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Brief Communication

Successful treatment by fecal microbiota transplantation for Japanese patients with refractory *Clostridium difficile* infection: A prospective case series

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Abstract We prospectively enrolled four Japanese patients with refractory *Clostridium difficile* infection (CDI) and were treated with a single fecal microbiota transplantation (FMT). The average age of the patients was 83.7 years. All patients had a successful clinical course for up to 3 months without any adverse events.

Introduction

Clostridium difficile (*C. difficile*) is a Gram-positive, spore-forming anaerobe that is colonized in 5%–15% of healthy adults and 50% or more of hospital patients without any symptoms.[1,2] Administration of antibiotics in patients with *C. difficile* colonization induces alterations of the gut microbiota (dysbiosis). Overgrowth of *C. difficile* and production of *C. difficile* toxin result in *C. difficile* infection (CDI). Vancomycin or metronidazole is used to treat CDI, but 15%–30% of patients experience recurrence of symptoms after discontinuation of antibiotics.[2] Fecal microbiota transplantation (FMT) can restore the diversity of the microbiota to that of healthy donors.

The first randomized, controlled trial of FMT for CDI was published in 2013 by van Nood et al.[3] They reported marked efficacy of FMT for 81% of patients with refractory CDI after the first infusion of donor feces through a nasoduodenal tube. Recently, systematic reviews and meta-analyses have established the efficacy and safety of FMT for refractory

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CDI.[4] Given these results, the latest guidelines for the management of CDI recommend FMT for relapsing CDI.[2]

Major outbreaks associated with the *C. difficile* NAP1/027 strain have been described since 2004, first in Canada, followed by the USA and Europe. In Japan, there are a few sporadic reports related to this strain. Most of the refractory cases of CDI are not caused by the NAP1/027 strain in Japan. FMT is an important option for treatment in refractory CDI. We report four cases of FMT for refractory CDI with successful clinical courses.

Methods

Study design and ethics

This was a single-center, open-label study to assess the efficacy and safety of FMT in patients with refractory CDI. The study was conducted from February 2013 to March 2017 at Shiga University of Medical Science Hospital. The research protocol was approved by the Ethics Committee of the Shiga University of Medical Science. The study was registered at the University Hospital Medical Information Network Center (UMIN0000020766).

Study participants

Patients ($\geq$2 years) with a relapse of CDI after at least one course of adequate antibiotic therapy ($\geq$10 days of vancomycin at $\geq$500 mg per day, or $\geq$10 days of metronidazole at $\geq$750 mg per day) were included. CDI was defined as diarrhea (three or more loose or watery stools per day for at least two consecutive days) with either a positive *C. difficile* toxin stool test or a positive *C. difficile* stool culture test and endoscopically confirmed pseudomembrane. The exclusion criteria were compromised immunity, pregnancy, use of antibiotics other than for *C. difficile* infection, and poor general condition precluding colonoscopy.

Fecal microbiota transplantation

Donors ($\geq$10 years and $\leq$60 years of age) were selected from family members or unrelated healthy volunteers and screened by stool and serological tests. The serological or blood tests were Hepatitis virus A, B, C, Human immunodeficiency virus-1 and -2, Human T-cell lymphoma virus, Cytomegalovirus, Epstein-Barr virus, *Entamoeba histolytica*, *Mycobacterium tuberculosis*, Syphilis, Parasite antibody panel (*Dirofilaria immitis*, *Toxocara canis*, *Ascaris suum*, Anisakis, Gnathostoma, *Strongyloides stercoralis*, *Paragonimus westetrmanii*, *Paragonimus miyazakii*, *Fasciola* spp., *Sparganosis mansoni*, *Cysticercus cellulosae*). The stool tests were Culture for enteric pathogens including Salmonella, Shigella, Vibrio, *Escherichia coli* O157, Campylobacter, *C. difficile* toxin, *C. difficile* antigen, Ova and parasite examination. Donor exclusion criteria included a history of antibiotic treatment during the 3 months before donation; a history of gastrointestinal illnesses, including inflammatory bowel disease, irritable bowel syndrome, chronic constipation, recent diarrhea episode, gastrointestinal cancer, or major gastrointestinal surgical procedures; and metabolic syndrome, obesity (body mass index $\geq$30 kg/m^2), hypertension, or moderate to severe malnutrition.

Patients ceased antibiotic treatment one day before FMT. Fresh donor feces (approximately 150 g) obtained within 6 h before FMT were dissolved in normal saline, homogenized and filtered to make a liquid slurry. Fecal material was administered to the cecum by colonoscopy following standard bowel preparation with polyethylene glycol (Niflec®, EA Pharma, Tokyo, Japan). All patients were required to come to our hospital twice, at the first visit and on the day of FMT.

Clinical outcomes

The primary endpoint was a clinical response at 3 months after FMT. Clinical symptoms were checked every 2 weeks up to 3 months. Fecal condition was evaluated by the Bristol Stool Scale. A *C. difficile* toxin test and stool culture were performed 3 months after FMT. Relapse was defined as diarrhea with a positive *C. difficile* toxin test. The fecal samples were obtained every 2 weeks up to 6 weeks. DNA was extracted from fecal samples and analyzed by polymerase chain reaction (PCR) and terminal restriction fragment length polymorphism (T-RFLP) as previously described.[5]

Results

The clinical course of Case 1 was shown in Fig. 1. The clinical data of the patients and the donors were summarized in Table 1. Except for Case 2, the diagnosis of CDI was based on clinical symptoms, such as diarrhea and fever, and a positive *C. difficile* toxin test. In Case 2, clinical symptoms and a positive stool culture for *C. difficile* were observed. However, testing for *C. difficile* toxin was consistently negative. The diagnosis of CDI was made from the colonoscopic findings of pseudomembrane.

In our case series, the recipients were all elderly, with average age of 83.7 years. All patients had a history of taking antibiotics prior to the onset of CDI and were refractory to multiple pulsed regimens of vancomycin or metronidazole.

The FMT procedure was done on an outpatient basis except for Case 2. This patient had to be admitted for 3 days for safety because of concomitant congestive heart failure. Colonoscopy for FMT was performed in all patients. In Case 3, the FMT was performed from the sigmoid colon, because this patient complained of pain during insertion. In all other cases, the colonoscope was inserted to the cecum and a liquid slurry was dispersed from the cecum.

All patients had successfully recovered from CDI. No recurrence of symptoms was observed after 3 months of FMT. No adverse events were observed.

Analysis of a fecal sample was done in Case 1. As shown in Fig. 1, the composition of the fecal microbiota was disturbed upon vancomycin treatment before FMT. The diversity of fecal microbiota increased after FMT.

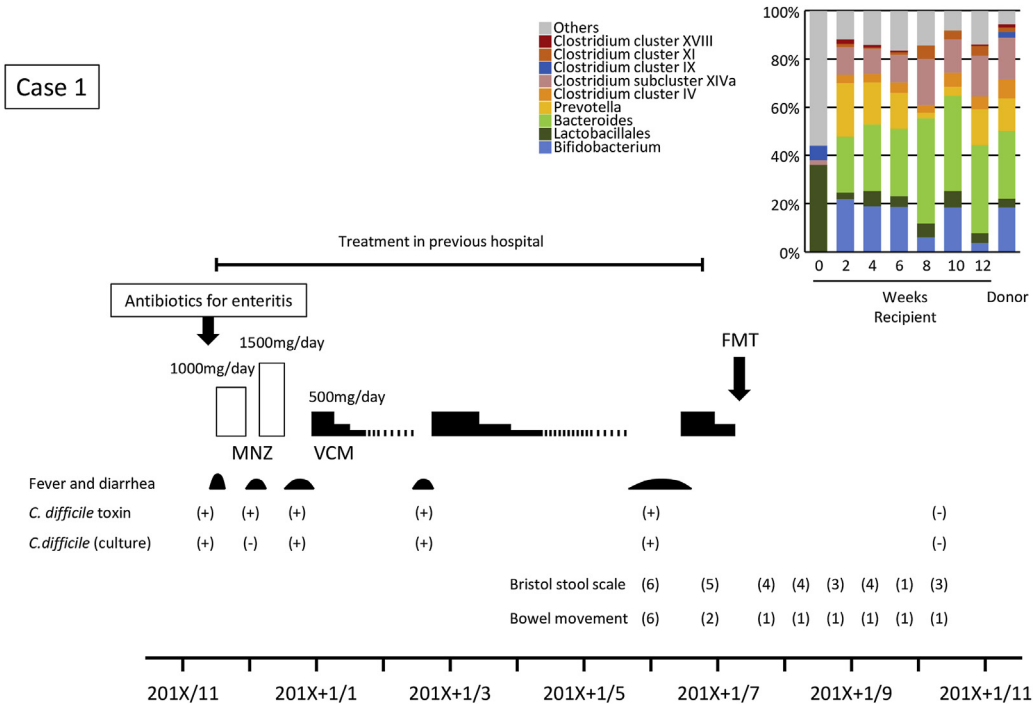

Figure 1. Clinical course and taxonomic profiles of Case 1. MNZ: metronidazole; VCM: vancomycin, *C. difficile*: *Clostridium difficile*, FMT: fecal microbiota transplantation.

Discussion

We present a case series of refractory CDI successfully treated with FMT. None of the patients relapsed within 3 months after FMT.

According to the guidelines of the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America (SHEA-IDSA),[1] testing for *C. difficile* toxin is the most important means of diagnosing CDI. However, the sensitivity of this test varies from 60% to 80%, depending on the conditions or the kits used for testing.[6] Therefore, CDI

Table 1 Summary of cases.

	Case 1	Case 2	Case 3	Case 4
Age (yr)	93	87	86	69
Sex	Female	Female	Female	Male
Prior antibiotics	+	+	+	+
Proton pump inhibitor	−	−	−	+
Naso-gastric tube	−	−	−	+
Prior ICU	−	−	−	+
Obesity	−	−	−	−
Post operation	−	−	−	−
Immunocompromised	−	−	−	−
Pseudomembrane	NA	+	+	−
Clostridium difficile toxin	+	−	+	+
Clostridium difficile (culture)	+	+	−	+
Number of pulsed session of antibiotics for symptoms	5	5	6	7
Period from onset to first visit to hospital (days)	208	127	189	178
Period from first visit to FMT (days)	30	18	18	25
Donor age (yr)	59	39	34	15
Number of the donors screened	1	2	2	3
Number of eligible donors	1	2	2	1
Relationship to the recipient	Son	Grandson	Granddaughter	Grandson
Reason of mismatch	−	−	−	IBS symptoms CD toxin indeterminant

ICU: intensive care unit, NA: not available, FMT: fecal microbiota transplantation.

cannot be ruled out based on a negative test for *C. difficile* toxin. In our Case 2, with a negative test for *C. difficile* toxin, the colonoscopic findings of pseudomembrane led to the diagnosis of CDI.[1,2,6]

Although the use of antibiotics is the most important risk factor for CDI, other host-related factors have been reported, such as use of a proton pump inhibitor, old age (≥65 years), history of treatment in the intensive care unit, placement of a nasogastric tube, recent operation, immunocompromise, and obesity.[6] In our cases, all patients had the risk factors of prior use of antibiotics and age ≥65 years. To date, there have been no outbreaks of the NAP1/027 strain in Japan. Therefore, host-related factors contribute to the onset of CDI rather than the microbial virulence of *C. difficile*.

The relationships between the route of FMT and the rates of symptom resolution without recurrence was reported by Drekonja et al.; upper gastrointestinal tract, colonoscopy, enema, and upper gastrointestinal tract and colonoscopy were 77%, 90%, 78%, and 100%, respectively.[4] FMT for adult patients with refractory CDI in Asia is reported mostly from South Korea. Two case series were reported from South Korea: 8 cases from the upper gastrointestinal tract, seven from colonoscopy and one from upper gastrointestinal tract and colonoscopy.[7,8] As for reports from Japan, there have been two reports of FMT for CDI.[9,10] One used nasojejunal tube and the other used colonoscopy. Among the 8 cases from the upper gastrointestinal tract in South Korea, 3 patients experienced vomiting or aspiration pneumonia. Considering the therapeutic effect and safety, administration using colonoscopy may be preferable.

There are also problems of the availability of donors. Recently, new approaches related to FMT have been reported: frozen preparation; frozen and oral, capsulized preparation; and sterile fecal filtrate transfer. Simpler treatment options will be available as the mechanisms of action become elucidated.

The limitations of this study were the fact that it was a prospective but single-center, open-label study and include a small number of subjects. The change of microbiota after FMT was analyzed only one case.

In conclusion, we have presented four cases of refractory CDI successfully treated with single FMT procedure. Further accumulation of cases and standardization of the procedure are anticipated.

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