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CORRELATION BETWEEN THE SEROPOSITIVITY OF CYTOTOXIN-ASSOCIATED GENE A *H. PYLORI* AND THE GASTRITIC SEVERITY DEGREE IN PATIENTS WITH DYSPEPSIA

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ABSTRACT

Background: The pathogenesis of dyspepsia associated with gastritis due to H. pylori infection remains unclear. Protein Cytotoxin-associated gene A (CagA) H. pylori is known to play an important role in the occurrence of more severe gastritis. Patients, infected with H. pylori with positive CagA were found to have a tendency to experience peptic ulceration and the development of gastric mucosal progression.

Objective: To analyze the association of seropositivity of CagA H. pylori with the severity of gastritis in dyspeptic patients.

Methods: This is a cross-sectional study involving all dyspepsia patients undergoing endoscopy and gastric biopsy in the endoscopic unit of the Internal Medicine Department of Dr Soetomo General hospital Surabaya. The assessment of the gastritis severity degree was performed using the updated Sydney System classification (inflammation, neutrophil infiltration, glandular atrophy, and intestinal metaplasia). As for the detection of CagA H. pylori was used serum serology.

Results: From all 34 patients with dyspepsia, 8 (23.5%) was positive of H.pylori patients and 4 (50%) patients were positive of CagA H.pylori. Inflammatory scores and neutrophil infiltration in the positive CagA H.pylori group were significantly higher than in the negative, H.pylori group and the positive Caga negative H.pylori group. While on the scores of gland atrophy and intestinal metaplasia, there was no difference between the three groups. There was no statistically significant association between CagA H. pylori seropositivity with inflammatory score, neutrophil infiltration, gland atrophy and intestinal metaplasia.

Conclusion: There was no statistically significant correlation between CagA H. pylori porosopositivity and the severity of gastritis according to Updated Sydney Systems in both the inflammatory categories, neutrophil infiltration, gland atrophy and intestinal metaplasia.

KEYWORDS: *Dyspepsia, gastritis severity degree, CagA, H. pylori*

INTRODUCTION

Dyspepsia is the most common complaint in health centers with a prevalence of 15-40%. Symptoms of dyspepsia are often associated with gastrointestinal abnormalities, one of which is gastritis caused by bacterial infection of Helicobacter pylori with a prevalence of about 60-70%. In recent years, *H. pylori* is known to have virulence factors that play a role in severe gastritis, one of which is Cytotoxic agentA (CagA) [*Wallander M, 2007, Graham D, Rugge M, 2010, Adisa JO, 2011, Suzuki H, 2011*].

Patients infected with *H. pylori* with CagA-positive tend to experience peptic ulceration and progression of gastric mucosal progression. Data sug-

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gest that H.pylori infection with CagA positive strains is present in almost 80-95% of peptic ulcer patients, whereas in patients with gastric adenocarcinoma the prevalence of H.pylori infection with CagA-positive is also quite high with the percentage of about 64% [*Meine G et al., 2011, Sahara S et al., 2012*]. Therefore, the detection of this protein becomes very important in the management of dyspeptic patients to prevent the occurrence of more severe complications. H.pylori-infected patients with CagA-positive were observed closely and evaluated whether or not the eradication therapy against *H. pylori* bacteria was successful.

CagA is a 128kD molecular weight protein encoded by the gene cagA and is found in about 50-60% of *H. pylori* strains. This protein mechanism can cause stomach cell destruction which is begun since the CagA protein enters the gastric epithelial cell cytoplasm through the Type Four Secretion System (TFSS) apparatus formed from the protein group encoded by the cag-pathogenecity island gene (cag-PAI). Some of the CagA near the surface of the cell will experience tyrosine phosphorilation by the enzymes Scr and Abl kinase, which then activate the SRC-homology 2 domain-containing protein tyrosinephosphatase2 (SHP-2). They ultimately induces inflammation and alters the morphology of gaster epithelial cells. Meanwhile, the non-phosphorylated CagA protein affects E-cadherin and Partitioning defective 1 (PAR-1). It damages the apical junction complex and decreases the polarity of gaster epithelial cells, thus destroying the association between gaster epithelial cells and inducing malignant processes [*Konturek S et al., 2006; Mehmood A, 2010; Wroblewski L et al., 2010; Tegtmeyer N et al., 2011*].

Until present, two investigations have been developed to detect CagA protein genotyping using the Polymerase Chain Reaction (PCR) method and serology of Immunoglobulin G (IgG). PCR is said to be more sensitive and specific than serology. A number of studies confirm that serological tests are good enough to be used to detect these proteins. In Indonesia, serology testing CagA *H. pylori* is still not routinely done. Data on *H. pylori* infection with seropositive Caga is also not much, as the relationship with the severity of gastritis. Therefore, the researcher aims to know between Cagop H.pylori seropositivities with the severity of gastritis, particularly in Dr. Soetomo General Hospital Surabaya.

Material and Methods

This is a cross-sectional analytic observasional conducted in Endoscopic unit of Division of Gastroenterohepatology, Department of Internal Medicine Dr. Soetomo General Hospital Surabaya during January-May 2013.

The samples in this study consisted of dyspeptic patients who met the inclusion criteria. There was no exclusion criteria obtained. The inclusion criteria were outpatient subjects with complaints of at least 3 months, minimum age of 20 years old, and willing to sign Informed Consent. We exclude patients who are pregnant or lactating, taking antibiotics, H2 receptor antagonists, proton pump inhibitors, misoprostol, sucralfat, bismuth, NSAIDs, steroids within 2 weeks, patients with upper and lower bleeding, patients with smoking history and alcohol, patients with gallbladder disease from physical and laboratory examination, and those having contra indications of endoscopic examination and gastric biopsies.

CagA H.pylori is a protein with a molecular weight of 140 *kDa*, encoded by the gene cagA and is a marker of the Cytotoxin-associated gene A Pathogenecity Island (cagPAI) gene. Patients who are infected with strains with positive CagA usually experience more severe gastritis. In this study the presence of CagA protein was detected by serologic examination by examining Imamoglobulin G anti CagA H. pylori. The tool used is EUROIMMUN (Medizinische Labordiagnostika GmbH, Germany). The result of the examination is in numerical form. The value of >22 *U/ml* indicated a positive result, while the value of ≤22 *U/ml* indicated a negative results.

The degree of gastritis severity is the extent of gastric mucosal damage begins with with chronic gastritis. In this study the degree of gastritis severity was assessed using the classification of the Updated Sydney System. Since we differentiated *H. pylori* into positive and negative groups, the assessment of gastritis severity only included four categories, namely chronic inflammation, PMN infiltration, atrophy, and intestinal metaplasia. Each category was then assessed using scores.

Qualified patients were given the explanations about the purpose and benefits of the examination, and were asked for consent to participate in the study by signing informed consent. Subsequently, general

data and other data were recorded according to the data collection form. Preparation and endoscopy as well as gastric mucosal biopsy for histopathological examination were performed. After obtaining positive H.pylori results on histology examination, an IgG anti CagA H.pylori from serum were examined. Serological examination of IgG anti *H. pylori* was carried out using ELISA method, which was done by taking blood samples after positive *H. pylori* histology examination results was obtained.

The collected data were processed and presented in the form of text, tables and graphs. Analysis of the subjects' general characteristics was conducted descriptively. Meanwhile, the relationship of seropositivity CagA *H. pylori* and the severity of gastritis was calculated using Mann-Whitney test since the independent variable in this study was nominal data and the dependent variable was ordinal data. The p value was significant when >0.05.

RESULTS

During the time of study, we obtained 34 dyspepsia patients who came to Endoscopy unit of Dr. Soetomo General Hospital Surabaya and meet the inclusion criteria. The subjects' characteristics are presented in Table 1.

Most subjects were female with 21 people (61.8%), while men were 13 patients (38.2%). The ages of most patients were over 40 with 22 patients (64.70%) and there 5 patients whose age is under 40 years (35.3%). The results of endoscopic examination in all subjects consisted of chronic superficial chronic gastritis (52.9%), erosiva gastritis (20.6%), peptic (ulcer 20.6%), duodenitis (5.6%) and tumor (5.9%).

In this study the patient was stated as positive for *H. pylori* infection when *H. pylori* germs were obtained on both histologic examinations performed by two different experts. there were 8 positive samples infected with *H. pylori* or about 23.5% of the total number of patients.

To detect *H. pylori* germs, two anatomical pathologists performed histological examination. In the results of tests performed by the first expert showed 14 patients infected with H. pylori, while the second expert obtained 9 patients positively infected with H. pylori. Kappa interobserver scor calculation showed the result of 0.59 with significance of 0.007. This suggests that the results from both experts have fair suitability level due to the Kappa score being more than 0.4. The measurement is considered to be suitable if the results of expert 1 and expert 2 showed Kappa score of >0.75. If the Kappa score is within the range of 0.4 to 0.75 then the suitability of the measurement is considered quite good.

TABLE 1.

Subjects' Characteristics

No.	Variable	Category	Frequency
1	Gender	Male	13 (38.2%)
		Female	21 (61.8%)
2	Age	20-29 years old	5 (14.7%)
		30-39 years old	7 (20.6%)
		40-49 years old	8 (23.5%)
		50-59 years old	9 (26.5%)
		>60 years old	5 (14.7%)
3	Ethnicity	Java	18 (52.9%)
		Madura	8 (23.5%)
		Flores	4 (5.90)
		Ambon	4 (11.8%)
		Sunda	2 (5.9%)
4	Endoscopy Result	Superficial Chronic Gastritic	25 (73.5%)
		Tumor/polip	2 (5.9%)
		Duodenitis	2 (5.9%)
		Peptic ulcer	7 (20.6%)
5	H.pylori Infection	Positive	8 (23.5%)
		Negative	26(76.5%)

Frequency of CagA H.pylori Seropositivity

In the H.pylori-infected group of patients, there were 4 patients with positive anti-Cagmon IgG (50%) as seen in table 2.

Histologic Overview of Patients with H.pylori-negative, H. pylori-positive with Caga-negative and H. pylori-positive with CagA-positive According to the Updated Sydney System Classification

In the group of patients with H. pylori-negative for inflammatory criteria, the highest hitologic

TABLE 2.

CagA serology frequency in H.pylori-infected patients

CagA	Frequency	Percentage
Positive	4	50
Negative	4	50

overview is found in the 1st score group. In the group of patients with CagA-positive and H.pylori-negative the obtained scores were 1 and 2. In the H. plory-positive positive and Caga H.pylori group the obtained scores were higher, i.e. 2 and 3.

In the criteria of ineutrophils nfiltration, the highest obtained scores of negative H.pylori group were 0 and 1. In the CagA-positive and H.pylori-positive group the obtained score was 2. In the Caga-positive and H.pylori-positive group the obtained scores were 2 and 3. In the criteria of atrophy of gland and intestinal metaplasia results, the results were not much different among the three groups with the scores ranging from 0 and 1. These results can be seen in table 3.

The association between CagA seropositivity and the severity of gastritis in H.pylori-infected dyspepsia

In the H.pylori-positive group, Mann Whitney test was performed to obtain the p-value to determine the relationship between seropositivity of CagA and the severity of gastritis according to the Updated Sydney System. Statistical test with Mann Whitney test found that the p values in chronic inflammatory category, neutrophil infiltration, gland atrophy and intestinal metaplasia were> 0.05 (p = 0.127; 0.127; 1.000; 0.371 respectively). Thus, it was concluded that there was no significant relationship between CagA seropositivity and the four categories.

Discussion

Dyspepsia is the most common complaint in health care centers with a prevalence of 15-40% with a manifestation of pain or discomfort localized to the persistent and recurrent upper abdomen [*Wallander M, 2007; Graham D, Rugge M, 2010*]. Dyspepsia that lasts longer and does not improve with standard therapy requires further evaluation one of which is endoscopic examination [*Wallander M, 2007; Graham D, Rugge M, 2010*].

The baseline data shows that most of dyspeptic patients are female (61.8%) with mean age of 32.4±13.1. In the previous study, female subjects also dominated (53.33%) with mean age of 34.2±14.3. Other studies found more dyspepsia in women of childbearing age [*Mauleti I, 2004; Wallander M, 2007; Turkay C et al., 2011*].

Endoscopic features in gmost patients in this study were superficial chronic gastritis with 25 patients (73.5%), and ulcers with 7 patients (20.6%). This is consistent with the study obtainin the most endoscopic images in dyspepsia patients is superficial gastritis. Other studies found 76.66% patients with gastritis and 23.33% patients with peptic ulcers [*Demiturk L, 2001, Mauleti I, 2004*].

Several studies on *H. pylori* in Indonesia conducted since 2000 have found various prevalence of bacterial infection. In a study examining 177 gastric biopsy preparations, there was 24% of *H.*

Table 3.

Histologic features according to the Updated Sydney System Classification on H. pylori-positive Caga-positive patients, H. pylory-positive CagA H-positive. and H.pylori-negative.

No	Updated Sydney System	Score	*H.pylori*-negative (n=26)	*H.pylori* -negative	
				CagA (-) (n=4)	CagA (+) (n=4)
1.	Inflammation	0	0 (0%)	0 (0%)	0 (0%)
		1	24 (76.4%)	1 (2.9%)	0 (0%)
		2	2 (5.9%)	3 (8.8%)	2 (5.9%)
		3	0 (0%)	0 (0%)	2 (5.9%)
2	Neutrophil infiltration	0	12 (35.3%)	0 (0%)	0 (0%)
		1	14 (41.2%)	0 (0%)	0 (0%)
		2	0 (0%)	4 (11.8%)	2 (5.9%)
		3	0 (0%)	0 (0%)	2 (5.9%)
3	Atrophy of the gland	0	22 (64.7%)	2 (5.9%)	2 (5.9%)
		1	4 (11.8%)	2 (5.9%)	2 (5.9%)
		2	0 (0%)	0 (0%)	0 (0%)
		3	0 (0%)	0 (0%)	0 (0%)
4	Intestinal Metaplasia	0	25 (67.6%)	4 (11.85)	3 (8.8%)
		1	1 (2.9%)	0 (0%)	1 (2.9%)
		2	0 (0%)	0 (0%)	0 (0%)
		3	0 (0%)	0 (0%)	0 (0%)

pylori infection. Another study found 9.5%, with the frequency of *H. pylori* infection being 36.5%. In this study, we obtained a H.pylori prevalence of approximately 23.5% in 34 dyspeptic patients. This result is higher than that in the previous studies [*Sumohardjo S, 2000, Mauleti I, 2004, Syam AF et al., 2005, Arinton IG, 2011*].

This difference in results may be due to the different methods used in detecting *H. pylori* infection and also due to the increased use of a number of antibiotics freely which can eradicate these bacteria thereby decreasing the incidence of *H. pylori* infection. Several tests can be performed to detect *H. pylori* infection, such as urea breath test, histology, serology, culture, PCR and immunohistology. In this study identification of *H. pylori* infection was carried out through histology examination which had 88.9% sensitivity and 98% specificity. [*Mauleti I, 2004, Syam AF et al., 2005, Arinton IG, 2011*].

In Indonesia there is not much data on the prevalence of CagA. It was found that 45% of patients with H.pylori infection were CagA positive with themimoblot method. 30% of biopsy specimens after PCR examination was a positive CagA strain and 88.6% of the biopsy specimens were positive strains of CagA [*Suata K, 1997; Mulyadi K, 1998; Sumohardjo S, 2000*]. In this study, 50% of patients infected with H. pylor demonstrated positive CagA IgA.

The histologic features of gastritis can be assessed by the classification of the Updated Sidney System by evaluating the presence or absence of gastric mucosal inflammation characterized by lymphocytes. Whether they are active or not was assessing through the presence of neutrophil infiltration in lamina propia, the presence of gland atrophy, intestinal metaplasia [*Rugge M et al., 2011*]. The presence of neutrophil infiltration indicates that the inflammation that occurs is acute and active. In gastritis associated with *H. pylori* infection, there is higher neutrophil infiltration compared to gastritis due to other cause. This is caused by the presence of antigens released by *H. pylori* bacteria that induce IL-8 secretion to activate and attract neutrophils to the site of infection. Neutrophil infiltration in the gastric mucosa may be used as a marker of *H. pylori* infection [*Fox JG, Wang TC, 2007*]. In this study it was found that neutrophil infiltration in the negative H.pylori group was mostly in the mild category (score 1), whereas in the positive H.pylori group were mostly in the medium and heavy category (score 2 & 3).

Mononuclear cell infiltration in histologic features of patients with gastritism suggests that the inflammation is chronic. In chronic H. pylori-related gastritis there was also increased mononuclear cell infiltration higher than gastritis caused by other causes. This is what causes the histologic picture of *H. pylori* infection called active chronic gastritis [*Rugge M et al., 2011*].

In this study, more inflammatory score in the H. pylori-negative group was found in score 1, whereas in the H. pylori-positive group, the scores were 2 and 3. Inflammatory scores for patients with positive *H. pylori* were higher than those in H. pylori-negative. Gastric atrophy can occur due to long-standing gastric mucosal inflammation. This process is strongly influenced by factors such as the presence of *H. pylori* infection, the presence of proinflammatory cytokine polymorphisms, and environmental factors, such as diet, smoking, alcohol and drugs. Gastric mucosal infection by bacteria other than *H. pylori* is also reported to induce the occurrence of atrophic gastritis [*Rugge M, Genta RM, 2005*]. This study found that atrophy in the H.pylori-negative group was included in category 1 with 4 patients. The same amount was also obtained in the positive H.pylori group of the same category (score 1).

Atrophic gastritis is an early lesion of malignancy by turning into metaplasia and dysplasia. Intestinal metaplasia is one of the most common metaplasia of the gastric mucosa, often preceded by atrophic gastritis. Like atrophic gastritis, the process of occurrence of metaplasia is also affected by three factors: bacterial or infectious factors, host factors and environmental factors. In addition, it takes avery long time for them to turn into metaplasia and dysplasia [*Rugge M, Genta RM, 2005, Rugge M et al., 2011*].

In this study intestinal metaplasia was obtained in 1 patient from H.pylori-negative group and 1 patient from H.pylori-positive group with mild category (score 1). This is in contrast to the results of other studies that obtained more intestinal metaplasia in patients with H. pylori-positive. Based on this, it can be concluded that the metaplasia occurring in the H.pylori-negative group may be due to

host factors and more dominant environments such as diet, other bacterial infections, etc. As in the H.pylori -positive group, there was only 1 patient with intestinal metaplasia. It might be because the H.pylori infection occurring in other patients was not long enough to cause gastric epithelial cell changes and it is difficult to know for the first occurence of H.pylori infection. H.pylori has a number of factors virulence that determines the malignancy of this bacteria. CagA is one of the most frequently studied virulence factors. H.pylori strains with CagA are positively associated with more severe gastric mucosal inflammation, peptic ulceration and changes towards malignancy [*Yamaoka Y, 2010*].

In this study, a group of patients with CagA-positive H. pylori-positive had higher neutrophil and inflammatory infiltration scores than those with CagA-negative H.pylori-positive, although not statistically significant (p = 0.127 and p = 0.127).

H.pylori infection usually first occurs at an early age and causes acute gastritis. In the absence of adequate therapy, this infection will persist throughout the life of the host. Chronic mucosal inflammation that occurs chronically affects gastric acid secretion. Increased levels of IL-1β are known to inhibit gastric acid secretion and increase the pH of the stomach. H.pylori infection that initially occurs gastric antrum, with increased pH of the stomach will grow to the extent of the gastric corpus and pangastritis occur. Increased pH of the stomach causes the stomach to achlorhydria and over time gland cells will be damaged and disappear which therefore called as gatritis atrophy [*Fox JG, Wang TC, 2007*].

In this study there was no difference in the occurrence of atrophic gastritis in the H.pylori-positive CagA-positive group and H.pylori-positive CagA-negative group. Statistical test results with Mann Whitney test also did not find any significant relationship between seropositivity of CagA with gland atrophy (p = 1.000). This suggests that the presence or absence of CagA protein has no effect on the occurrence of atrophic gastritis, although it must be reconfirmed with larger samples.

The role of *H. pylori* infection in the pathogenesis of gastric malignancy through the onset of atrophic gastritis and intestinal metaplasia. This process takes a long time, which is up to tens of years. In this study, intestinal metaplasia was obtained in only 1 patients from the *H. pylori* -ositive CagA-positive group in the mild category. The result of statistical test showed no significant relationship between seropositivity of CagA with the occurrence of intestinal metaplasia (p = 0.371).

The results of this study showed that the presence or absence of CagA protein does not seem to affect the severity of gastritits according to the Updated Sydney System in patients with H.pylori-infected dyspepsia in Dr. Soetomo General Hospital. Some points that can explain the difference in these results are firstly, too few samples of patients with H. pylori-positive and therefore it requires reconfirmation in subsequent studies with larger samples. Secondly, the possibility of mutations in the cagA gene may not work properly and can not induce the inflammation properly. Third, the presence of other H.pylori virulence factors that play a role in gastritis rather than CagA. Fourth, suspected host and environmental factors are more influential than H.pylori bacterial infection factor in the occurrence of severe gastritis.

Conclusion

There was no significant association between CagA H.pylori paraserocytosis and chronic inflammation, neutrophil infiltration, gland atrophy and intestinal metaplasia.

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