CASE REPORT

Fatal Diarrheal Disease Caused by Vibrio cholerae O67 in a Patient with Myelodysplastic Syndrome

Shinobu Tamura1, Fumitaka Taniguchi1, Chiaki Nakamoto2, Hiromichi Nakamoto3, Eiji Arakawa1, Takahiko Fukuchi1, Yoshio Nakano1 and Tokuzo Fujimoto1

Abstract

A 71-year-old man with myelodysplastic syndrome (MDS) receiving treatment with azacitidine developed extensive watery diarrhea for three consecutive days. As a result of high-grade dehydration, the patient was urgently admitted to the hospital and fluid replacement therapy was initiated. However, the patient’s diarrhea did not improve. Vibrio cholerae non-O1/non-O139 was detected in a fecal culture. On the fourth day, the patient died due to circulatory collapse. An autopsy revealed extensive necrosis of the intestinal mucosa. Vibrio cholerae non-O1/non-O139-induced diarrheal disease often develops in patients with hepatic cirrhosis and has a serious clinical course. We herein report a fatal outcome of Vibrio cholerae O67 infection in an immunocompromised MDS patient.

Key words: Vibrio cholerae non-O1/non-O139, bacterial diarrheal disease, myelodysplastic syndrome, azacitidine, intestinal virulence factors


Introduction

While Vibrio cholerae is classified into more than 200 species based on the O-antigen of lipopolysaccharide, only two serogroups, i.e., O1 and O139, including the cholera toxin induce epidemic cholera (1). The other serogroups are known as Vibrio cholerae non-O1 (nontoxigenic Vibrio cholerae) and extensively inhabit the aquatic environment around Southeastern Asia (2, 3). Similar to Vibrio cholerae O1 and O139, some Vibrio cholerae non-O1/non-O139 are enteropathogenic and have been reported to cause cholera-like epidemics (2-4). Among afferent infectious diseases, these serogroups are one of the main causative organisms of bacterial diarrheal disease (5). Several studies have reported that some Vibrio cholerae non-O1/non-O139 induce bactere mia in patients with underlying diseases, including hepatic cirrhosis and hematological disorders, which can become serious (6-8). The number of cases of Vibrio cholerae non-O1/non-O139-induced bacterial diarrheal disease in developing countries has increased in recent years (2-5). In Japan, isolation of Vibrio cholerae non-O1/non-O139 has also been reported in various regions of river water as well as fish and shellfish (5). In the United States, Vibrio cholerae non-O1/non-O139 infections are associated with a higher mortality rate (2.6-5%) compared with Vibrio cholerae O1/O139 (0-0.5%), according to recently reported surveillance data from 1996 to 2010 (9).

According to a report by Yamai et al., Vibrio cholerae non-O1/non-O139 with the cholera toxin accounts for only 2% of the 1898 strains identified to date (10). Nevertheless, some patients with the same severe diarrhea as that caused by Vibrio cholerae O1 and O139 have been reported, which suggests the presence of intestinal virulence factors other than the cholera toxin (3, 8). We herein present a case in which an immunocompromised patient with myelodysplastic syndrome (MDS) who received azacitidine developed Vibrio cholerae non-O1/non-O139-induced bacterial diarrheal disease that resulted in a fatal outcome.

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Table. Laboratory Data

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<th>Complete Blood Count</th>
<th>AST</th>
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<tr>
<td>White Blood Cells</td>
<td>4,100/μL</td>
<td>58IU/L</td>
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<td>Neutrophil</td>
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<tr>
<td>Lymphocyte</td>
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<tr>
<td>Monocyte</td>
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<td>Eosinophil</td>
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<tr>
<td>Basophil</td>
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<tr>
<td>Red Blood Cells</td>
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<tr>
<td>Hemoglobin</td>
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<td>9.4g/dL</td>
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<tr>
<td>Hematocrit</td>
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<tr>
<td>Platelet</td>
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<table>
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<tr>
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<th>Base Excess</th>
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<tbody>
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<td>Cr</td>
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<td>-26.7mM/L</td>
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<tr>
<td>BUN</td>
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<tr>
<td>Sodium</td>
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<td>PaCO2</td>
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<td>Potassium</td>
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<td>HCO3</td>
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<td>O2</td>
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<tr>
<td>Phosphorus</td>
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<td>Lactic Acid</td>
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</table>

**Case Report**

A 68-year-old man was referred to our hospital for pancytopenia. He was diagnosed with refractory cytopenia with unilineage dysplasia classified according to the World Health Organization classification system and was defined as low-risk according to the International Prognostic Scoring System. After receiving the diagnosis of MDS, he was followed up at our Outpatient Department. Two years previously, he began to receive regular transfusions, due to the progression of anemia, and chemotherapy with azacitidine, a molecular-targeted drug, due to MDS, at an alternative dosing schedule of 75 mg/m²/day subcutaneously for five days every four weeks (11). Seven courses of azacitidine therapy for MDS were completed, and the transfusions were no longer required. At that time, the patient was able to walk to the Outpatient Department without complaints or marked changes in his blood cell count (white blood cells: 2,000/μL, with 60% neutrophils, hemoglobin: 9.5 g/dL, platelet count: 6.5×10^4/μL).

Five days after the consultation, he suddenly developed watery diarrhea. The diarrhea became exacerbated, with the patient experiencing 20 episodes of diarrhea in one day. Since his symptoms did not improve for three successive days, the patient was transferred to the Emergency Department at our hospital via ambulance. During the medical interview conducted on admission, the patient explained that he had not travelled overseas and had not eaten fish or shellfish before the onset of diarrhea. He used mountain water as his daily water supply. A physical examination performed on admission revealed hypotension without fever. The patient presented with oral dryness and poor skin turgor; however, he did not have abdominal tenderness. Laboratory examinations revealed high-grade dehydration and metabolic acidosis with large amounts of watery diarrhea (Table). Simple computed tomography (CT) disclosed a small amount of intestinal fluid, although it did not show any infectious foci in other regions. Due to the patient’s high-grade dehydration, fluid replacement with normal saline solution was initiated from the time of admission. Various cultures, including those of fecal and blood specimens, were performed. Despite the administration of fluid replacement with normal saline solution at 250 mL/hr, the patient’s laboratory results did not improve. Large amounts of watery diarrhea persisted. On the same day, the patient entered the intensive care unit (ICU) because further fluid replacement and electrolyte correction were required. The levels of electrolytes were checked every hour, and the infusion of normal saline solution was increased to 1,000 mL/hr. On the second day, we introduced continuous hemodiagnosis because the patient’s renal function would take a long time to recover. In the early morning of the third day, the patient developed abdominal distension and pain, and the large amounts of watery diarrhea persisted. The laboratory results obtained at that time showed a white blood cell count of 1,300/μL (with 72% neutrophils), a hemoglobin level of 10.8 g/dL, a platelet count of 3,000/μL, and a creatinine level of 3.77 mg/dL, a blood urea nitrogen level of 57.6 mg/dL, and a creatine kinase (CK) level of 218 IU/L and a C-reactive protein level of 11.2 mg/dL. The patient exhibited a fever, and a single dose of 1 g of cefazidime was administered. When CT was performed again, paralytic ileus accompanied by large amounts of watery diarrhea was diagnosed (Fig. 1B). Although ileus tube insertion succeeded in relieving the patient’s symptoms, the amount of drainage through the ileus tube was high (more than 3,000 mL/day). The patient’s metabolic acidosis did not improve, the levels of lactate acid and CK increased to 66 and 3,630 IU/L, respectively, and circulatory failure and a decreased level of consciousness were noted. These findings indicated the presence of intestinal necrosis or perforation; however, the patient was unable to undergo surgery due to his poor general condition. Simultaneously, a fecal culture obtained on the first day was found to be positive for *Vibrio cholerae* non-O1/non-O139. On the fourth day, despite the administration of catecholamines, the patient developed serious circulatory failure. He did not have stable vital signs and ultimately died in the ICU.

An autopsy was performed on the same day. The macroscopic findings showed extensive erosion and necrosis extending from the jejunum through the descending colon (Fig. 2A, B). The microscopic findings indicated necrosis of the entire intestinal mucosa and diffuse submucosal hemorrhage; however, no microthrombi suspicious of disseminated intravascular coagulation were noted (Fig. 2C). At a later date, we evaluated the O-serogroup and intestinal virulence factors. The serogroup of *Vibrio cholerae* non-O1/non-O139 was O67. Regarding intestinal virulence factors, the cholera...
toxin was not found; however, a polymerase chain reaction (PCR) assay detected hly (hemolysin (El Tor type)), hap (matrix metalloprotease) and rtxA (repeat toxin) (Fig. 3).

**Discussion**

It is well known that bacterial diarrheal disease caused by *Vibrio cholerae* non-O1/non-O139 can become serious in patients with underlying diseases. Hepatic cirrhosis, in particular, can readily cause *Vibrio cholerae* non-O1-induced bacteremia, with a mortality rate of approximately 50% (6, 8, 12). Hematological diseases have also been reported to cause *Vibrio cholerae* non-O1/non-O139-induced bacteremia (6, 13, 14). Although blood cultures were obtained in our case, *Vibrio cholerae* non-O1/non-O139 was not detected. Although this case did not progress to bacteremia, the patient developed extensive intestinal necrosis that resulted in a fatal outcome. One reason why this patient experienced a serious clinical course was that he was an immunocompromised host with MDS, was receiving azacitidine and had an impaired local immunological defense mechanism in the intestines. The side effects of azacitidine, including myelosuppression and infection, were mild to moderate and manageable with the alternative dosing schedule (11). For seven months, our patient received chemotherapy without experiencing serious adverse events. However, when his condition became serious on the third day, the neutrophil count increased slightly. These findings suggest that *Vibrio cholerae* O67 taken into the intestines was proliferating at that time.

Second, the identified *Vibrio cholera* O67 possessed some intestinal virulence factors that were considered to be serious. Hemolysin, repeat toxin, heat-stable enterotoxin and Shiga-like toxin have been identified to be intestinal virulence factors in addition to the cholera toxin (3). A molecular analysis of *Vibrio cholerae* non-O1/non-O139 isolated in Calcutta, India in 2003 frequently detected the El Tor type hemolysin and rtxA gene and found that these toxins acted cytotoxically (3). The El Tor type hemolysis toxin exerts a cytolitic effect on intestinal epithelial cells and mucosal cells, consistent with the clinical presentation of *Vibrio cholerae* non-O1/non-O139 (15). The intestinal toxin, RtxA, is thought to destroy the cytoskeleton and exhibits the same effects as El Tor type hemolysin (16). In this case, the hly and rtxA genes were detected, both of which became presumably potent intestinal virulence factors. Matrix metalloprotease
produced from *Vibrio cholerae* has been shown to intensify intestinal toxic effects (17). In this case, the *hap* gene, one of these proteases, was also detected and may have been involved in the acceleration of other virulence factors. Other causes of the patient’s fatal outcome are likely to include the virulence factors we identified in this case.

Third, despite the administration of fluid replacement for high-grade dehydration, paralytic ileus and intestinal necrosis rapidly progressed. On the third day of admission, the patient’s abdominal symptoms suddenly appeared and the laboratory results showed increased levels of lactic acid and CK as well as metabolic acidosis, findings that are indicative of severe intestinal ischemic changes. Our autopsy findings showed that the intestinal blood flow was inadequate from the jejunum through the descending colon without thromboembolic occlusion of the mesenteric arteries or veins. The segmental intestinal ischemic changes observed in this case appeared to be associated with nonocclusive mesenteric ischemia (NOMI) due to vascular spasms (18, 19). NOMI is primarily involved in states of hypoperfusion, including circulatory failure, surgery, hemodialysis and sepsis. On admission, the patient was also at high risk of NOMI, as previously reported (18, 19). In addition, the intestinal ischemia was thought to have worsened due to dehydration lasting for a long period of time prior to admission. Therefore, we should have initiated fluid replacement as early as possible when the diarrheal disease first developed.

Antibiotics comprise adjunctive therapy in patients with *Vibrio cholerae* non-O1/non-O139-induced diarrheal disease due to its rare occurrence. On the other hand, the administration of antibiotic therapy for *Vibrio cholerae* non-O1-induced bacteremia associated with hepatic cirrhosis decreases the mortality rate (21). In addition, providing early antibiotic therapy for bacteremia in a cirrhotic patient resulted in a good outcome, as reported by Petsaris et al. (12). The *Vibrio cholerae* O67 isolates obtained in this case were sensitive to most of the antibiotics tested. Although the patient developed renal failure, the condition may not have become serious if antibiotics had been administered at an early stage.

We encountered an autopsy case of MDS complicated by bacterial diarrheal disease caused by *Vibrio cholerae* non-O1. Despite treatment with fluid replacement and ICU management, the patient ultimately developed intestinal necrosis, which led to a fatal outcome. Our findings revealed that *Vibrio cholerae* non-O1/non-O139 can be a lethal pathogen in immunocompromised patients receiving chemotherapy, such as azacitidine. In such patients, *Vibrio* infection should be suspected in cases of large amounts of watery diarrhea and high-grade dehydration, for example, due to *Vibrio cholerae* O1/O139, and physicians should initiate aggressive rehydration and empiric antibiotic therapy as early as possible.

The authors state that they have no Conflict of Interest (COI).

### References


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