). The PCR was used for detecting *Helicobacter pylori* genes and *CagA* strain. Topographic localization of *Helicobacter pylori* was classified as gastric antrum and corpus.

Results: There were 33 (86.8%) *CagA*-positive strains of 38 patients with *Helicobacter pylori*-positive genes. There were no significant differences between topographic localization of *Helicobacter pylori* - either in the gastric antrum ($\rho = 0.14$, $p = 0.40$) nor in the corpus ($\rho = 0.27$, $p = 0.10$) and the *CagA* status of *Helicobacter pylori*.

Conclusion: *CagA* gene status of *Helicobacter pylori* does not determine chronic gastritis grading and gastric topographic localization.

Keywords: chronic gastritis, *cagA* gene, *Helicobacter pylori*, gastric antrum, gastric corpus.

INTRODUCTION

It is estimated that more than half of people in the world has been infected by *Helicobacter pylori* (*H. pylori*).¹ The prevalence of *H. pylori* in Indonesia is more than 80%.² *H. pylori* infection may cause gastritis which includes dyspepsia 40%, gastric ulcer 15% and gastric cancer 2% patients, respectively.² *H. pylori* is one of the risk factors that trigger gastric carcinogenesis cascade, known as Correa cascade. This cascade is initiated by chronic gastritis, followed by gastric atrophy, intestinal metaplasia and dysplasia and finally develops a gastric cancer.³

Genetically, there is polymorphism of *H. pylori* in human population.⁴⁻⁹ *Cag* island and *vacA* strains of *H. pylori* are examples for *H. pylori* polymorphism, which may affect the clinical outcome and balance.¹⁰⁻¹¹

The goal of this study was to discover the topographic localization of *CagA*-positive strains of *H. pylori* and its correlation with histopathological scoring of gastritis compared to *CagA*-negative strains of *H. pylori*.

MATERIALS AND METHODS

The protocol was approved by the Institutional Review Board of the Margono Soekarjo hospital, Purwokerto; while the study was conducted on dyspeptic patients at Department of Internal Medicine, Faculty of Medicine, University of Jenderal Soedirman/ Margono Soekarjo hospital, Purwokerto between 2 January and 31 December 2005.

According to Rome II Criteria, dyspepsia is defined as chronic or recurrent pain or discomfort referable to

upper gastrointestinal tract. Epigastric discomfort is a negative subjective feeling, a mild pain which may be accompanied by other various symptoms including rapid satiety, bloating and nausea.¹² Prominent epigastric pain or discomfort suggests dyspepsia, whereas esophageal reflux has other prominent symptoms such as heartburn or acid regurgitation.¹³

Criteria for eligibility in this study were patients with uninvestigated dyspepsia, age ≥ 45 years and had signed informed consent form. The exclusion criteria were patients with alarm symptoms (family history of upper-gastrointestinal malignancy, unexplained weight loss, GI bleeding, progressive dysphagia, odynophagia, unexplained iron-deficiency anemia, persistent vomiting, palpable mass or lymphadenopathy and jaundice),¹⁴ symptoms of gastroesophageal reflux, patients who have been under regular medication of non-steroid anti-inflammatory drugs (NSAIDs), proton pump inhibitors, histamine H₂-receptor antagonists, and antibiotics for at least 4 weeks prior to the study, history of upper GI tract surgery and pregnancy. Body mass index (BMI) was calculated as body weight (in kilograms)¹⁵ divided by the square of the height (in meters).

In all patients, endoscopy and biopsy evaluation were performed on gastric antrum and gastric corpus, which included two specimens for each location. A group of specimens had paraffination, cut and hematoxylin-eosin staining for histopathological examination.¹⁶ Histopathological scoring was based on Chen et al method by evaluating the score of chronic, atrophic, and intestinal metaplasia inflammation infiltrate. Score 0 = normal, 1 = mild, 2 = moderate, 3 = severe.¹⁷

The other group of specimens was evaluated for *H. pylori* detection by PCR method, using PCR reagent kit: PCR Core System (Promega #M7660, Promega Corp. Madison WI, USA). We used primer of UreC/glmM with a sequence of

ureC1 5'-GGATAAGCTTTTAGGGGTGTTAGGGG-3'
ureC2 5'-GCTTGCTTTCTAACACTAACGCGC-3'
(Invitrogen, Tokyo Japan)

and amplification target of 294 bp. Amplification procedures were as follows: samples were treated by 30 cycles of incubation at 94°C for 1 minute for denaturation of ds-DNA, followed by annealing of

primers at 45°C for 1 minute, and extension of nascent strands by DNA polymerase by incubation at 72°C for one minute. For detecting CagA gene, we used primer of

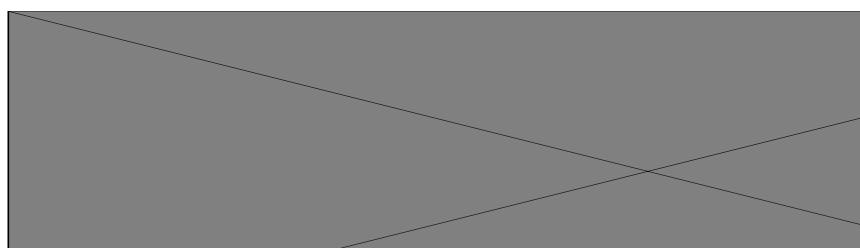
cagA1 5'-ATAATGCTAAATTAGACAACCTTGAGCGA-3'
cagA2 5'-AGAAACAAAAGCAATACGATCATTC-3'

and amplification target of 298 bp. Amplification procedures were as follows: denaturation at 95°C for 30 seconds, annealing at 54°C for 1 minute, extension at 72°C for 1.5 minute. Each reaction had 35 cycles using MyCycler machine (Biorad 170-9703, Biorad Corp USA). PCR products were analyzed by using Biorad Minisub DNA Cell (Biorad, USA) with 2% agarose gel and Tri Borate buffer at constant voltage of 100 V for approximately 1 hour. DNA strands were observed by ethidium bromide staining and ultra violet light. The size of DNA bp was determined by using Low DNA Mass Ladder (Invitrogen, USA). Gel image was recorded on Polaroid film by using MP4+ Camera System (Polaroid, USA). The result was considered as positive-*H. pylori* if there was DNA strand with the size of approximately 294 bp on cagA 298 bp.¹⁸

Descriptive statistical analysis for categorical variables were expressed as frequency distribution and proportion (n and %), while continuous numeric variables were expressed as mean value and standard deviation (SD). Data normality was analyzed by Kolmogorov-Smirnov test, whereas data correlation was analyzed by Spearman test. The difference of histopathologic grading was analyzed by Mann-Whitney U. A p value of < 0.05 , 95% confidence interval and 80% power were taken as the limit of significance. Statistical analysis was conducted by using SPSS version 11.5 for Windows computer program.

RESULTS

Of 448 dyspeptic patients, 104 subjects were included in the study and 38 subjects were *H. pylori*-positive. Female subjects were greater in amount of 20 patients (52.6%) compared to male subjects. The ratio of female to male subjects was 10:9. There were 33 subjects (86.8%) with CagA-positive among *H. pylori*-positive patients. There was no significant difference ($p > 0.05$) of age, gender and body mass index (BMI) between both groups of patients with CagA-positive and CagA-negative strains of *H. pylori* (table 1).



Patient distribution of cagA *H. pylori* in various chronic gastritis grading in antrum are shown in figure 1 and in chronic gastritis grading in gastric corpus are shown in figure 2.

Figure 1. Patient distribution of cagA *Helicobacter pylori* in chronic gastritis grading in antrum

Figure 2. Patient distribution of cagA *Helicobacter pylori* in chronic gastritis grading in gastric corpus

Based on Spearman correlation test, there was no correlation between chronic gastritis grading, either in antrum or gastric corpus, and cagA-positive and cagA-negative strain of *H. pylori* (table 2)

Table 2. Correlation of chronic gastritis grading in antrum and gastric corpus with cagA status

DISCUSSION

Helicobacter pylori strains and the cag pathogenicity island (PAI) have been known as virulent phenotype.¹⁹ CagPAI gene is an area of 40-kb DNA containing about 30 genes which may code type IV secretion system.²⁰ CagA is one of cagPAI genes which most commonly found in *H. pylori*.^{21,22} CagA protein is translocated into gastric epithelial cells and experiencing tyrosine phosphorylation.^{20,23}

Phosphorylated-cagA has important roles in gastric carcinogenesis,²⁴ interleukin (IL-8) synthesis by gastric epithelial cells,^{19,21,25} renewing of surface cells and pedestal forming.^{26,27} IL-8 is important for chemotaxis and neutrophils activation.²² Furthermore, it also associated with the grade of neutrophils infiltration in gastric mucosa infected by *H. pylori*.²⁸ Colonization of cagA-positive strains of *H. pylori* is correlated to increased risk of peptic ulcer and gastric cancer.²⁹ Infection of certain *H. pylori* are varied based on socio-economic condition, patient's age, and the country development status. CagA strains of *H. pylori* predominate in developing countries and it is almost about 100% in some Asian countries.

By PCR examination, *H. pylori*-positive is detected in 38 subjects and there are 33 (86.8%) subjects with cagA-positive strains of *H. pylori*; while in some Asian country, there are almost 100% cagA-positive strains of *H. pylori*.³⁰⁻³⁴ Similar results are found in Latin America, i.e. about 50-80%³⁵ and in Kuwait 87%.³⁶ Lower rate are found in Egypt 79%,³⁷ Turkey 61.7%,³⁸ and Srilanka 47.5%.³⁹

CagA-positive strains of *H. pylori* is associated with increased intensity of gastric inflammation, increased cytokine level especially IL-8 and disease progression of peptic ulcer and gastric cancer. Therefore, cagA-positive strains of *H. pylori* may generate higher immune response compare to the cagA-negative strains.⁴⁰ Atherton et al, conclude that colonization density of cagA-positive strains of *H. pylori* in antrum is significantly higher than the cagA-negative strains.⁴¹ However, in this study, we found no significant correlation of topography of chronic gastritis *H. pylori* in antrum and gastric corpus in cagA-positive strains of *H. pylori* compare to

cagA-negative strains. Similar result has been reported by Twisk et al⁴² and it has been proven that cagA status of *H. pylori* does not determine patient's clinical status. Therefore, cagA status of *H. pylori* can not be used in clinical diagnosis.⁴³

CONCLUSION

In this study, the cagA status of *Helicobacter pylori* does not correlated to chronic gastritis grading. Furthermore, the topographic distribution of *H. pylori* is not determined by cagA status of *Helicobacter pylori*

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