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

**Yudith A.A. Rezkitha¹, Muhammad Miftahussurur¹,²,³, Iswan A. Nusi², Ummi Maimunah², Pangestu Adi¹, Yoshio Yamaoka³**

¹ Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.
² Department of Internal Medicine, Faculty of Medicine Universitas Airlangga - dr. Soetomo Teaching Hospital, Surabaya, Indonesia.
³ Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu, Japan.

Corresponding Author:
Muhammad Miftahussurur, MD, PhD. Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu-City, Oita 879 5593, Japan. email: miphto@yahoo.co.id.

**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 ad immunohistochemistry). Analysis using sequencing based polymerase chain reaction found the patient infected by strain producing CagA and vacA s1m1. Further analysis using 7 housekeeping genes confirmed that the strain categorized to hspEAsia group. The patient was given continuous intravenous infusions of proton pump inhibitor and standard triple therapy regimens for 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 gastrointestinal 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 countries\(^7,9\) 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 *H. pylori* 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...
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

![Gastric ulcer](image1)

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

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

<table>
<thead>
<tr>
<th>Genes</th>
<th>Name of Primer</th>
<th>Primer sequences (5’→3’)</th>
<th>PCR product (bp)</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA</td>
<td>cagOMF AGCAAAAGCGACCTTGGAAA</td>
<td>521</td>
<td>95°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cagOMR AGTGCTCAAGCTCGTAAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vacA</td>
<td>s1/s2 VAI-F ATGGAATACAACAAACACAC</td>
<td>259/268</td>
<td>94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAI-R CTGCTTGAATGCGCCAAAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m1/m2 VAG-F CAACTGTCCAATACAGGAG</td>
<td>567/642</td>
<td>94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAG-R GCGTCAAATAATCCAAAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>atpA atpA-F GGAATCGGTAAACGCACG</td>
<td>821</td>
<td>94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>atpA-R CTGAAACGCCAACAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>efp efp-F GGCAATTGTGATGCGAGG</td>
<td>642</td>
<td>94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>efp-R CTGCCTCTTCAAGATCTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mutY mutY-F GTGTTGTTAYGGTGAACATTACA</td>
<td>639</td>
<td>94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mutY-R CTTAAGCCTGTGTTCTTACCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ppa ppa-F GGGAATTGCAATGTTTGG</td>
<td>676</td>
<td>94°C, 1 min; 53°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ppa-R GTGGGTATTTACGTGTTAAATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>trpC trpC-F TAGAATGCCAAAAACGATCCG</td>
<td>950</td>
<td>94°C, 1 min; 58°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>trpC-R TAAGGGCCGACACTTATTTTCGCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ureI ureI-F AGGTATCTGTAAGGTGCCG</td>
<td>875</td>
<td>94°C, 1 min; 53°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ureI-R GTTTTATACCCTTTAGATTGCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>yphC yphC-F CACGCCTTTTTTGACTAAAAAC</td>
<td>950</td>
<td>94°C, 1 min; 55°C, 1 min; 72°C, 1 min (35 cycles)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yphC-R CATYACCCTCAATGATGC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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, 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. 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. 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. 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. In general, strains with cagA positive...
(especially East-Asian-type \textit{cagA}) and \textit{vacA} \textit{s1m1} are considered to be more virulent and induced higher inflammation and/or atrophy than \textit{cagA}-negative, Western-type \textit{cagA}, and/or other \textit{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 \textit{H. pylori}, were rare in the indigenous populations but were common among the more recently arrived Chinese and Indian immigrants.\textsuperscript{18} It is suggested that the Chinese Indonesian might become a high-risk population of \textit{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 surveillance\textsuperscript{19} and also reported to give more detailed information about human population structure than the method using human microsatellite or mitochondrial DNA.\textsuperscript{20} Recently, the genomic diversity within \textit{H. pylori} populations was examined by employing the MLST method using 7 housekeeping genes (\textit{atpA}, \textit{efp}, \textit{mutY}, \textit{ppa}, \textit{trpC}, \textit{ureI}, and \textit{yphC}).\textsuperscript{21-23} At present, \textit{H. pylori} strains can be divided into seven population types on the basis of geographical associations and designated as follows: \textit{hpEurope}, \textit{hpEastAsia}, \textit{hpAfrica},\textsuperscript{1} \textit{hpAfrica2}, \textit{hpAsia2}, \textit{hpNEAfrica}, and \textit{hpSahul}.\textsuperscript{23} \textit{hpEurope} includes almost all \textit{H. pylori} strains isolated from ethnic Europeans, including people from countries colonized by Europeans. \textit{hpEastAsia} is common in \textit{H. pylori} isolates from East Asia also includes subpopulations, i.e. \textit{hspMaori} (Polynesians, Melanesians, and native Taiwanese), \textit{hspAmerind} (Amerindians), and \textit{hspEAsia} (East Asians). \textit{hpAsia2} strains have been isolated in South, Southeast, and Central Asia. \textit{hpAfrica1} includes 2 subpopulations, \textit{hpWAfrica} and \textit{hpSAfrica}; \textit{hpAfrica2} is very distinct and has only been isolated in South Africa. \textit{hpNEAfrica} is predominant in isolates from Northeast Africa. \textit{hpSahul} strains are isolated from aborigines of Australia and highlanders in New Guinea.\textsuperscript{24} This case report patient was categorized as \textit{hspEAsia}, which suggested that his descendants were likely from East Asian region. It is also confirmed the previous study that the \textit{hspEAsia} group mostly contained East-Asian-type \textit{cagA} and \textit{vacA} \textit{s1m1}.\textsuperscript{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.\textsuperscript{25} Although it is still controversy about the ability of \textit{H. pylori} infection as the initial or primary cause of the GU, there is no doubt of the value of \textit{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 \textit{H. pylori} infection is superior to ulcer healing drugs and reduces recurrent bleeding by 17% compared with ranitidine or omeprazole. The use of IV \textit{PPI} is perhaps best established in the treatment of complicated PUD, and has largely replaced the use of \textit{H2RA}. A meta-analysis of 24 randomized controlled trials with 4373 patients, comparing IV or oral \textit{PPI} with placebo or \textit{H2RA} in bleeding PUD, reported that \textit{PPI} treatment in PUD bleeding reduces rebleeding and surgery compared with placebo or \textit{H2RA}. Several studies have looked at the efficacy of \textit{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 \textit{>6}.\textsuperscript{26}

According to current guidelines, standard triple therapy containing a \textit{PPI} and two antibiotics, amoxicillin and clarithromycin or metronidazole, is still the first-line regimen for treatment of \textit{H. pylori} infection.\textsuperscript{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. Unfortunately Indonesia only has old local antimicrobial resistance data, around 10 years ago and collected from 1 city which cannot be generalized across Indonesia. First-line treatment should be recommended on the basis of an understanding of the local prevalence of *H. pylori* antimicrobial resistance. The local antibiotic resistance surveillance update, selection of appropriate first-line regimen and detail evaluation of patient prior antibiotic usage are essensial 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.

**REFERENCES**


