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Unique EPIYT Motif CagA Gene Helicobacter pylori Bali Isolates as The Cause of Hematemesis Melena in a Male Duodenal Ulcer Patient in Wangaya General Teaching Hospital Denpasar Bali

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ABSTRACT

52 years old male, Balinese, patient came to the hospital due to hematemesis one day prior to admission. In the emergency room, the doctor stabilizing patient condition with prompt treatment and giving intravenous proton pump inhibitor. Upper gastrointestinal endoscopy is done after improving of patient condition. We found a solitary giant ulcus in the bulbous duodenum area. Duodenal biopsy was performed and the Pathologist found rod-shaped microorganisms, confirming *Helicobacter pylori (H. pylori)* involvement. Polymerase Chain Reaction and sequencing of *H. pylori* revealed an EPIYA-ABD motif with unique motifs EPIYT on second EPIYA sequence. This is the first time in Bali we have sequencing of *H. pylori* gene, then isolate of *H. pylori* named then *as H. pylori* Bali 03 isolate.

Standard protocol of *H. pylori* eradication is conducted by administration of triple-drug therapy consisting of proton pump inhibitor (PPI), two antibiotics such as clarithromycin and amoxicillin, and for 14 days. Patient discharged from hospital in stable condition with successful treatment of *H. pylori.* Esophagogastroduodenoscopy reevaluation was done in the next 3 months later and revealed completely ulcer resolved.

Keywords: *Helicobacter pylori*, isolate Bali 03, Endoscopy

INTRODUCTION

Peptic ulcer is a chronic disease due to imbalance of protective and aggressive factors in the gastrointestinal area. *H. pylori* is the one of the aggressive factor found by Marshall and Warren. *H. pylori* infects almost 50% of the world population. Clinical presentation varies from asymptomatic to gastric cancer may appear.[1] We would like to present one case that *H. pylori* isolates Bali 03 as the cause of duodenal ulcer.

CASE REPORT

A male, 52 years old from Denpasar, came to the hospital with chief complaint of hematemesis since one day before admission. He felt nausea and abdominal discomfort since 3 months before admission. Pain was felt in the center of the abdomen after eating, bloating reported, and it worsen in the middle of the night. The patient reports use of analgetic and antacid to release the pain but it did not help the symptoms. History of non-steroidal inflammatory drug (NSAID) consumption was denied. History of weight loss was denied. History of alcohol and smoking was denied. History of previous illnesses and chronic diseases such as diabetes mellitus and cardiovascular diseases were denied. No history of gastric cancer or chronic disease in the family.

Based on physical findings we found stable blood pressure, 130/70 mmHg, respiratory rate 18 times/minute, pulse rate 89 beep/ minute, Abdominal examination revealed pain on palpation of gastric area, inspection, and auscultation were within normal limits. No anemia sign was found. Laboratory results were within normal limits with white blood cell (WBC) 9 x 10^3 gr/dl, hemoglobin 10 gr/dl, platelet 156 x10^3 gr/dl. Liver function and kidney function tests were also within normal limits. The patient diagnosed with hematemesis due to suspected of peptic ulcer. In the emergency room, the doctor in charge performed initial examination, giving intravenous fluid of ringer lactate infusion, intravenous PPI 40 mg, and maintenance dose by using syringe pump of PPI 8 mg/hour. Patient is fasted for 4 hours to evaluate the bleeding and scheduled for esophagogastroduodenoscopy after admission to the ward.

One day after admission the patient condition was stable. No more hematemesis, but melena reported. No sign of anemia found. Vital sign was normal. Gastric cooling was clear, and the the nasogastric tube was released from the nose. Patient ready to do esofagogastroduodenoscopy. Esophagogastroduodenoscopy revealed esophagus was normal, a solitary giant ulcer in the bulbous duodenum with Forrest III classification (Figure 1).

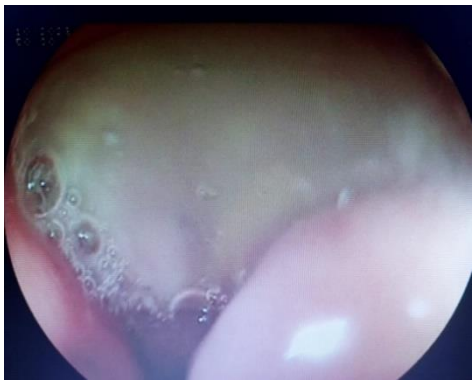

Figure 1. Solitary giant ulcer in the bulbous duodenum area.

Duodenal biopsy was obtained and then examined by pathologist. Histopathology report shown discontinuity of epithelial duodenal mucosa and many polymorphonuclear and lymphoplasmacytic cells (Figure 2). Spherical and rod-like organism, suggesting *H. pylori* was also found.

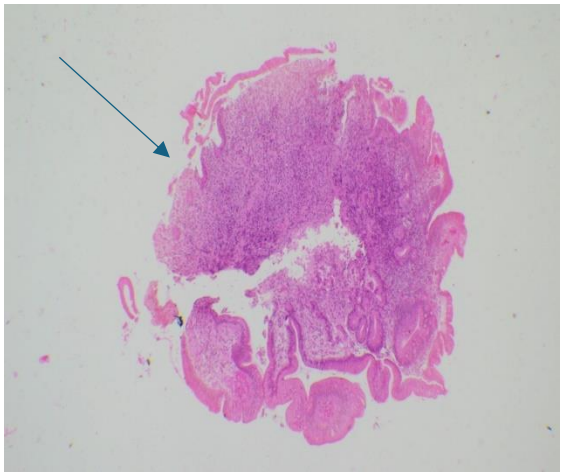

Figure 2. Histopathology shows discontinuity of the epithelial duodenal layer (blue arrow).

We sent the duodenal biopsy to the Biomedical laboratorium at West Nusa Tenggara Regional Hospital to do the culture of gastric biopsy. The bacteria was growth in the blood agar, the shape is small round, white to yellowish color. Polymerase Chain Reaction and sequencing of *H. pylori* revealed an EPIYA-ABD motif with unique motifs EPIYT on second EPIYA sequence. This is the first time in Bali we have sequencing of *H. pylori* gene, then isolate of *H. pylori* named then *as H. pylori* 03 Bali isolate. So the diagnosis of this patient confirmed as Hematemesis due to duodenal ulcer area due to *H. pylori* Bali 03 isolate.

Standard protocol of *H. pylori* eradication conducted by administration of triple-drug therapy consisting of Omeprazole 20 mg BID, Amoxicillin 1 gram BID, and clarithromycin 500 mg BID for 14 days. Patient discharged from hospital in stable condition with successful

eradication of *H. pylori.* Reevaluation of esophagogastroduodenoscopydone in the next 3 months later and revealed completely ulcer resolved with normal in the bulbous duodenum area (Figure 3).

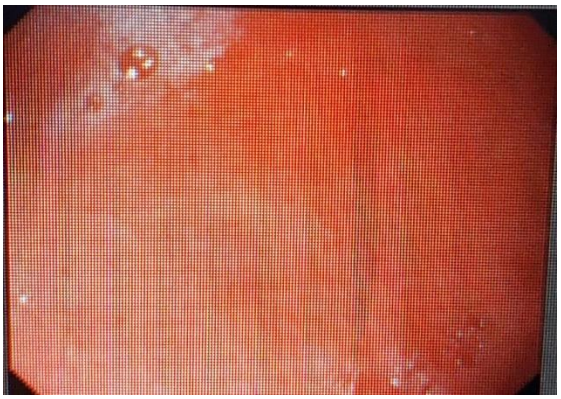

Figure 3. Reevaluation endoscopy after 3 months shown the bulbus duodenum area was normal.

DISCUSSION

Peptic ulcer disease (PUD) may present as asymptomatic or with symptoms. Peptic ulcer disease presents with symptoms that include pain in the gastric area, especially after meals. Sometimes we also find such as fullness sensation in the stomach, discomfort and another symptoms. In severe cases, symptoms include melena, hematemesis, and loss of body weight.[1]

In our patient, he presents with hematemesis and abdominal pain occurring directly after eating. PUD Etiology includes *H. pylori* infection, alcohol, steroids, stress, bile acids, NSAIDs, pepsin, smoking, and changes in gastric mucin consistency. Other causes include Zollinger-Crohn, Ellison syndrome, chronic liver disease, and similar symptoms of stomach cancer. PUD symptoms are commonly nonspecific and diagnosis based on anamnesis. Patient may present with epigastric pain, nausea, flatulence, and heartburn. In the case of posterior ulcer, symptoms of pain radiating to the back may occur.[2]

Peptic ulcers may relieved spontaneously and the symptoms may be intermittent. Complications may be life-threatening and occur abruptly such as perforation. These symptoms most commonly found in geriatric patients with history of NSAID consumption. Some of the most serious complications bleeding and perforation. Bleeding can be both gradual and abrupt. If abrupt bleeding occurs, it causes black, tarry stools and hypovolemic shock. Only small number of people with a peptic ulcer require surgery. Perforation usually causes sudden abdominal pain and some cases require surgery.[2]

If we suggest the diagnosis is peptic ulcer, we must evaluate for alarm symptoms such as anemia, hematemesis, melena, or heme-positive stool suggests bleeding, obstruction, anorexia or weight loss suggests cancer and severe, spreading upper abdominal pain suggests perforation.

In our case, we conducted esophagogastroduodenoscopy (EGD) after stabilizing the patient. Duodenum biopsy was performed to evaluate the cause of the ulcer. We found the *H. pylori* is the primary cause of ulcer because the characteristic of duodenal ulcer is big, solitaire, and also from the anamnesis there were no history of taking analgetic or NSAID in a frequent times. Before EGD performed, we stabilized the patient's condition with intravenous fluid, giving of proton pump inhibitor infusion of esomeprazole bolus 40 mg and maintenance by giving dose of 8 mg/hour until 72 hours with syringe pump, antacid, and sucralfate syrup also given.

Duodenal biopsy also conducts culture of biopsy, then the bacteria growth. Polymerase chain reaction performed and the result revealed a 640 bp single band, confirmed *H. pylori*. This isolate was then named *H. pylori* Bali 03 (BLI03) isolate (Figure 4).

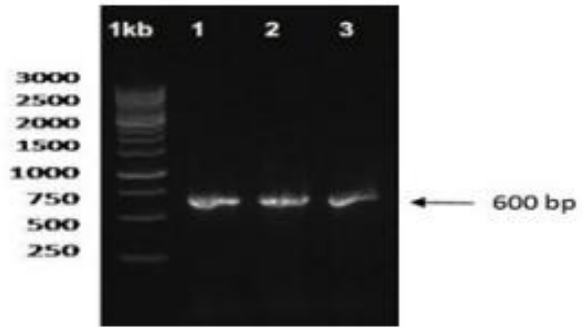

Figure 4. Electrophoresis of 640 bp CagA. PCR products amplified from *H. pylori* genomic DNA Bali isolates.[3]

We sequenced the isolate in Singapore to know the sequence of the deoxyribonucleic acid (DNA) and the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs of cytotoxin-associated antigen A (CagA) of the BLI03 isolates. *Helicobacter pylori* Bali 03 isolate examined was positive for CagA gene. Substitution A to T in EPIYA motif is indicated in red eclipse (Table.1). It was found that low variation of the amino acid sequence C-terminal contained EPIYA motif of CagA BLI03 isolates. BLI03 isolates have EPIYA ABD motifs with unique EPIYA motifs on second EPIYA sequence.[3]

Table 1: CagA of *H. pylori* Bali 01 (BLI 03) isolates, the EPIYA motifs. [3]

Species/Abbrv	***** ***** ***** ** * ***** ****** ** **
1. H._pylori_BLI-01	NGLKNSEEPIYAQVNKKKAGQAASPEEPIYTQVAKKVNARIDRLNK
2. H._pylori_BLI_02	NGLKNSEEPIYAQVNKKKAGQAASPEEPIYTQVAKKVNARIDRLNK
3. H._pylori_BLI_03	NGLKNSEEPIYAQVNKKKAGQAASPEEPIYTQVAKKVNARIDRLNK
4. GU173854_(Philippines)	NGLKNNEEPIYAQVNKKKAGQAASPEEPIYAQVAKKVSAKIDQLNE
5. FJ458163_(Korea)	NGLKNNTEPIYAKVNKKKAGQAASPEEPIYAQVAKKVSAKIDQLNE
6. EU369652_(Indian-Malay)	NGLKN--EPIYAQVNKKKAGQAASHEEPIYAQVAKKVNAKIDRLNQ
7. giAB017923_(Jepang)	NGLKNNTEPIYAQVNKKKAGQATSPEEPIYAQVAKKVSSKIDQLNE
8. KF028589_(China)	NGLKNNTEPIYAQVNKKKAGQATSPEEPIYAQVAKKVSAKIDQLNE
9. DQ306710_(Central_China)	NGLKN--EPIYAQVNKKKAGQATSPEEPIYAQVAKKVSAKIDQLNE
10. LC007101(Indonesia)	NGLKNNTEPIYAQVNKKKIGQVASPEEPIYAQVAKKVSAKIDQLNE
11. GU173879_(Thailand)	NGLKN--EPIYAKVNKKKTGQAASLEEPIYAQVAKKVNAKIDRLNQ

Phylogenetic analysis and determination of the EPIYA pattern include CagA 32 region nucleotide sequences of 3 isolates of which are 3 Bali isolates and 8 isolates from other countries retrieved from GeneBank (Figure 5). To determine the phylogenetic relationship between Lombok isolates and those from other countries the nucleotide sequences of CagA 32 region was multiple aligned and Maximum Likelihood phylogenetic tree was constructed using the CLUSTALW program.

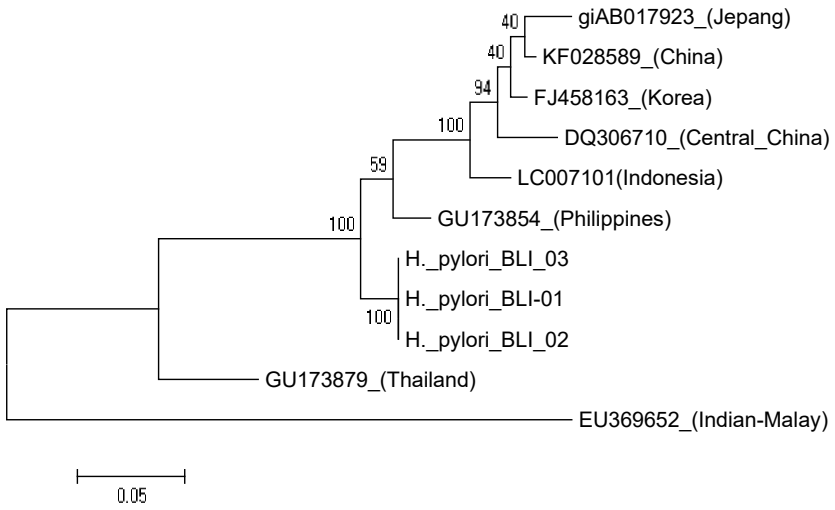

Figure 5. Phylogenetic tree based on the cagA3' variable regions of *H. pylori* Bali isolates compared with 8 reference strains. Maximum Likelihood trees of 5% partial conservation sequence of *H. pylori* isolate Bali BLI_03.[3]

H. pylori is a negative gram anaerobic bacterium that colonizes the human stomach and causes gastric diseases such as chronic gastritis and peptic ulcers.[4] Infecting mostly 50% of the world's population. *H. pylori* status among patients influenced by age, living conditions, population density, and socioeconomic status. Transmission is usually through fecal-oral or

oral-oral, with more cases from the latter and usually occurring among members of the same family, such as parents and children.[5-6]

Most *H. pylori* infections are asymptomatic, but most people with an infection have variable degrees of gastritis, that may become severe symptoms over time, with an estimated 25 to 30% of infected individuals developing dyspepsia, peptic ulcer, and even gastric cancer. Data shows that 70% of gastric ulcers and 80% of duodenal ulcers caused by *H. pylori.* Peptic ulcers are the most prevalent gastrointestinal disorders in the world, cause chronic gastritis, ulcer, and further may cause intestinal bleeding. Increasing risk in previous history of infection, although previous successful eradication therapy, recurrence rate of 4% for gastric ulcer. The gut microbiome is a dynamic environment influenced by several factors, such as host lifestyle, long using of PPI, antibiotic treatment, and *H. pylori* infection. infection as well as eradication of *H. pylori* and the interaction within the gut microbiota can change the microecological balance, influence the onset and progression of gastrointestinal disorders. *H. pylori* is capable of adapting to the gastric acid environment and interact with host cell receptors in order to make a colony in the gaster.[7]

There are several mechanisms in which *H. pylori* can cause successful colonization in the gastric environment and then migration to duodenum. After colonizing the stomach, it can form biofilms, interfere with metabolic pathways, induce neuroimmune mechanisms, and cause imbalance in gastric barrier homeostasis. *H. pylori* has many factors such as urease, bacterial shape, and flagella which mediate its movement to successfully colonize the gastric and duodenal mucosa. After entering the gastric and duodenal mucosa, *H. pylori* releases an abundance of intracellular urease, around 10% of its protein production.[6,7] Urease causes hydrolysis resulting in production of ammonia and carbamate, further breaking down into ammonia and carbonic acid. With the presence of water, ammonia then neutralizes stomach acidity, resulting in an almost neutral environment around *H. pylori,* easing its ability to infiltrate the gastric mucosa. The bacterial shape of *H. pylori* allows it to move through gastric contents and adapt to the acidic environment. It uses its flagella to move, allowing bacteria to enter the gastric mucosa. There are 4 to 8 sheathed flagella on single or both poles of the bacterium. The flagella adapt its movements based on the media in which the bacterium is located. Some studies shown that several mutations in genes that encode specific flagellar proteins such as fliD, FlaA and FlaB impair the proper motility of *H. pylori*, which can reduce or even cease its capacity to colonize the gastric mucosal layer.[5-7] It interacts with surface

adhesin mucin 5 (MUC5AC), which helps colonization on gastric mucosa. Increased production of MUC5AC may ease bacterial infiltration. These adhesins also contain neutrophil-activating proteins, further stimulating production of free radicals, leading to tissue damage as well as production of IL-8.[6,8]

There are chemoreceptors involved such as T1pA, B, C, and D, CheA kinase and coupling proteins, that also important for bacteria colonization. Adhesion molecules and surface receptors of gastric cells are also important in the interaction between bacteria and host. One of these molecules is the blood group antigen-binding adhesin A (BabA). Bacteria with high BabA expression are more virulent and can increase risk of duodenal ulcer and gastric adenocarcinoma.[8]

CagA is a bacterial protein that induces specific modifications in the morphology of epithelial cells while also altering cell polarity. Other non-CagA virulence factors include VacA (vacuolating cytotoxin). This factor increases ability of *H. pylori* to change gastric homeostasis by forming acidic vacuoles in the gastric epithelial cells cytoplasm. It disturbs the integrity of cell components, causing it to collapse. It promotes activation and suppression of immune response, induces immune tolerance, persistent *H. pylori* infection, enhancing the risk of gastritis, peptic ulcer, and cancer. Another factor is DupA, which has higher acid resistance towards *H. pylori* and increaseingIL-8 within mucosa of gaster. All these proteins and factors play a role in accelerating mucosal inflammation, polymorphonuclear leukocyte infiltration further increasing risk of gastritis and duodenal ulcers.[8-11]

CONCLUSION

We must think the possibility of *H. pylori* infection if we find a solitaire giant ulcer in the gaster, The gastric biopsy should be performed to know the cause of peptic ulcer until there is no data may exclude the *H. pylori* infection as the cause of ulcer. Eradication of *H. pylori* should performed immediately to cure the ulcer as well as no *H. pylori* no ulcer theory.

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