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FAECAL MICROBIOTA TRANSPLANTATION FOR *CLOSTRIDIUM DIFFICILE* INFECTION USING A LYOPHILIZED INOCULUM FROM NON-RELATED DONORS: A CASE SERIES INVOLVING 19 PATIENTS

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Faecal microbiota transplantation (FMT) has been reported to be effective in treating relapsing of refractory *Clostridium difficile* infections, although some practical barriers are limiting its widespread use. In this study, our objective was to evaluate the rate of resolution of diarrhea following administration of lyophilized and resolved FMT via a nasogastric (NG) tube. We recruited 19 patients suffered from laboratory-confirmed *C. difficile* infection. Each of them was treated by lyophilized and resolved inoculum through a NG tube. One participant succumbed following the procedure due to unrelated diseases. Out of 18 cases, 15 patients reportedly experienced a resolution of the symptoms. One patient was treated with another course of antibiotics, and two of the non-responders were successfully retreated with another course of FMT utilizing a lyophilized inoculum. Notably, no significant adverse activities were observed. In accordance to our clinical experiences, a patient will likely benefit from FMT treatment including lyophilized inoculum.

Keywords: *Clostridium difficile* infection, faecal microbiota transplantation, lyophilization, nasogastric tube

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Introduction

Clostridium difficile is a Gram-positive, obligate anaerobic, spore-forming bacterium. It was first described as a member of commensal flora in 1935; however, since the late 70s, it has been recognized as the most important causative agent of antibiotic-associated diarrhea and pseudomembranous colitis [1, 2]. In recent years, *C. difficile* infection (CDI) has become a major clinical entity [3–5]. Due to a hypervirulent strain (NAP1/BI/021), which emerged in the early 2000s, CDI tends to be less responsive to conventional therapy and both of the proportion of recurrent and severe episodes have risen [6, 7]. Recently published papers suggest that faecal microbiota transplantation (FMT) could be a reasonable option among these clinical circumstances [8–12]. Compared with many other medical procedures, FMT is not complicated and is considered cost-effective. Most of the clinicians are performing FMT using freshly prepared samples, which has a limited viability, usually estimated up to 6 h. Furthermore, proper donor screening is a long procedure precluding the use of FMT in acute cases. These logistical barriers of the procedure inspire clinicians to investigate the possibility of the use of pooled, standardized samples. According to the published data of Hamilton et al. [13], the use of frozen and thawed FMT was not found inferior when compared with the use of fresh FMT, and this data were confirmed in a paper written by Lee et al. [14]. In this study, our aim was to evaluate the possibility of the use of lyophilized specimen for FMT.

Methods

Patient indications

In this study, participants were recruited from the patients referred to the Infectious Diseases Ward of the 1st Department of Internal Medicine of University of Pécs. Subjects with refractory or recurrent active CDI were included. Active CDI was defined as a diarrhea (>3 loose stools per day) including a positive stool test result of *C. difficile* toxin enzyme-linked immunosorbent assay. A recurrent episode was defined as the presence of diarrhea and a positive *C. difficile* stool assay within 2–6 weeks from the initial episode. Refractory CDI was defined as failure of a 2-week vancomycin therapy with or without an alternative antibiotic. Major exclusion criteria included acute abdomen, signs of perforation, recent operation with the opening of the upper or lower gastrointestinal (GI) tract, upper GI tract hemorrhage, signs of ileus, and uncontrollable vomiting.

Donor determination and screening

Our donors were healthy, non-pregnant adults. In our clinical practice, each of our donors served as a volunteer. It is immediately clear that proper donor screening is essential to avert the likely transmission of communicable diseases. Our essential strategy included an oral interview and a physical examination of the donor in the screening process. Many authors recommend using a simple questionnaire (e.g., FDA Full-Length Donor History Questionnaire [15]) for identifying potential risk factors considered undetectable in the laboratory. We used this questionnaire and adapted it to the local circumstances. Individuals with any significant medical history, for use of any antibiotics, or with a history of any extensive travel in the preceding 6 months, drug abusers, and individuals with high-risk sexual behavior were excluded. Recently published papers suggest that the intestinal microbiota plays a very important role in many chronic diseases, such as Crohn's disease and ulcerative colitis, metabolic syndrome, cancer, obesity [16], and irritable bowel syndrome [17]. The exclusion of donors with any evidence of autoimmune or other chronic conditions should be considered, until the exact role of the intestinal microbiota in these conditions is substantiated in reference to the donors.

Volunteers underwent a physical examination and general laboratory screening tests. Donated faeces were screened for parasites, enteropathogenic bacteria, and *C. difficile*, whereas the serum was screened for HIV-1 and HIV-2, EBV, CMV, hepatitis A, B, and C viruses and *Treponema pallidum*.

Preparation of the sample

However, there are no evidence based-recommendations of FMT; meta-analysis suggests that a larger volume of faeces will increase the success rate and reduce the relapse rate [18–20]. Most authors recommend the use of normal saline, but some have used milk in view of the finding that patients with recurrent CDI excrete lower amounts of short-chain fatty acids into their faeces as compared with healthy controls [21, 22].

The donated faeces were transported to our department within 2 h of passage. A 60-g sample was homogenized in a mortar and suspended in 200 ml of normal saline (0.9%). The suspension was then filtered through 4 × 4 cm sheets of sterile gauze into another container, and 100 ml of the filtrate was acquired in a sterile container. All of the steps of the preparation were performed in a laminated flow box and sterile devices were used. Next, the filtrate was frozen at –20 °C for 24 h in a thin layer, and over a large surface area within a plastic container. Following

all the above, it was then lyophilized with a Heto Drywinner Freeze Dryer, Biolab Inc. in $-40\text{ }^{\circ}\text{C}$, under vacuum. The lyophilized transplant material (TM) was stored for a maximum of 8 weeks at normal temperature ($21\text{ }^{\circ}\text{C}$). Prior to the FMT procedure, the lyophilized material was dissolved in 100 ml of sterile distilled water.

Recipient preparation

Generally, if and when the condition of the patient permitted, discontinuation of antibiotics 4 days prior to the procedure was required. Participants were asked to fast 8 h prior and 3 h following the FMT. In serious cases, this period was reduced to 1 day. In each case, thorough purging was applied on the day prior to the intervention, to effectively increase the success of the procedure, by flushing away residual faeces, antibiotics, toxins, *C. difficile* bacteria, and spores to the maximum attainable extent. The patients received a double dose of proton pump inhibitor intravenously in the morning of the planned procedure. This increases the chance of survival of the transplanted flora by reducing the gastric acid output. About 1 h prior to the transplantation, 20 mg of *metoclopramide* was intravenously administered, to prevent vomiting and to facilitate bowel movement to promote the delivery of the transplantation material to the colon.

FMT delivery

In accordance with recently published results [10, 23–26], our experiences revealed that FMT via upper GI tract is also a highly effective procedure [27]. NG tubes were inserted prior to the procedure including 100 ml of solution, which was flushed in. During upper GI tract delivery all of the patients were set in a semi-sitting position throughout the procedure and 1 h afterward. Next, the patients were encouraged to resume both normal diets and physical activities.

Cure rate

Primary cure rate is defined as the percentage of cases in which the symptoms are considered resolved without recurrence within 6 weeks following the FMT, and the secondary cure rate as the percentage of cases in which the symptoms are resolved following a second FMT [28]. IDSA/SHEA guidelines do not recommend *C. difficile* testing in patients who do not have symptoms, because patients can be colonized with *C. difficile* and do not develop the disease [29, 30].

All of our patients were hospitalized and discharged three or more days following FMT. Possible adverse events were elicited by the medical staff of our ward, based on a self-report of the patients. Fever, GI symptoms, headache, fatigue, and rash were the main symptoms evaluated.

Results

Table I summarizes the demographic characteristics and clinical outcomes of the 19 participants. Between August 2013 and June 2015, we performed FMT with lyophilized TM on a total of 19 patients, of whom nine (47.36%) were females. The mean age was 67.73 years, with a range of 38–94 years. In all cases, the diagnosis of *C. difficile* colitis had been confirmed by positive stool test results for *C. difficile* toxin. The average number of recurrent infections was 2.0

Table I. Demographic characteristics of and clinical outcomes for patients

Patient	Age (years)	Sex	<i>C. difficile</i> test results before FMT	Severe episode of CDI at FMT	Recurrent episodes of CDI before FMT	Clinical outcome after first FMT	Clinical outcome after second FMT
1	75	F	Positive	Yes	4	Resolution	NA
2	38	F	Positive	Yes	3	Treatment failure	Resolution
3	94	F	Positive	Yes	0	Resolution	NA
4	82	M	Positive	Yes	1	Resolution	NA
5	65	M	Positive	Yes	2	Resolution	NA
6	51	M	Positive	Yes	1	Resolution	NA
7	75	M	Positive	No	3	Death	NA
8	67	F	Positive	No	1	Resolution	NA
9	62	M	Positive	Yes	2	Resolution	NA
10	70	F	Positive	Yes	0	Resolution	NA
11	79	M	Positive	Yes	4	Resolution	NA
12	62	M	Positive	Yes	4	Resolution	NA
13	85	M	Positive	Yes	1	Resolution	NA
14	70	M	Positive	Yes	5	Resolution	NA
15	58	F	Positive	Yes	2	Resolution	NA
16	57	F	Positive	Yes	0	Treatment failure	Resolution
17	67	F	Positive	Yes	2	Resolution	NA
18	73	F	Positive	No	1	Treatment failure	NA
19	57	M	Positive	Yes	2	Resolution	NA

(range: 0–5). All of the patients had received at least one course of metronidazole/vancomycin. These treatments had been provided by the medical staff within our ward. In three cases, FMT was carried out to treat the first episode of FMT, because of the severity of the infection and the failure of preceding antibiotic treatment. In 16 cases (84.21%), the patients had severe CDI during the transplantation. All participants were admitted at least 1 day prior, and were hospitalized for at least three additional days following the procedure.

Patient 7 suffering from severe comorbidity (chronic obstructive pulmonary disease and polyvascular disease) and who was in a very poor general condition succumbed 2 days following the procedure. Among the surviving 18 patients, 15 (83.33%) reported having a normal stool within 48 h following the FMT. Three patients proved to be non-responders. Patients 2 and 16 underwent a second transplantation, and following a second treatment, the clinical resolution of the diarrhea was reported in both cases. Patient 18 developed diarrhea within 5 days following the stool transplantation, and additional stool tests for *C. difficile* toxin was found to be positive. She was treated by an additional course of fidaxomicin and clinical resolution of her symptoms was reported within 4 days.

We experienced in which FMT through NG tubes, using a freeze-dried specimen, has the primary cure of 83.33%, whereas the secondary cure rate was found to be 100%.

No serious adverse events or recurrences were observed. None of the patients reported vomiting within 24 h of administration. Mild adverse events, e.g., bloating, abdominal cramps, and nausea, were reported only in three cases (16.67%).

Discussion

Today, *C. difficile* is a cause of antibiotic-associated diarrhea and pseudo-membranous colitis. The severity of the disease ranges from mild cases, where discontinuation of antimicrobials and supportive care could be efficient, to severe life-threatening cases. It has a relevant impact of morbidity and mortality in the elderly. Recommended therapies for CDI include oral administration of metronidazole or vancomycin. Reported data suggest that antibiotic treatment has a clinical efficiency >90%. Recurrent episodes of CDI are generally treated with an additional course of metronidazole or vancomycin. However, some patients develop a chronic pattern of recurrent CDI. Limited experience with FMT in Europe and the United States suggests it may likely be the solution in treating these individuals.

Our former clinical experiences demonstrated FMT via the upper GI tract, using freshly prepared suspension, is indeed an effective procedure to treat CDI. In

the implementation of our treatment, the overall primary cure rate of upper GI tract FMT is reportedly 82.43%, whereas the overall secondary cure rate was 94.59% [27]. During this study, we experienced the same efficiency. These results are not inferior to those which have been reported in meta-analytical studies [18, 19]. We prefer to use NG tubes in our practice, largely due to the advantage in which it requires less patient preparation, clinical time, less patient inconvenience, and considerably inexpensive when compared with other routes of administration. This method does not require the specialized skills of endoscopic procedures and it can be performed at the patients' bedside. However, it is important to note that this method has several limitations. The most important concerns are the limited viability of the samples and the long screening procedure of the donors on each occasion. Moreover, many of our patients are elderly without any healthy living relatives. These facts inspired us to investigate an alternative method for FMT. According to the recorded data of Hamilton et al., which were confirmed by Lee et al., the use of frozen samples is as effective as the use of freshly prepared suspension. This method requires a refrigerator to store the specimen. The papers published by Kupletskaya [31] and Antheunisse [32] suggest that bacteria can retain their viability after a freeze-drying (lyophilization) procedure. This data inspired us to investigate the possibility and effectiveness of this procedure.

Although we experienced an excellent clinical outcome, it is important to note that this study has major limitations, such as the small sample size and the lack of placebo or active comparator. The design of a multicenter study involving larger numbers of patients and with active comparators should be considered in the future. If our data are to be reproduced, it will likely serve toward making FMT accessible to a wider population of patients. In addition, encapsulating the freeze-dried specimen may very well prove to be a promising future prospect. The other major limitation is the problem of long-term follow up. All study participants were contacted by phone, both 6 weeks and 6 months following the FMT. They were asked to complete a short questionnaire concerning their bowel movements, abdominal pain, and general well-being, recurrent episode of CDI, antibiotic intake since FMT, and any new medical conditions occurring since receiving FMT. No new medical conditions were reported. However, it is important to note that these answers were not confirmed by a health care professional (e.g., a general practitioner or a nurse), and no control stool tests were done.

Conclusions

Despite deficiencies in the design of this study, our results seem to adequately support that FMT, using a lyophilized material through NG tube

administration, is a rational treatment option for patients with recurrent or refractory CDI. Controlled trials need to be done to further prove the effectiveness of this method, and to determine the ideal procedure in which to treat recurrent or antibiotic refractory CDI in the most effective measure possible.

Funding Sources

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Conflict of Interest

The authors work in a University Hospital, where FMT is a treatment option for patients suffering from recurrent or severe CDI. No authors have active patents, patents under application, or intention to file for patents, related to the techniques used in this study. No other disclosures are reported.

Ethics

All FMT procedures were carried out with the written ethical approval (permission number: 16014) of the appropriate Scientific and Ethics committee (Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottság, ETT-TUKEB), and the written informed consent was taken from all patients participated in the study.

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