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An East-Asian-type *cagA* *Helicobacter pylori* Infected Patient with Clinical Manifestation of Gastric Ulcer

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ABSTRAK

Kami melaporkan sebuah kasus seorang laki-laki, 72 tahun, etnis Tionghoa dengan keluhan utama buang air besar berwarna hitam lembek. Pemeriksaan fisik menunjukkan warna pucat pada konjungtiva palpebra yang dikonfirmasi dengan hasil hitung darah lengkap. Pemeriksaan gastroduodenoskopi menemukan adanya ulkus berukuran 3 mm di antrum (Forrest stage III). Infeksi *H. pylori* dinyatakan positif berdasarkan lima metode berbeda (urinary antibody tests, rapid urease test, kultur, histologi dan imunohistokimia). Analisis dengan sequencing berbasis polymerase chain reaction didapatkan bahwa pasien terinfeksi oleh strain berjenis East-Asian-type *cagA* dan *vacA s1m1*. Analisis lanjutan dengan menggunakan tujuh housekeeping gen mengkonfirmasi bahwa strain tersebut tergolong dalam kelompok *hspEAsia*. Pasien diberikan infus intravena kontinyu pompa proton inhibitor dan standar triple therapy regimens untuk terapi eradikasi *H. pylori*.

Kata kunci: *Helicobacter pylori*, ulkus peptikum, East-Asian-type *cagA*, *vacA*.

ABSTRACT

We reported a male, 72 yo, Chinese ethnic with chief complaint black mushy defecation. Physical examination revealed pale on conjunctival palpebra which confirmed as anemia on complete blood count. Gastroduodenoscopy revealed a 3 mm ulcer at the antrum (Forrest stage III). *H. pylori* infection was positive based on five different test methods (urinary antibody tests, rapid urease test, culture, histology and immunohistochemistry). Used polymerase chain reaction-based sequencing, we found the patient infected by *CagA* producing, East-Asian-type *cagA* and *vacA s1m1*-strain. Further analysis using 7 housekeeping genes confirmed that the strain categorized in to *hspEAsia* group. The patient was given continuous intravenous infusions of proton pump inhibitor and standard triple therapy regimens eradication of *H. pylori*.

Keywords: *Helicobacter pylori*, gastric ulcer, East-Asian-type *cagA*, *vacA*.

INTRODUCTION

Gastric ulcer (GU), part of peptic ulcer disease (PUD), is a deep lesion penetrating through the entire thickness of the gastro intestinal mucosa and muscularis mucosa with a diameter of at least 0.5 cm.¹ *Helicobacter pylori* infection plays an important role in the pathogenesis of GU, causes more than 90% of cases when non steroidal anti-inflammatory agents (NSAID) are excluded.² Several studies reported that *cagA* and *vacA*, the best studied being virulence factors of *H. pylori*, are a risk factor of GU.^{3,4} Nomura et al.³ reported that subjects with seropositivity for both *H. pylori* and *CagA* had an odds ratio of 4.4 (95% CI: 1.8, 10.5) for GU. In addition, in vitro study reported that *cagA* with an EPIYA-D segment (East-Asian-type *cagA*) has a higher binding affinity to Src homology-2 domain-containing phosphatase 2 (SHP2) than *cagA* with an EPIYA-C (Western-type *cagA*). Further, individuals infected with East-Asian-type *cagA* strains reportedly have an increased risk of PUD compared with those with Western-type *cagA* strains.⁵

On the other hand, the gene encoding *vacA* displays allelic diversity including the signal (s) regions s1 and s2 and middle (m) regions m1 and m2. Based on in vitro experiments, s1m1 strains have the highest cytotoxicity because they consistently induce cell vacuolation, followed by s1m2 strains (in which cell vacuolation is not consistently induced) and s2m2 strains, which have no cytotoxic activity due to a failure to induce cell vacuolation.⁶ In agreement with in vitro data, many studies examining populations in Western countries⁷⁻⁹ have shown that individuals infected with *vacA* s1 or m1 *H. pylori* strains have an increased risk of PUD compared with those with s2 or m2 strains.

In contrast with several Southeast Asian countries where have high prevalence of *H. pylori* infection; e.g., the *H. pylori* infection rate reported ranged from 54.1–76.1% in Thailand, most researchers reported low prevalence of *H. pylori* infection in Indonesia; 0–11.2% by the urea breath test and 5.7–12.8% by histology.¹⁰ Interestingly the highest *H. pylori* prevalence on Surabaya, Indonesia was found in patients from the Chinese Indonesian population instead

of patients from the Javanese population,¹⁰ although the prevalence of *H. pylori* infection in Indonesians of Chinese descent was lower than that of Chinese non-immigrants.¹¹ Moreover multilocus sequence typing (MLST) of seven housekeeping genes from different geographical, ethnic, and/or linguistic origins identified a high incidence of gastric cancer was found in regions prevailed by hp East-Asia strains (including China, Japan and Korea) than other population.¹² We reported a Chinese Indonesian patient was infected with An East-Asian-type *cagA H. pylori* with clinical manifestation GU.

CASE ILLUSTRATION

A 72 years old male, Chinese Indonesian ethnic (father, mother and grandparents are Chinese ethnic), was admitted to the hospital for having black mushy defecation since 3 days ago. He also had abdominal discomfort since 5 months ago, aggravated by meals without radiating pain and temporary relief by antacids.

Physical examinations revealed the conjunctival palpebra were pale and no sign of jaundice. There was no lymph nodes enlargement. The thorax and abdominal examination was normal. The extremities were a few pale.

Complete blood count revealed hemoglobin 8 g/dl, hematocrit 23%, leukocytes 7300/uL, granulocytes 75%, platelets 378.000/uL. The biochemical analysis were SGOT 11/uL, SGPT 8/uL, albumin 3.86 g/dl, total bilirubin 0.52 mg/dl, direct bilirubin 0.16 mg/dl, BUN 17 mg/dl, creatinine 0.98 mg/dl, random blood glucose 105 mg/dl, sodium 137 mEq/L, potassium 3.7 mEq/L, chloride 107 mEq/L and HbsAg negative. *H. pylori* urinary antibody test result was positive.

Chest X-ray showed the heart and lungs were normal. Abdominal ultrasonography showed the liver size within normal limit and there was no splenomegaly. Gastroduodenoscopy revealed a 3 mm ulcer at the antrum which categorized on Forrest stage III (**Figure 1**). Biopsy was taken three from antrum used for *H. pylori* culture, rapid urease test (CLO-test), and histological examination of specimens and one from the corpus for histological examination. The result was positive for CLO-test. Histology examination confirmed by immunohistochemistry showed

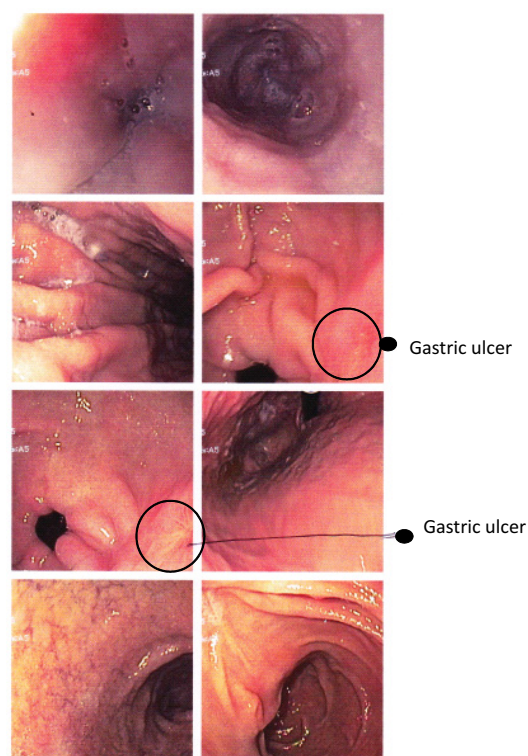


Figure 1. Antral gastric ulcer (Forrest III) was confirmed by gastroduodenoscopy examination which maybe a cause of melena.

H. pylori positive with density 1 both in the antrum and corpus. Based on the updated Sydney system; the degree of neutrophil activity, inflammation, atrophy and intestinal metaplasia were 1, 2, 1, 0, respectively for antrum and 1, 1, 0, 0, respectively for body. According to the operative link on gastritis assessment (OLGA) system, the degree of gastritis scores was stage 1.

The *H. pylori* bacteria culture were growth, we termed as the SBYPUD-1 strain. The polymerase chain reaction (PCR) amplification of *cagA* conserved region was *CagA* producer, whereas *vacA* genotype was s1m1. The lists of primers are shown in **Table 1**. Direct sequencing of a conserved region of *CagA* identified EPIYA-A, -B and -D segments, therefore categorized in to East-Asian-type *cagA* (**Figure 2**). The sequences of seven housekeeping genes confirmed this strain categorized in to hspEAsia group (**Figure 3**).

The patient was given soft and low fiber diet, 80 mg intravenous bolus Pantoprazole followed by infusion 8 mg/h. He was also given *H. pylori*

Table 1. The primers used for detecting virulence factors of *H. pylori*

Genes	Name of Primer	Primer sequences (5'→3')	PCR product (bp)	PCR conditions
<i>cagA</i>	cagOMF	AGCAAAAAGCGACCTTGAAA	521	95°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)
	cagOMR	AGTGGCTCAAGCTCGTGAAT		
<i>vacA</i>				
s1/s2	VAI-F	ATGGAAATACAACAAACACAC	259/268	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)
	VAI-R	CTGCTTGAATGCGCCAAAC		
m1/m2	VAG-F	CAATCTGTCCAATCAAGCGAG	567/642	
	VAG-R	GCGTCAAAATAATTCCAAGG		
<i>atpA</i>	atpA-F	GGACTAGCGTTAAACGCACG	821	94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)
	atpA-R	CTTGAAACCGACAAGCCCAC		
<i>efp</i>	efp-F	GGCAATTTGGATGAGCGAGCTC	642	94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)
	efp-R	CTTCACCTTTTCAAGATACTC		
<i>mutY</i>	mutY-F	GTGTTGTAGYTGGAACTTTACAC	639	94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)
	mutY-R	CTTAAGCGTGTGTYTTTCTAGG		
<i>ppa</i>	ppa-F	GGAGATTGCAATGAATTTAGA	676	94°C, 1 min; 53°C, 1 min; 72°C, 1 min (35 cycles)
	ppa-R	GTGGGGTTAARATCGTTAAATTG		
<i>trpC</i>	trpC-F	TAGAATGCAAAAAGCATCGCCCTC	950	94°C, 1 min; 58°C, 1 min; 72°C, 1 min (35 cycles)
	trpC-R	TAAGCCCGCACACTTTATTTTCGCC		
<i>ureI</i>	ureI-F	AGGTTATTCGTAAGGTGCG	875	94°C, 1 min; 53°C, 1 min; 72°C, 1 min (35 cycles)
	ureI-R	GTTTAAATCCCTTAGATTGCC		
<i>yphC</i>	yphC-F	CACGCCTATTTTTTACTAAAAAC	950	94°C, 1 min; 55°C, 1 min; 72°C, 1 min (35 cycles)
	yphC-R	CATTYACCCTCCCAATGATGC		

	EPIYA-A	EPIYA-B
Consensus	E P I Y A K V N K K K A G Q A T S P E E P I Y A Q V A K K V S A K I D Q L N E A A S	E P I Y A Q V A K K V S A K I D Q L N E A A S
SBYPUD-1	E P I Y A Q V N K K K A G Q V A S P E E P I Y A Q V A K K V N A R I D R L N K I A S	E P I Y A Q V A K K V N A R I D R L N K I A S
	EPIYA-D	
Consensus	A I N R K I D R I N K I A S A G K G V G G F S G A G R S A S P E P I Y A T I D F D E A N Q A G	
SBYPUD-1	T I N A K V D Q L N K T A S A S K G V G G F S G A G R S A S P E P I Y A T I D F D E A N Q A G	

Figure 2. Sequence analysis of *CagA* structural polymorphisms of SBYPUD-1 strain. Sequences of A, B and D segments almost similar with segments in East-Asian-type *cagA* consensus.

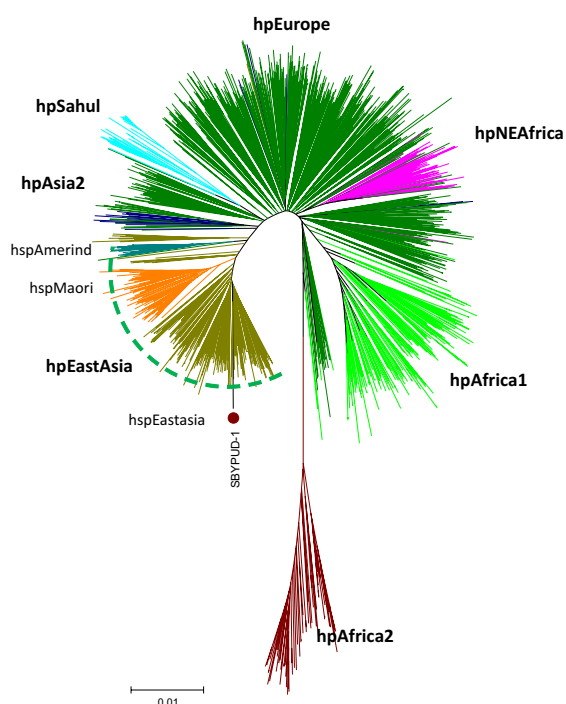


Figure 3. Phylogenetic tree of SBYPUD-1 based on the seven housekeeping genes of *H. pylori*. Sequence data sets of the seven housekeeping genes of 1,126 strains with different genotypes were obtained from the pubMLST database (62 from hpAsia2, 493 from hpEurope, 76 from hpNEAfrica, 50 from hpSahul, 28 from hpAfrica2, 279 from hpEastAsia, and 138 from hpAfrica1) then compared to SBYPUD-1. Neighbor-joining trees were constructed in MEGA v.5.05 using Kimura-2 parameters. The scale bar indicates genetic distance.

eradication therapy consisted of Pantoprazole 2x20 mg, Clarithromycin 2x500 mg, Amoxicillin and 2x1 g for 7 days. The black mushy defecation was stop on 3rd day of admission.

DISCUSSION

Although *H. pylori* was discovered more than 30 years ago by Warren and Marshall.¹³,

which method should be considered as a gold standard for detection of *H. pylori* infection remains unclear. Culture from biopsy specimens has the potential of leading to a high sensitivity, given that only one bacterium can multiply and provide billions of bacteria. However, both strict transport conditions and careful handling in the laboratory are necessary. On the other hand rapid urease test, such as the CLO test, can be useful as a rapid diagnostic method. However, these results can also be affected by the bacterial load.¹⁴ Histological diagnosis depends on the density of *H. pylori* biopsy sites; thus, these tests can occasionally show false negative results and very much dependent on the expertise of the pathologists. Immunohistochemistry staining could increase histology accuracy due to using a specific *H. pylori* antibody which has the highest sensitivity and specificity and better interobserver agreement compared to histochemical stains.¹⁵ Therefore the combination of two or more tests should be applied to determine the accurate prevalence of infection.

Although *H. pylori* infection is a major factor to severe gastroduodenal disease, the infection remains latent in the majority of infected patients, and only a minority of individuals with *H. pylori* infection ever develop the disease.¹⁶ The difference of *H. pylori* infection rate is not enough to explain the difference of the incidence of gastric cancer around the world. In addition to host and environmental factors, as a part, the difference of the incidence of gastric cancer irrespective of *H. pylori* infection rate can be explained by the difference of virulence factors.¹⁷ In general, strains with *cagA* positive

(especially East-Asian-type *cagA*) and *vacA* s1m1 are considered to be more virulent and induced higher inflammation and/or atrophy than *cagA*-negative, Western-type *cagA*, and/or other *vacA* types. In fact, the investigators from the early and mid-twentieth century in Malaysia and Java, Indonesia reported that gastric cancer and PUD typically associated with *H. pylori*, were rare in the indigenous populations but were common among the more recently arrived Chinese and Indian immigrants.¹⁸ It is suggested that the Chinese Indonesian might be become a high-risk population of *H. pylori* associated disease even in Indonesia.

MLST characterized isolates of bacterial and fungal species using nucleotide sequences of internal fragments of housekeeping genes. This method is finding a place in clinical microbiology and public health by providing data for epidemiological surveillance¹⁹ and also reported to give more detailed information about human population structure than the method using human microsatellite or mitochondrial DNA.²⁰ Recently, the genomic diversity within *H. pylori* populations was examined by employing the MLST method using 7 housekeeping genes (*atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI*, and *yphC*).²¹⁻²³ At present, *H. pylori* strains can be divided into seven population types on the basis of geographical associations and designated as follows: hpEurope, hpEastAsia, hpAfrica,¹ hpAfrica2, hpAsia2, hpNEAfrica, and hpSahul.²³ hpEurope includes almost all *H. pylori* strains isolated from ethnic Europeans, including people from countries colonized by Europeans. hpEastAsia is common in *H. pylori* isolates from East Asia also includes subpopulations, i.e. hspMaori (Polynesians, Melanesians, and native Taiwanese), hspAmerind (Amerindians), and hspEAsia (East Asians). hpAsia2 strains have been isolated in South, Southeast, and Central Asia. hpAfrica1 includes 2 subpopulations, hspWAfrica and hspSAfrica; hpAfrica2 is very distinct and has only been isolated in South Africa. hpNEAfrica is predominant in isolates from Northeast Africa. hpSahul strains are isolated from aborigines of Australia and highlanders in New Guinea.²⁴ This case report patient was categorized as hspEAsia, which

suggested that his descents were likely from East Asian region. It is also confirmed the previous study that the hspEAsia group mostly contained East-Asian-type *cagA* and *vacA* s1m1.¹⁷

Most patients with PUD present with abdominal discomfort, pain or nausea. The pain is located in the epigastrium and usually does not radiate. However, these symptoms are neither sensitive nor specific. Classically, GU pain is aggravated by meals, whereas the pain of duodenal ulcers is relieved by meals. Hence, patients with GUs tend to avoid food and present with weight loss, while those with duodenal ulcers do not lose weight.²⁵ Although it is still controversy about the ability of *H. pylori* infection as the initial or primary cause of the GU, there is no doubt of the value of *H. pylori* eradication leading to long-term healing of GU. Eradication of this bacterium improves GU recovery and is a primary and secondary prophylaxis to reduce the risk of recurrent ulcer bleeding. A meta-analysis suggested a remission rate of 97% of GU after successfully eradicating infection compared to 61% in patients with persistent infection. In addition, treatment of *H. pylori* infection is superior to ulcer healing drugs and reduces recurrent bleeding by 17% compared with ranitidine or omeprazole. The use of IV PPI is perhaps best established in the treatment of complicated PUD, and has largely replaced the use of H2RA. A meta-analysis of 24 randomized controlled trials with 4373 patients, comparing IV or oral PPI with placebo or H2RA in bleeding PUD, reported that PPI treatment in PUD bleeding reduces rebleeding and surgery compared with placebo or H2RA. Several studies have looked at the efficacy of PPIs, given in a combination of oral, IV bolus (defined as administration with an IV push at regular intervals) and high dose IV continuous infusion forms (usually preceded by an 80 mg bolus IV push, followed by an infusion at 8 mg/h), in achieving and maintaining this pH target goal of >6.²⁶

According to current guidelines, standard triple therapy containing a PPI and two antibiotics, amoxicillin and clarithromycin or metronidazole, is still the first-line regimen for treatment of *H. pylori* infection.²⁷⁻³⁰ However, in recent years,

the efficacy of legacy triple regimens has been seriously challenged and eradication rates lower than 70% are now reported in many countries.³¹ Unfortunately Indonesia only has old local antimicrobial resistance data, around 10 years ago and collected from 1 city which cannot be generalized across Indonesia.³² First-line treatment should be recommended on the basis of an understanding of the local prevalence of *H. pylori* antimicrobial resistance.^{33,34} The local antibiotic resistance surveillance update, selection of appropriate first-line regimen and detail evaluation of patient prior antibiotic usage are essential to combat *H. pylori* antibiotic resistance in Indonesia.

CONCLUSION

In addition to the positivity of *H. pylori* infection, the severity of gastroduodenal disease outcome could be explained by the difference of *H. pylori* virulence factors.

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