Detection of *Helicobacter pylori* CagA gene and Its Association with Endoscopic Appearance in Balinese Dyspepsia Patients

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ABSTRACT

Background: *Helicobacter pylori (H. pylori) infection causes various abnormalities in the stomach. Only particular strain can cause severe problems in the stomach. CagA is a microbial virulent factor which is associated with more severe stomach problems, such as: peptic ulcer and stomach cancer. We would like to know the prevalence of CagA in Balinese population, and the association of H. pylori CagA status with the severity of endoscopic appearance in dyspepsia patients.*

Method: *Study design being used was analytic cross sectional study, involving 71 dyspepsia patients who underwent upper gastrointestinal endoscopic examination in Surya Husada Hospital and Balimed Hospital in June-December 2013. Sample was chosen in consecutive manner. Later, polymerase chain reaction (PCR) examinations of the stomach mucous biopsy tissue to determine H. pylori infection status and CagA status were performed. Further, Chi-square test was used to identify the difference in proportion of H. pylori and CagA between mild and severe endoscopic appearance.*

Results: *In this study, we found that the prevalence of H. pylori infection was 22.5% using PCR examination. Prevalence of CagA positive in H. pylori positive was 62.5%. There was significant association between status of H. Pylori infection and severity of endoscopic appearance (p = 0.038; OR= 2.67; 95% CI = 1.18-6.05). Status of CagA in H. pylori infected patients was not associated with the severity of endoscopic appearance. Additionally, there was significant association between patients' age and severity of endoscopic appearance.*

Conclusion: *The prevalence of CagA in H. pylori positive was 62.5%. H. pylori infection was associated with severity of endoscopic appearance and CagA status in H. pylori infected patients was not associated with severity of endoscopic appearance.*

Keywords: *Helicobacter pylori, CagA, endoscopic appearance*

ABSTRAK

Latar belakang: *Infeksi Helicobacter pylori (H. pylori) mengakibatkan berbagai kelainan pada lambung. Hanya jenis tertentu yang dapat menimbulkan kelainan yang berat pada lambung. CagA merupakan faktor virulensi kuman yang dikaitkan dengan kelainan lambung yang lebih berat seperti ulkus peptikum dan kanker lambung. Peneliti ingin mengetahui prevalensi CagA pada populasi suku Bali, dan hubungan status CagA H. pylori dengan beratnya gambaran endoskopi pada pasien dispepsia.*

Metode: *Penelitian dengan rancangan potong lintang analitik, melibatkan 71 pasien dispepsia yang dilakukan pemeriksaan endoskopi saluran cerna atas di Rumah Sakit (RS) Surya Husada dan RS Balimed pada periode Juni-Desember 2013. Sampel dipilih secara konsekutif. Kemudian dilakukan pemeriksaan reaksi berantai polimerase pada jaringan biopsi mukosa lambung untuk menentukan status infeksi H. pylori dan status CagA. Kemudian dilakukan uji chi-square untuk mengetahui perbedaan proporsi H. pylori dan CagA antara gambaran endoskopi ringan dan berat.*

Hasil: *Pada penelitian ini didapatkan prevalensi infeksi H. pylori 22,5% dengan pemeriksaan reaksi berantai polimerase. Prevalensi CagA positif pada H. pylori positif 62,5%. Terdapat hubungan yang bermakna antara status H. pylori dengan beratnya gambaran endoskopi (p = 0,038; OR= 2,67; 95% CI = 1,18-6,05). Status CagA pasien terinfeksi H. pylori tidak berhubungan dengan beratnya gambaran endoskopi. Dan terdapat hubungan yang bermakna antara umur pasien dengan beratnya gambaran endoskopi.*

Simpulan: *Prevalensi CagA pada H. pylori positif adalah 62,5%. Infeksi H. pylori berhubungan dengan beratnya gambaran endoskopi dan status CagA pasien terinfeksi H. pylori tidak berhubungan dengan beratnya gambaran endoskopi.*

Kata kunci: *Helicobacter pylori, CagA, gambaran endoskopi*

INTRODUCTION

Recently, *Helicobacter pylori* (*H. pylori*) infection is recognised as a health problem across the globe. *H. pylori* colonisation in human's stomach occurs in up to 50% of worldwide population and 70% of these are found in developing countries.[1,2] The prevalence of infection in South East Asia is also high, where in Thailand the prevalence was 54.1-76.1%.[3] Meanwhile, seroepidemiology in Indonesia showed the prevalence of *H. pylori* infection is approximately 36-46.1%.[4] In Bali, we have performed several studies in different populations. A study in a rural-based community which was performed in Tabanan showed prevalence of 43%, while another study in Denpasar revealed a prevalence of 41.2%.[5]

H. pylori infection is the most common cause of chronic gastritis, and is closely related to peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer.[1-3,6,7] In 1994, International Agency for Research on Cancer (IARC) and World Health Organization (WHO) classified *H. pylori* as a definite carcinogenic group.[8] The development of gastric carcinoma involves several factors, including *H. pylori* virulence level, host genetic characteristic, environment factor and or host-microbe specific interaction. *H. pylori* heterogeneity has been studied to identify the possibility of virulence factor because most *H. pylori* infection did not cause cancer.[9] CagA *Helicobacter pylori* is the first bacterial oncoprotein which has been identified for its association with human cancer.[10] Patient who is infected by strain containing CagA, has an increased risk to develop gastric cancer compared to those infected by strain which did not contain this oncoprotein.[11] CagA is the main virulence factor of *H. pylori*. Recent publication on the role of virulence factor to abnormalities happening in gastric mucosa in Indonesia which was performed in 5 big islands, Sumatra, Java, Borneo, Sulawesi, and Papua concluded that most infection that happened were infection of CagA strain East Asia type, but with low virulence.[12] Currently, there is no publication on the role of CagA virulence factor to the severity of gastric mucosa abnormality in Balinese population.

Based on the aforementioned background, we established formulation of research problems as follows: what is the prevalence of CagA *H. pylori* in dyspepsia patients and how is the association of *H. pylori* infection and CagA with endoscopic appearance in Balinese dyspepsia patients. This study aimed to identify the prevalence of *H. pylori* infection using PCR method in Balinese dyspepsia patients, to identify the prevalence of CagA in Balinese dyspepsia patients, to reveal the association of *H. pylori* status with endoscopic appearance, and to discover the association *of* CagA *H. pylori* status with endoscopic appearance.

METHOD

This was a study using analytic cross sectional design to identify the association between CagA *H. pylori* status and the endoscopic appearance of dyspepsia patients. It was conducted in Endoscopy Unit Surya Husada Hospital and Balimed Bali Hospital and Mataram Biomedik Laboratory. This study was performed for 6 months from June-December 2013. Target population of this study was all Balinese dyspepsia patients in Denpasar. Accessible population of this study was dyspepsia patients in Balimed Hospital and Surya Husada Hospital who underwent upper gastrointestinal

endoscopy examination. Samples of this study were dyspepsia patients in Balimed Hospital and Surya Husada Hospital who fulfilled inclusion and exclusion criteria. Inclusion criteria included patients with dyspepsia complaints, agreed to underwent endoscopy and gastric biopsy, aged 18 years old or more, and Balinese. Patients who refused to participate in the study, those with unstable hemodynamic condition, those who consumed PPI drugs in the last 2 weeks, NSAID drugs or antibiotics in the last 1 month.

Sample size was determined by using formula quoted from Madiyono et al, with zα 1.96, prevalence of Hp CagA+: 80%, with desired level of absolute precision of 10%; thus, based on the calculation using those numbers above, we found that the minimal number of sample needed was 69 samples.[13,14] Samples were chosen using consecutive sampling technique. Patients with dyspepsia complaints who visited Balimed Hospital and Surya Husada Hospital for treatment and fulfilled the inclusion and exclusion criteria were included as samples, until the number of required sample was fulfilled.

Variables in this study were classified as follows: independent variable was CagA *H. pylori* status. Dependent variable was endoscopic appearance. Confounding variables: age, education level. Diagnosis of *H. pylori* was performed using PCR technique, to detect UreC and CagA gene. Endoscopic appearance was based on the results of endoscopic examination of upper gastrointestinal tract, which could be in the form of antrum superficial gastritis, superficial pangrastritis, erosive gastritis, gastric ulcer, duodenal ulcer, polyp or tumour and could be normal mucosal appearance. Severe endoscopic appearances were considered if in endoscopic examination, there was presence of erosion, ulcer, tumour, or polyp. Mild endoscopic appearances included superficial gastritis appearance or normal appearance.

Before this study was conducted, the researcher asked for permission to the Director of Balimed Hospital and Surya Husada Hospital, Denpasar, and established cooperation with nurses in the endoscopy unit. Study sample was collected through consecutive sampling. Dyspepsia patients who came to undergo endoscopic examination were explained about this study, if agreed they signed an informed consent. Patients performed preparation for endoscopic examination including fasting 6 hours before the procedure being performed. Endoscopic examination was performed by a gastroenterologist. During endoscopy, biopsy of patients' gastric mucosa was performed, one from the corpus area and one from the antrum area. Results of endoscopic examination were presented in the form of pictures with description of endoscopic appearance being found. Gastric mucosal biopsy was then preserved in buffer media, for further PCR examination in Mataram Biomedik Laboratory. Collected data was analysed using computer software.

RESULTS

In this study, 71 samples who fulfilled inclusion and exclusion criteria were included. Thirty-eight individuals (53.5%) were males and the other 33 were (46.5%) females. Patients who participated in this study were between 21-70 years old. Samples' age was normally distributed (Kolmogorov Smirnov; p = 0.2). Patients who were included in this study were all Balinese living in Bali; endoscopic examination was performed in 2 private hospitals in Denpasar. PCR examination of *H. pylori* was performed in Mataram Biomedik Laboratory. Complete sample characteristics were presented in Table 1.

Table 1. Subjects' characteristics data

Variable	n (%)
Sex	
Male	38 (53.5)
Female	33 (46.5)
Age (years)	
20-29	8 (11.3)
30-39	20 (28.2)
40-49	15 (21.1)
50-59	14 (19.7)
60-70	14 (19.7)
Education	
Primary school	13 (18.3)
Junior high school	4 (5.6)
High school	38 (53.5)
University	16 (22.5)
Endoscopic appearance	
Superficial antrum gastritis	43 (60.6)
Superficial pangastritis	12 (16.9)
Gastritis erosive	7 (9.9)
Gastric ulcer	4 (5.6)
Duodenal ulcer	3 (4.2)
Gastric polyp	2 (2.8)

In this study, we found 16 (22.5%) patients from 71 samples were positive for PCR UreC examination, and found 10 (62.5%) individuals with positive CagA from 16 patients with positive PCR results. To perform analysis of study results, endoscopic appearance (EA) was classified into 2 main group: mild abnormality and severe abnormality. Superficial antrum gastritis 43 (60.6%) and superficial pangastritis 12 (16.9%) were classified into mild abnormality, while erosive gastritis 7 (9.9%), gastric ulcer 4 (5.6%), duodenal ulcer 3 (4.2%) and gastric polyp 2 (2.8%) were classified into severe abnormality. To define which variables influence the endoscopic appearance, we performed analysis to age, sex, and education level variables of the samples. The analysis results could be seen in Table 2.

Table 2. Association of sex, age, and education level with endoscopic appearance (EA)

Variable	Severe EA (n = 16) n (%)	Mild EA (n = 55) n (%)	p
Sex			
Male	9 (56.3)	29 (52.7)	
Female	7 (43.8)	26 (47.3)	0.80
Age (mean ± SD)	51.50 ± 12.48	43.45 ± 13.0	
Education			0.032
Primary School	3 (18.8)	10 (18.2)	
Junior high school	3 (18.8)	1 (1.80)	0.06
Senior high school	8 (50.8)	30 (54.4)	
University	2 (12.5)	14 (25.5)	

EA: endoscopic appearance

From the results of Chi-square test, there was no significant difference between proportion of sex towards endoscopic appearance. Similarly, in education level, there was no significant difference of education level proportion to endoscopic appearance. Meanwhile, to evaluate age mean difference, since the data was normally distributed, unpaired t-test was performed. There was significant mean difference in severe endoscopic appearance compared to the group with mild endoscopy appearance (p = 0.032; 95% CI: 5.42-25.36).

Association of *H. pylori* PCR with patients' endoscopic appearance could be seen in table 3.

Table 3. Association of PCR examination results and endoscopic appearance

PCR Examination	Severe EA (n = 16)	Mild EA (n = 550)	p
PCR (+)	7 (43.8)	9 (16.4)	0.038
PCR (-)	9 (56.3)	46 (83.6)	

PCR: polymerase chain reaction; EA: endoscopic appearance

From the table above, we could see significant difference of PCR (+) proportion between severe EA (43.8%) and mild EA (16.4%). Additionally, the odds ratio was OR = 2.67; 95% CI=1.18-6.05.

Samples with positive PCR UreC examination were further followed up for detection of CagA gene. Results of CagA status could be seen in table 4 below.

Table 4. Proportion of CagA in each endoscopic appearance groups in patients with PCR (+) results

Endoscopic appearance	PCR (+)	CagA (+)	CagA (-)
Severe EA	7	5 (71.4%)	2 (29.6%)
Mild EA	9	5 (55.6%)	4 (44.4%)

PCR: polymerase chain reaction; EA: endoscopic appearance

From the table above, we could see that CagA positive was performed in 5 from 9 PCR (+) in mild endoscopic appearance (superficial gastritis), and 5 from 7 PCR (+) in severe EA (3 from 4 PCR (+) in erosive gastritis, 1 from 2 PCR (+) in gastric ulcer and 1 from gastric polyp). Relationship between CagA status and severity of endoscopic appearance could be seen in Table 5. From that cross table, Chi-square test was performed. Because there was more than 1 cell having expected value < than 5, then the Chi-square test criteria was not fulfilled. Therefore, Fisher test was performed. From Fisher test, we obtained p = 0.63.

Table 5. Association of CagA status and severity of endoscopic appearance

Cag A Examination	Severe EA (n = 7)	Mild EA (n = 9)	p
CagA (+)	5 (71.4%)	5 (55.6%)	0.63
CagA (-)	2 (28.6%)	4 (44.4%)	

EA: endoscopic appearance

From the table above, the proportion of CagA (+) in severe EA (71.4 %) was higher compared to mild EA (55.6 %); this difference was not statistically significant (p = 0.63; OR = 1.5; 95% CI= 0.41-5.45). From these results of analysis, it could be concluded that there was no significant association between CagA status of *H. pylori* infected patients and severity of the patients' endoscopic appearance.

DISCUSSION

In this study, we obtained that the prevalence of *H. pylori* infection using PCR method was 22.5%. Meanwhile, using IgG Anti *H. pylori* examination, we found that the prevalence was 18.3%. These results were quite low compared to the previously performed studies using different methods of examination. Generally, prevalence of *H. pylori* reaches up to 80% in developing countries in middle-aged population, while in developed countries the prevalence was 20-50%. In children, the incidence of *H. pylori* infection was quite low, but will increase up to 20% in the age of 40 and 50% in the age of 60.[15]

The result of this study was similar to the study performed by Darya in 2009 which found that the prevalence of *H. pylori* infection in gastritis patients in Sanglah Hospital was 18.8% using immunochromatography method.[16] Target population in both studies was similar, which was dyspepsia patients who visited each hospitals for treatment. Meanwhile, other studies had different target population, which was general population who had dyspepsia complaints got higher results. A study in rural area in Tabanan obtained a prevalence of 43%, while another study in Denpasar found a prevalence of 41.2%.[5] Latest study in Indonesia attained a prevalence of 33.6%.[12]

PCR is a very good diagnostic tool to detect the presence of *H. pylori* infection. In the study performed by Fonseca et al, it was found that the sensitivity and specificity to detect *H. pylori* using ureA PCR was 93.3 and 95.8%, meanwhile if culture is used, the result showed 100% sensitivity and specificity.[17] Additionally, compared to *H. pylori* examination using immunohistochemical analysis and campylobacter like

organism (CLO) test, PCR examination can identify more samples. This is proven by study performed by Weiss et al which found 23 *H. pylori* positive samples using PCR but negative using immunohistochemical analysis or even using CLO test.[18]

A study in Netherland showed that in order to detect *H. pylori,* PCR examination has the highest sensitivity of 99.4%, followed by histological examination (92.2%), culture (89.5%) and CLO test (89.0%). For their specificities, these 3 examinations had specificity of more than 98%. This study also showed that the positive results obtained from CLO test ($p = 0.001$) and culture ($p = 0.031$) was very influenced by bacterial density. However, this is not the case in examination using PCR method ($p = 0.130$). Therefore, effect of mistakes during sample collection for PCR examination was very small, because it did not require a large amount of sample for examination using PCR method.[19]

In this study, we observed that age was associated with the severity of endoscopic appearance. The older patients showed more severe endoscopic appearances. In accordance to the results of previous studies. Prevalence of normal gastric mucosa was said to decrease in line with the addition of age, either with or without the presence of *H. pylori* infection.[20] In older patients, there will be a decreased capacity of defensive factors in gastric mucosa to maintain gastric mucosal integrity. It may relate to the decrease in the production of mucous and bicarbonate. In healthy old individuals, it is also found that there is a decrease in prostaglandin concentration compared to younger individuals; this is known to be the result of significant decrease of cyclooxygenase enzyme activity. Therefore, it is easier to experience abnormality compared to younger age.[21]

H. pylori strain which contains CagA gene is said to cause infection with worse clinical symptoms. Although CagA is not *H. pylori* infection determinant, identifying the presence of this gene may help to determine the prognosis and association of infection with the occurring disease.[17,19]

In this study, we found 62.5% patients with positive PCR had CagA gene. This result was in accordance to the previous study in which this gene was reported to be found in approximately 60-70 % from *H. pylori* isolates in Europe.[22] Other study found a prevalence of 68.3%, while a study conducted by Fonseca reported a prevalence of 53%, where 6 (75%) patients suffered from erosive gastritis and 2 (25%) patients suffered from superficial gastritis.[17,23] Another study even indicated a lower prevalence of 40.8%.[24]

In this study, we found significant association between *H. pylori* infection and the severity of endoscopic appearances (OR = 2.67; 95% CI = 1.18-6.05). This result was in accordance with the available literature. *H. pylori* is the aetiology of most duodenal and gastric ulcer. Individual who is infected by *H. pylori* will have risk of 3-25% to suffer from peptic ulcer in his life. There is a robust evidence that *H. pylori* infection increases the risk of gastric cancer. *H. pylori* infection significantly increases the risk of gastric MALT lymphoma and 72-98% of patients with gastric MALT lymphoma were infected by *H. pylori*.[25] *H. pylori* infection causes impairment of epithelial cells through various mechanisms. Epithelial cell impairment can result from reactive oxygen or nitrogen species which is produced by activated neutrophils.[26] Epithelial impairment is the initial stage in the formation of abnormality in gastric mucosa. The presence of inflammatory process which is induced by *H. pylori* and the role of host immune response will cause various abnormalities in gaster, starting from gastritis, ulcer, until gastric cancer.

In this study, CagA gene in *H. pylori* infected patients was not significantly associated with severity of endoscopic appearance. This result concurred with the results reported in a study performed by Viana et al, who found that there was no significant difference between clinical manifestations and CagA status. However, they found that the prevalence of peptic ulcer was more common in CagA EPIYA-ABCC/ABCCC strain compared to CagA EPIYA-ABC strain.[24] This showed that microbial virulence was not only determined by the presence of *CagA* gene, but also by the role of EPIYA motif in the CagA gene.

Recent study found that the virulence level of *H. pylori* was determined by genotype of EPIYA motif in CagA. Patients with EPIYA Western type (ABC) motif had milder clinical appearance compared to EPIYA East Asian type (ABD) because EPIYA ABD motif would produce more IL-8 from gastric epithelial cells compared to EPIYA ABC motif.[27] In addition to CagA, there are several microbial virulence factors which need to be considered that influence the severity of gastric abnormality, such as VacA, OipA, IceA, BabA. VacA type s1/m1 is the most active type which has the potential to cause more severe abnormality.[28]

Infection of gastric mucosa by *H. pylori* is marked by the production of proinflammatory cytokine, particularly interleukin 8 (IL-8), which is a potent neutrophil activation chemokine. Cag PAI is involved in the induction of gastric IL-8 production. A study showed that CagA was involved in the induction of

IL-8 depend on the strain. IL-8 level induced by CagA East Asian type was higher compared to those induced by CagA Western type.[29] This probably underscores more severe clinical manifestation to happen due to infection of *H. pylori* strain CagA East Asian type.

A Study performed in 5 big islands in Indonesia revealed that the prevalence of CagA was 97.7% from patients with positive *H. pylori*, with dominant of EPIYA East Asian motif, but clinical symptoms which appear mostly were mild. This was thought to be caused by EPIYA gene East Asian type which was found in Indonesia had deletion in 6 bp, thus it had different type to the EPIYA gene East Asian type.[12] Bali Island which is located in Indonesia, probably had CagA strain similar to this study, thus also had CagA strain with EPIYA East Asian motif with mild clinical appearances.

CONCLUSION

In this study, we found prevalence of CagA in *H. pylori* infection was 62.5%. *H. pylori* status was associated with severity of endoscopic appearance. Meanwhile, CagA status from *H. pylori* was not associated with severity of endoscopic appearance. Further study is needed to identify the EPIYA motif from *H. pylori* strain with positive CagA to determine the virulence of *H. Pylori* strain.

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