ORIGINAL ARTICLE

INTESTINAL RESEARCH

Long-Term Clinical Outcome of *Clostridium difficile* Infection in Hospitalized Patients: A Single Center Study

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Background/Aims: Antibiotic usage and increasingly aging populations have led to increased incidence of *Clostridium difficile* infection (CDI) in worldwide. Recent studies in Korea have also reported increasing CDI incidence; however, there have been no reports on the long-term outcomes of CDI. We therefore investigated the long-term clinical outcomes of patients with CDI, including delayed recurrence, associated risk factors and mortality. **Methods:** Hospitalized patients diagnosed with CDI at Seoul Paik Hospital between January 2007 and December 2008 were included. Their medical records were retrospectively investigated. 'Delayed recurrence' was defined as a relapse 8 weeks after a successful initial treatment. Multivariate logistic regression analysis was performed to identify risk factors for the delayed recurrence. Kaplan-Meier curves were used to analyze mortality rates. **Results:** A total of 120 patients were enrolled; among them, 87 were followed-up for at least 1 year, with a mean follow-up period of 34.1±25.1 months. Delayed recurrence was observed in 17 patients (19.5%), and significant risk factors were age (over 70 years, *P*=0.049), nasogastric tube insertion (*P*=0.008), and proton pump inhibitor or H_2-blocker treatments (*P*=0.028). The 12- and 24-month mortality rates were 24.6% and 32.5%, respectively. No deaths were directly attributed to CDI. **Conclusions:** Delayed recurrence of CDI was not rare, occurring in 19.5% of the study population. Although CDI-related mortality was not reported, 2-year (32.5%) mortality rate of CDI patients implies that a CDI diagnosis may predict severe morbidity and poor prognosis of the underlying disease. **(Intest Res 2014;12:299-305)**

Key Words: *Clostridium difficile*; Recurrence; Mortality; Risk factors

INTRODUCTION

Clostridium difficile infection (CDI) is a major cause of diarrhea in hospitalized patients. CDI incidence has been increasing owing to increase of elderly patients with co-morbidities as well as the use of antibiotics, including newly developed quinolone and cephalosporin.[1-3] Furthermore, recent reports of increasing CDI treatment failures and recurrence rates[2,4] as well as severe infections resulting in death and various complications[5-7] have raised concerns regarding the association with hypervirulent strains (e.g., BI/NAP1/ribotype 027).[8-10] Despite increased treatment failures, CDI treatment continues to have a high primary treatment success rate of over 80%[4,11] but reports of recurrent cases making recurrence an important issue for severe chronic patients and elderly patients.

According to the national wide multi-center research by IBD research group of Korean Association for the Study of Intestinal Diseases (KASID), 17.2 CDI cases per 10,000 hospitalized patients occurred in 2004, increasing to 27.4 cases per 10,000 in 2008, a 1.6 fold increase in 5 years.[12] In a single center study, Lee et al., reported a drastic increase of CDI from 2003 to 2008, from 15 CDI cases per 10,000 hospitalized patients in 2003 to 18, 36, 101, 61, and 71 cases in 2004, 2005, 2006, 2007, and 2008 respectively.[13] Along with the

more experiences of CDI, new clinical outcomes have been reported, such as CDI recurrence rate[14-16] and hypervirulent strains in Korea.[17,18]

The reported CDI recurrence rates vary by different studies, but approximately 15–30% of patients experience recurrence within 8 weeks; the second recurrence rate among patients with the first recurrence has been reported to be 40%. Lastly, the infection recurrence rate for patients with at least twice CDI recurrences is approximately 45–65%.[19-24] Up to 28% of patients experienced CDI recurrence within 90 days of treatment.[25] The CDI recurrence rates in Korea are still relatively low (8.9%) compared to other western countries.[12] However, these incidence rates only include early CDI recurrences, within 8 weeks of treatment; thus, the long-term recurrence rate through clinical observations remains unknown.

We have previously reported CDI incidence and recurrence rates of CDI at a single center,[13] but the results were limited, reporting only early recurrence rate within 8 weeks of treatment; thus, it was unclear which chronic repetitive aspects of CDI recurrence which is recent concern. We therefore investigated patients recently treated for CDI, their long-term recurrence rates and clinical progress, thereby contributing to a better understanding of the clinical aspects of chronic CDI.

METHODS

1. Study Subjects

Patients aged 18 years or older hospitalized at Seoul Paik Hospital diagnosed with CDI between January 2007 and December 2008 were included in this study. Patient medical records and examination results including age, sex, duration of hospital stay, intensive care unit admission, nasogastric tube insertion, underlying diseases, diarrhea, antibiotics used prior to CDI diagnosis, and serum albumin levels at diagnosis were retrospectively investigated. Patient deaths and CDI recurrence after successful treatment were also investigated. The study protocol and consent form exemption were approved by the clinical study committee of Seoul Paik Hospital.

2. Methods

1) CDI Diagnostic Criteria
CDI was diagnosed based on the following criteria: patients passed soft stools more than twice daily for 2 days or 6 times per 36 hours since admission, or had diarrhea. The patients need fulfill one or more; (1) stool samples positive for either *C. difficile* toxin A or B or identification of *C. difficile* in the stool, or (2) specific pseudomembranous colitis observed via lower gastrointestinal endoscopy. CDI recurrence was defined as a CDI relapse diagnosed using the above criteria, with diarrhea accompanying fever or abdominal pain after successful treatment. Recurrence diagnosed within 8 weeks of an initial successful treatment was defined as 'early recurrence'; cases diagnosed after more than 8 weeks were considered 'delayed recurrence'.

2) Detection of *C. difficile* Toxin
In order to detect *C. difficile* toxins, enzyme-linked fluorescent based VIDAS® *C. difficile* Toxin A & B (CDAB) were utilized. Based on the manufacture's instruction, a patient was considered positive, equivocal, and negative if relative fluorescence value (RFV) is higher than 1, in between 0.4–1.0, and lower than 0.4, respectively.

3) *C. difficile* Culture Test
C. difficile was cultured by inoculation of patient diarrhea samples on cycloserine cefoxitin fructose agar and cultured for 48–72 hours under anaerobic conditions. Suspected strains were identified by spore staining and using a Vitek ANA anaerobic bacteria identification card (bioMérieux, France).

4) Diagnosis of Pseudomembranous Colitis
Pseudomembranous colitis induced by *C. difficile* was identified by the presence of multiple whitish-yellow raised pseudomembranes, a typical lesion, via colonoscopy or sigmoidoscopy.

3. Statistical Analysis

Statistical analyses were performed using PASW Statistics for Windows (Version 18.0, SPSS Inc., Chicago, IL, USA). Frequency, percentile, and mean of patient characteristics were analyzed. Risk factors of early recurrence and mortality in a total of 120 cases were analyzed by using multivariate logistic regression analysis. The percentage of delayed recurrence was calculated for 87 patients who were able to be evaluated for clinical progress and survival/mortality after 1 year. The risk factors for delayed recurrence were investigated by performing multivariate logistic regression analysis. Lastly, recurrence and mortality rates of patients with CDI were depicted on a Kaplan-Meier curve.

RESULTS

1. Patient Characteristics and Clinical Aspects

A total of 120 patients were diagnosed with CDI at Seoul Paik Hospital over 2 years (January 2007 through December 2008). The mean follow-up period was 28.2±25.6 months (0.3–66.3 months); after 1 year, clinical progress and survival/death were confirmed in 87 patients (72.5%). The mean follow-up period for these 87 patients was 34.1±25.1 months (0.5–66.3 months) while the mean period for the remaining patients was 3.6±3.2 months.

The mean age of patients with CDI was 62.2±16.1 years (18–92 years) and there were 66 male and 54 female patients (55% and 45%, respectively). When diagnosed, the mean hospitalization period was 65.8±63.6 days. Thirty-one patients were diagnosed with malignant tumors (25.8%), and 8 received chemotherapy within 1 month (6.7%). Nine patients were treated for chronic kidney disease (7.5%) and 53 patients received treatment in the intensive care unit (44.2%). Fifty-five (45.8%) and 50 patients (41.7%) had nasogastric tube insertion and proton pump inhibitor (PPI) or H_2-blocker treatments, respectively. Of surgery patients (59 patients, 49.2%), 32 had gastrointestinal operations (Table 1).

Prior to the CDI diagnosis, 17.1% of patients had not received antibiotic treatments, while 30.0%, 21.4%, and 31.4% of patients had been previously prescribed 1, 2, or more than 2 antibiotics, respectively. The mean antibiotic treatment period was 15.5±14.7 days. Antibiotics suspected of inducing CDI, including redundant combinations, were cephalosporin (61.7%), quinolone (39.2%), carbapenem (22.5%), aminoglycoside (17.5%), penicillin (9.2%), and others (14.2%); the cephalosporin class was most prevalent.

2. CDI Early Recurrence Rate

After successful CDI treatment, there were 23 cases of early recurrence (19.2%), within 8 weeks of treatment end. Six cases were reported within 2 weeks of primary treatment, while 7 and 10 cases were reported between 2 to 4 weeks and 4 to 8 weeks, respectively. Multivariate logistic regression revealed nasogastric tube insertion (P=0.026) and PPI or H_2-blocker treatment (P=0.048) to be risk factors of early recurrence, whereas age, sex, underlying diseases including malignant tumors, number of antibiotics, and duration of antibiotic treatment did not influence the early recurrence rate (Table 2).

3. CDI Delayed Recurrence Rate

Among 87 patients available for survival/death and clinical follow-up 1 year after CDI diagnosis, there were 17 cases (19.5%) of delayed recurrence 8 weeks after successful treatment. Kaplan-Meier curve analysis showed a recurrence rate of 31% after 60 months (Fig. 1). In particular, of 19 early

Table 1. Characteristics of Patients With *Clostridium difficile* Infection

Variable	n=120
Age (yr)	62.2±16.1
Female	54 (45.0)
Prior hospital stay (days)	65.8±63.6
Number of antibiotics (none/1/2/≥3, %)	17.1/30.0/21.4/31.4
Serum albumin (g/dL)	3.0±0.6
Underlying disease	
Malignancy	31 (25.8)
Chemotherapy	8 (6.7)
Chronic kidney disease	9 (7.5)
Surgical history	59 (49.2)
ICU admission	53 (44.2)
Nasogastric tube insertion	55 (45.8)
Anti-gastric acid agent*	50 (41.7)
Early recurrence[†]	23 (19.2)

Variables are presented as mean±SD or n (%).
*Medication of proton pump inhibitor or H_2-blocker.
[†]Early recurrence is defined as a relapse within 8 weeks from an initial successful treatment.
ICU, intensive care unit.

Table 2. Risk Factors of Early Recurrence in Patients With *Clostridium difficile* Infection

Parameter	OR	95% CI	*P*–value
Female	1.040	0.207–5.212	0.962
Age ≥70 yr	1.203	0.242–5.975	0.822
Malignancy	0.260	0.025–2.663	0.257
Anti-gastric acid agent*	5.416	1.015–28.892	0.048
Nasogastric tube insertion	8.734	1.290–59.149	0.026
Number of antibiotics			0.986
One	0.906	0.072–11.326	0.939
Two	1.329	0.086–20.643	0.839
More than two	1.203	0.139–10.429	0.867

Early recurrence is defined as a relapse within 8 weeks of successful initial treatment.
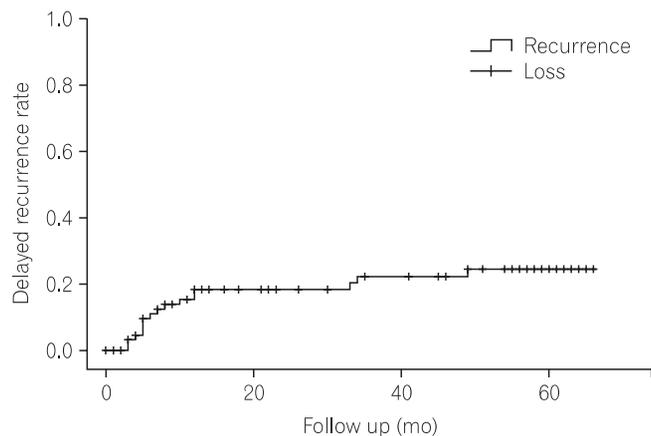*Treatment with proton pump inhibitor or H_2-blocker.

Fig. 1. Kaplan-Meier curve of delayed recurrence in patients with *Clostridium difficile* infection.
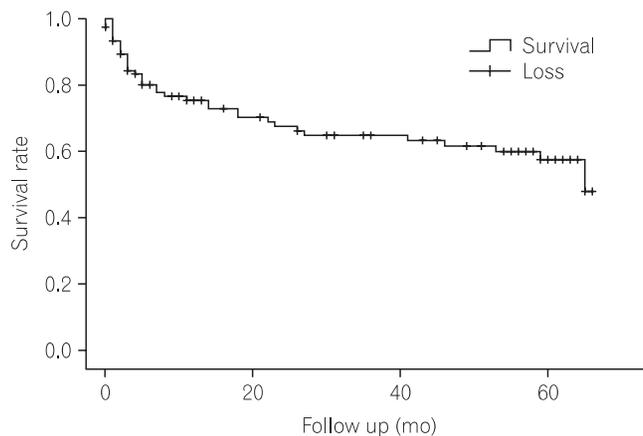

Fig. 2. Kaplan-Meier survival curve in patients with *Clostridium difficile* infection.

Table 3. Risk Factors of Delayed Recurrence in Patients With *Clostridium difficile* Infection

Parameter	OR	95% CI	*P*-value
Female	2.678	0.349–20.568	0.344
Age ≥70 yr	4.434	1.009–89.909	0.049
Malignancy	0.619	0.050–7.609	0.708
Anti-gastric acid agent*	1.118	1.012–1.234	0.028
Nasogastric tube insertion	40.111	2.646–608.140	0.008
Number of antibiotics			0.242
One	0.219	0.006–8.464	0.415
Two	1.043	0.034–32.317	0.981
More than two	0.079	0.004–1.692	0.104

Delayed recurrence is defined as a relapse after 8 weeks of successful initial treatment.
*Treatment with proton pump inhibitor or H_2-blocker.

recurrence cases, 8 re-occurred after 8 weeks, a recurrence rate 42.1% among patients with early recurrence, a finding comparable to previous reports.[20,21,24]

In multivariate logistic regression analysis, significant risk factors for delayed recurrence included age (more than 70 years, OR 4.434, *P*=0.049), nasogastric tube insertion (OR 40.111, *P*=0.008), and PPI or H_2-blocker treatment (OR 1.118, *P*=0.028). Eleven patients were administered PPI medication, but PPI medication did not show statistically significant association. In addition, a history of surgery, underlying diseases, number of antibiotics, medication duration, and hospitalization period did not influence the rate of delayed CDI recurrence (Table 3). These results do not agree with other reports.[15,16,26,27]

4. CDI Patient Survival Rates

The survival rate of 120 cases of CDI patients were 75.4%, 67.5%, and 47.9% in 12 months, 24 months, and five years. During the hospitalization, there were 20 cases of deaths after the diagnosis albeit any deaths were not definitely attributed to CDI (Fig. 2). A total of 40 deaths were confirmed.

Among these deaths, 10 and 7 patients had underlying malignant tumors and chronic kidney disease, respectively. A surgical history prior to CDI diagnosis was reported in 17 patients (10 patients who underwent gastrointestinal tract surgery). Twenty-five patients who died had nasogastric tubes, and 21 and 8 patients had been treated in the intensive care unit and had early CDI recurrence, respectively. Among these clinical features, low serum albumin level was the only significant risk factor for death (*P*=0.026).

Causes of death included sepsis (10 patients), pneumonia (7 patients), tuberculosis (1 patient), acquired immunodeficiency syndrome (1 patient), hepatorenal syndrome (1 patient), stroke (2 patients), ventricular tachycardia (1 patient), terminal cancer (6 patients: hepatic cancer, 1 patient; lung cancer, 1 patient; gastric cancer, 2 patients; lymphoma, 2 patients), gastrointestinal tract bleeding (3 patients), and unknown reasons (8 patients). Among unknown causes of death, fever and diarrhea also occurred in 2 patients, raising the possibility of death caused by CDI.

DISCUSSION

In western countries, outbreaks of hypervirulent CDI strains (BI/NAP1/ribotype 027) have been reported since 2002[8] and its mortality rate has been increasing recently.[28,29]

Particularly, in Quebec Canada, there was a five-fold increase in CDI between 1994 and 2003 along with noticeable increment of severe infections accompanying toxic megacolon, shock, and perforation.[28,29] Similarly in Korea, it has been reported for critical pseudomembranous colitis induced by hypervirulent strains (BI/NAP1/ribotype 027)[17]; in following epidemiological studies, the detection rate for such hypervirulent strains was 0.6% but their clinical significance is still unclear.[18,30]

The CDI recurrence rate after successful treatment is relatively high (15–30%), and it is more likely to recur when patients had previously experienced recurrence (45–65%).[20,21,24] Musher et al., reported a long-term CDI recurrence rate of up to 28% among patients with no relapse within 90 days of initial treatment, indicating that chronic CDI recurrence may be an important health care issue in the future.[25]

The CDI recurrence rate in Korea was reported to be 10.8–21.4%;[14,31] however, these incidence rates measure only early recurrence, within 8 weeks of treatment. Therefore, the long-term recurrence rates remain unknown. In this regard, the present study is significant because we investigated not only the recurrence rate of CDI within 8 weeks of treatment (19.2%) but we also measured the delayed recurrence rate beyond 8 weeks after treatment (19.5%) via long-term clinical observations (mean, 34.1 months), although the study was performed in a single institution. In addition, we found a repeated recurrence of 42.1%, a result in agreement with other reports.[19,24]

Potential recurrence mechanisms for CDI relapse include incomplete eradication of *C. difficile* or re-infection with new *C. difficile* stains. In addition, other known CDI recurrence trigger conditions include: (1) patients older than 65 years of age, (2) continuous antibiotic usage, (3) failure to provide proper treatment, and (4) suppressed patient immune systems.[32-35] However, re-infection with new strains is believed to be mainly responsible for CDI recurrence (48–75%).[14,34-36] In this respect, it is meaningful that nasogastric tube insertion increases the risk of CDI up to 9-fold as manipulation of contaminated instruments can transfer *C. difficile* strains from hospital environments.[37] The results of this study reinforce these previous findings; specifically, CDI recurrence 8 weeks after treatment (i.e., delayed recurrence) was significantly associated with age (older than 70 years), medication (PPI or H_2-blocker), and nasogastric tube insertion.

In our study, both of delayed recurrence and 5-year recurrence by Kaplan-Meier analysis showed high recurrence rates (19.5% and 31%, respectively). This finding emphasizes the need to develop effective treatments for repetitive CDI

recurrence. When CDI recurs, the initial antibiotics are typically re-administered if they were effective against the first infection since antibiotic resistances (e.g., metronidazole and vancomycin) are not common in Korea. However, for cases of repeated recurrence (more than twice), long-term administration of low doses of vancomycin following full dose vancomycin therapy, with or without pulse therapy has been suggested.[2] In addition to standard antibiotics, non-absorbing toxin-conjugating agents (e.g., cholestyramine) are intended to facilitate excretion of *C. difficile* toxins. In addition, oral administration of probiotics may speed recovery of disrupted intestinal microflora. Recently, fecal microbiota transplantation showed a very high success rate (92%) and low recurrence rate (4%), making it a promising alternative to conventional treatments.[24,38]

Musher et al. addressed the significance of chronic CDI recurrence and raised the possibility that CDI could be utilized as a prognostic indicator given the high mortality (34%) 6 months after diagnosis.[25] In the present study, the mortality rate among patients diagnosed with CDI was 24.6%, 32.5%, and 52.1% after 12 months, 24 months, and 5 years after diagnosis, that cannot be easily ignored. Although no deaths were directly attributed to CDI, it is regarded as a strong prognostic indicator for deaths from other underlying diseases.

At the time when chronic CDI recurrence has been emerging as a serious global problem, this study is meaningful as it is the first study, to our knowledge, to shed further light on clinical aspects of CDI through long-term observation in Korea. In particular, the delayed recurrence rate was found to be noticeably high, warranting development of novel methods to resolve the issue. Basically, unnecessary use of broad-spectrum antibiotics should be avoided and more emphasis should be placed on hygiene management such as washing hands. In addition, more active preventive measurements should be discussed; for instance, an aseptic method for nasogastric tube insertion might be worthwhile to consider for patients with a history of CDI.[14] Lastly, additional investigations are needed to distinguish if recurrence was caused by the same strain or re-infection by other strains by analyzing samples from patients with chronic recurrence. This type of analysis could also be used to identify severe CDI due to hypervirulent strains.

The present study has several limitations. Firstly, we calculated the delayed CDI recurrence rate (19.5%) in 87 patients; these cases included patients who were available for follow-up 1 year after CDI treatment, or who had died. In other words, of the 87 patients, 20 patients (23.0%) died during

hospitalization after CDI diagnosis; therefore, the group of 87 patients may not accurately reflect features associated with recurrence among surviving patients. Owing to this potential bias, we further investigated the delayed recurrence rate using Kaplan-Meier curve analysis and found a higher recurrence rate (31%). Secondly, because this study was conducted retrospectively in relatively small sample size (n=87), it is possible that potential risk factors were overlooked in the analysis. Furthermore, the number of CDI deaths might be underestimated because analyses for hypervirulent strains have not yet been performed.

In conclusion, in this study, we found early recurrence (defined as relapse within 8 weeks of the treatment of CDI) and delayed recurrence rates (defined as relapse after 8 weeks of the treatment of CDI) of 19.2% and 19.5%, respectively. The delayed recurrence rate for patients who had experienced early recurrence was particularly high (42.1%). The mortality rate is also high at 2 years after CDI diagnosis (32.5%), suggesting that CDI diagnosis may be an important prognostic indicator for deaths from underlying diseases.

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