

Clostridium difficile Infection at Diagnosis and during the Disease Course of Pediatric Inflammatory Bowel Disease

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Purpose: *Clostridium difficile* colonization and infection are commonly associated with poor outcomes in patients with pediatric inflammatory bowel disease (PIBD). We aimed to investigate the prevalence of *C. difficile* colonization and infection at the time of diagnosis and to evaluate risk factors associated with the development of *C. difficile* infection during the course of PIBD treatment.

Methods: We retrospectively enrolled a total of 59 children who were newly diagnosed with PIBD at the tertiary medical center. All patients underwent *C. difficile* toxin assays and cultures initially and at every follow-up during the disease course. Kaplan-Meier survival analysis and Cox regression test were used for statistical analysis.

Results: Initial cultures for *C. difficile* were positive in 13 (22.0%) of 59 PIBD patients, whereas initial toxin assays were positive in 3 patients (5.1%). During treatment, *C. difficile* cultures converted to positive in 28 (47.5%) in addition to 13 patients who were initially culture-positive, and *C. difficile* toxins converted to positive in 13 (22.0%) in addition to 3 originally toxin-positive patients. Antibiotic usage alone was significantly associated with the development of *C. difficile* colonization ($p=0.011$), and the length of hospitalization was associated with the development of *C. difficile* infection ($p=0.032$).

Conclusion: *C. difficile* colonization and infection occur frequently during the disease course of PIBD. Antibiotic usage and longer hospital stay were significant risks factors for the conversion of *C. difficile* status in PIBD patients undergoing treatment.

Key Words: *Clostridium difficile*, Infection, Inflammatory bowel diseases, Child

INTRODUCTION

Clostridium difficile is an anaerobic, gram-positive spore-forming bacillus causing a wide spectrum of

diseases. Symptoms of *C. difficile* infection can range from mild, self-limited diarrhea to severe colitis with cramping, hematochezia, pseudomembrane formation, and intestinal perforation [1]. *C. difficile* col-

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onizes the colon although the colonization itself is not invasive. Exotoxins (toxin A and toxin B) produced by the *C. difficile* bacillus mediate tissue injury and inflammation and cause symptoms associated with *C. difficile* infection [1].

Imbalances or alterations in gut microbiota composition (dysbiosis) have been reported in patients presenting with inflammatory bowel disease (IBD) [2], and IBD is associated with an increased prevalence of *C. difficile* infection [3]. Reportedly, the prevalence of *C. difficile* infection in pediatric patients diagnosed with IBD ranges between 3.5% and 69% [2,4-10].

C. difficile colonization and infection are common complications of IBD and are associated with poor outcomes [4,5]. Toxin producing *C. difficile* infection can trigger or worsen an IBD flare-up. Early diagnosis of *C. difficile* infection in IBD patients is thus very important. However, it is particularly difficult because clinical symptoms of *C. difficile* infection are indistinguishable from those of IBD flare-up [11]. Most of our current knowledge regarding *C. difficile* colonization and infection in pediatric IBD (PIBD) patients is limited because most data have been obtained from adult studies and are associated with number of confounding factors [4,12,13]. Despite its clinical significance in PIBD, to date, *C. difficile* infection in PIBD has rarely been studied.

Therefore, the aims of our study were to investigate the prevalence of *C. difficile* colonization and infection in PIBD patients at the time of initial diagnosis and to evaluate the risk factors associated with *C. difficile* colonization and infection in PIBD patients during the disease course and treatment.

MATERIALS AND METHODS

Subjects

We enrolled a total of 59 children and adolescents who were newly diagnosed PIBD at the Seoul National University Bundang Hospital between March 2011 and May 2017. These patients were selected from the pediatric gastrointestinal disease database of our tertiary medical center. All enrolled patients underwent

C. difficile testing at the time of initial diagnosis and at every follow-up throughout the disease course. Demographic and clinical data were reviewed retrospectively in each patient.

This study was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB no. B-1603-338-101).

Diagnosis of inflammatory bowel disease

Diagnosis of Crohn disease (CD) and ulcerative colitis (UC) was based on clinical symptoms and signs, endoscopic and histopathological findings, as well as radiological investigations including magnetic resonance enterography (MRE). All enrolled patients underwent complete diagnostic work-up including colonoscopic examination with ileal intubation, upper gastrointestinal endoscopy, and MRE to evaluation of small intestine. Multiple biopsies were obtained in each patient from each segment of the gastrointestinal tract for histological evaluation.

Diagnosis of *Clostridium difficile* infection

All enrolled patients underwent *C. difficile* toxin assay testing and cultures. Toxin assay testing was performed using a stool enzyme immunoassay (EIA) kit (VIDAS[®] *C. difficile* Toxin A & B assay; BioMérieux, Marcy-l'Etoile, France). Cultures were performed using a specific stool culture medium (ChromID[®] *C. difficile*; BioMérieux).

C. difficile colonization was defined as a positive stool culture for *C. difficile* with a negative toxin immunoassay. *C. difficile* infection was defined as a condition wherein both the stool culture and toxin immunoassay for *C. difficile* were positive.

Statistical analysis

Data are presented as mean ± standard deviation (SD) for parametric continuous variables and median (range) for nonparametric continuous variables. All data were analyzed using the IBM SPSS Statistics ver. 22.0 software program (IBM Co., Armonk, NY, USA).

The chi-squared test was used for statistical analysis of categorical variables compared between the

groups. The Mann-Whitney U test for nonparametric analysis was used for analysis of continuous variables between the groups.

Kaplan-Meier survival analysis was performed to analyze the status of *C. difficile* during the disease course of PIBD. *C. difficile* colonization was defined as the event during the disease course on Kaplan-Meier survival analysis. Positive conversion of *C. difficile* culture and *C. difficile* toxin were set as a primary outcome of the Kaplan-Meier survival analysis in the present study. Kaplan-Meier survival curves were generated and statistical significance was analyzed.

Cox regression tests were used to analyze of risk factors associated with *C. difficile* colonization or infection during the treatment course of PIBD. Patients who initially tested positive for *C. difficile* toxin or culture were excluded from the Cox regression analysis. Using the R program, the Cox hazard model was created by plotting a graph of the relative log of the hazard ratio. All *p*-values < 0.05 were considered statistically significant.

Table 1. Baseline Clinical Features of the Patients Recruited

Variable	IBD (n=59)
Male gender	38 (64.4)
Age (y)	13.5±2.6
Initial <i>C. difficile</i> culture positivity	13 (22.0)
Initial <i>C. difficile</i> toxin positivity	3 (5.1)

Values are presented as number (%) or mean±standard deviation. IBD: inflammatory bowel disease, *C. difficile*: *Clostridium difficile*.

Table 2. Comparison of Clinical Factors between Children with CD and Those with UC

Variable	CD (n=41)	UC (n=18)	<i>p</i> -value
Male gender	27 (65.9)	11 (61.1)	0.726
Age (y)	13.5±2.5	13.4±2.8	0.833
Initial <i>C. difficile</i> culture positivity	10 (24.4)	3 (16.7)	0.735
Initial <i>C. difficile</i> toxin positivity	2 (4.9)	1 (5.6)	1.000
Colon involvement	29 (70.7)	18 (100.0)	0.012
No. of patients who used antibiotics during the disease course	5 (12.2)	1 (5.6)	0.656
No. of patients who underwent operation during the disease course	4 (9.8)	0 (0)	0.303
The total duration of admission (d)	38.1±41.0	15.3±18.0	0.025
Frequency of disease flare during the disease course	1.3±0.8	1.1±1.0	0.459

Values are presented as number (%) or mean±standard deviation. CD: Crohn disease, UC: ulcerative colitis, *C. difficile*: *Clostridium difficile*. *p*-value less than 0.05 was set to be statistically significant.

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RESULTS

Patient characteristics

Our study included 59 children and adolescents (38 boys, 21 girls; mean age±SD, 13.5±2.6 years) who were newly diagnosed with IBD and underwent *C. difficile* testing at their first visit. Demographic and clinical data of the PIBD patients recruited are listed in Table 1.

The status of *C. difficile* infection of patients with pediatric inflammatory bowel disease at the time of diagnosis and during the disease course

C. difficile cultures were positive at the time of initial diagnosis in 13 patients (22.0%) of 59 children with PIBD, whereas the *C. difficile* toxin assay at the time of diagnosis was positive in only 3 patients (5.1%) of 59 PIBD patients (Table 1).

Thirteen patients (22.0%) were initially culture-positive at the time of diagnosis, and an additional 28 patients (47.5%) showed conversion to positive cultures during the disease course of IBD. Thus, *C. difficile* cultures were positive in a total of 41 (69.5%) patients with PIBD.

Only 3 patients (5.1%) were initially toxin-positive at the time of diagnosis, and toxins assay tests converted to positive result in an additional 13 patients

(22.0%) during the course of PIBD. Thus, *C. difficile* toxin assays were positive in a total of 16 (27.1%) patients with IBD.

Clinical features and the status of *C. difficile* infection in pediatric patients diagnosed with Crohn disease and ulcerative colitis at the time of diagnosis and during disease course

Among the 59 newly diagnosed PIBD patients, 41 patients (69.5%) had CD and 18 patients (30.5%) had UC. Factors that can affect the status of *C. difficile* infection in patients diagnosed with IBD such as colonic involvement, antibiotics use, history of operations, length of hospitalization, and frequency of disease flare-ups were listed and compared between the patients with CD and those with UC (Table 2). Only the length of hospitalization revealed a statistically significant difference between the groups (Table 2).

Kaplan-Meier survival analysis was performed in patients with CD and UC, respectively, for *C. difficile* colonization during IBD treatment, which revealed no statistically significant difference between those with CD and UC ($p=0.332$) although *C. difficile* colonization with culture positivity developed in both groups during the disease course of IBD (Fig. 1).

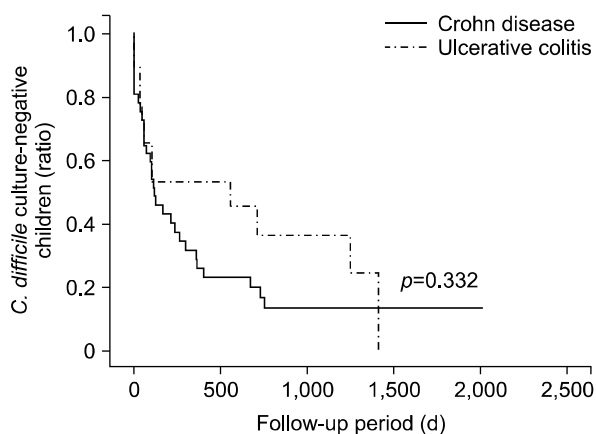


Fig. 1. Survival curve for *Clostridium difficile* colonization with culture positivity during the disease course of inflammatory bowel disease in children with Crohn disease and those with ulcerative colitis, respectively. As *C. difficile* colonization was defined as the event for survival analysis, y axis of the graph indicates the ratio of *C. difficile* culture-negative children.

In the same way, Kaplan-Meier survival analysis was also performed in patients with CD and UC for *C. difficile* infection during the disease course of IBD, which revealed no statistically significant difference between those with CD and UC ($p=0.672$) although *C. difficile* infection with both toxin and culture positivity developed in both groups during the disease course (Fig. 2).

Factors affecting the development of *C. difficile* colonization and infection during the treatment of pediatric inflammatory bowel disease

Based on the results obtained from univariate analysis, Cox regression analysis was performed using significant variables such as the type of IBD, colonic involvement, antibiotic use, history of operations, length of hospitalization, and the frequency of disease flare-ups as possible risk factors associated with the development of *C. difficile* colonization and infection. Use of antibiotics was the sole significant risk factor associated with fecal conversion to *C. difficile* colonization ($p=0.011$) (Table 3). Length of hospitalization was the sole risk factor associated with fecal conversion to *C. difficile* infection in PIBD pa-

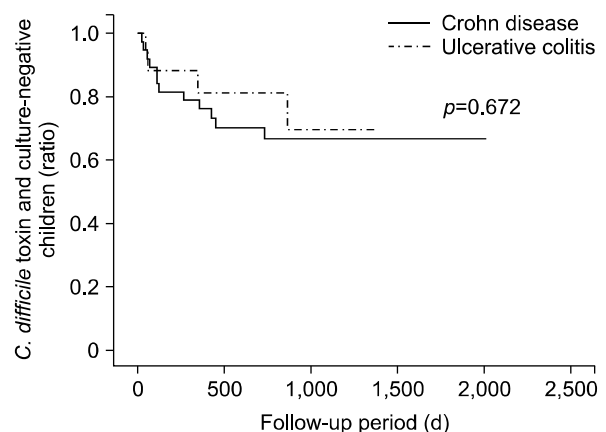
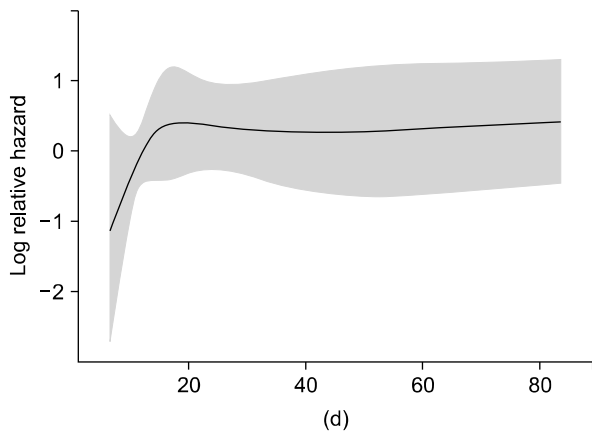


Fig. 2. Survival curve for *Clostridium difficile* infection with toxin and culture positivity during the disease course of inflammatory bowel disease in children with Crohn disease and those with ulcerative colitis, respectively. As *C. difficile* infection was defined as the event for survival analysis, y axis of the graph indicates the ratio of *C. difficile* toxin and culture-negative children.

Table 3. Risk Factors for Developing *Clostridium difficile* Colonization and *C. difficile* Infection during the Disease Course of Pediatric Inflammatory Bowel Disease

	Variable	Exp(B)	95% confidence interval	p-value
<i>C. difficile</i> colonization	Antibiotic usage	3.754	1.360-10.366	0.011
<i>C. difficile</i> infection	The duration of admission	1.018	1.002-1.035	0.032

p-value less than 0.05 was set to be statistically significant.

**Fig. 3.** Relative hazard ratio graph (log scale) of *Clostridium difficile* infection according to total hospitalization period in children with inflammatory bowel disease.

tients ($p=0.032$) (Table 3, Fig. 3).

DISCUSSION

In the present study, we evaluated the status of *C. difficile* infection at the time of initial diagnosis of PIBD and investigated the risk factors associated with *C. difficile* colonization and infection during the disease course and IBD treatment. Our results revealed that initial culture positivity at the time of diagnosis was observed in approximately 22% of patients with PIBD, whereas *C. difficile* toxin positivity at the time of initial diagnosis was noted only in approximately 5% of patients with PIBD. Furthermore, *C. difficile* cultures converted to positive results in approximately 47.5% of PIBD patients during the disease course of PIBD in those receiving treatment. Thus, approximately 70% of PIBD patients eventually demonstrated *C. difficile* culture positivity at least once. Additionally, *C. difficile* toxin assays also con-

verted to show positive results in 22.0% of the patients during the disease course of PIBD. Thus, approximately 27.1% of PIBD patients finally demonstrated toxin positivity at least once. According to a previous study, *C. difficile* colonization and infection were observed to be more prevalent in patients with PIBD than children without PIBD, and subsequent conversion to *C. difficile* colonization and infection occurred frequently during PIBD treatment even in those with a negative *C. difficile* status at the time of diagnosis [14].

Recent studies reported a significantly increased prevalence of *C. difficile* infection in patients with IBD [9,15-17], particularly in the pediatric age group, ranging between 16 and 69% [2,6,18,19]. The high prevalence of *C. difficile* colonization and infection in IBD patients could be attributed to the fact that dysbiosis is a risk factor for the development of both, IBD and *C. difficile* infection [20,21]. Our study showed a positive *C. difficile* colonization rate of 22.0% and a *C. difficile* infection rate of 5.1% with both culture and toxin positivity at the time of initial diagnosis and total rates (infection rate at the time of diagnosis combined with the conversion rate during the disease course) were 69.5% and 27.1%, respectively. This significant discrepancy observed between related studies could be because of the extremely low sensitivity of the toxin immunoassay used to detect *C. difficile* infection and other unidentified local factors [22,23]. Another study revealed that the sensitivity of the *C. difficile* toxin assay in immunocompromised patients was very low, and this can be similarly applied to IBD patients treated with methotrexate and thiopurines [24].

Previous studies have demonstrated that IBD itself is an independent risk factor for *C. difficile* in-

fection in both adult and pediatric populations [2,11,12]. In this study, the prevalence of *C. difficile* colonization was significantly higher in patients with PIBD than other children at the time of initial diagnosis [14]. Additionally, *C. difficile* colonization and infection were not related to the type of IBD in our study – this finding was consistent with the results reported by other previous studies [8,9].

Because IBD is a chronic gastrointestinal disease, its treatment continues without interruption as the disease runs a life-long course. Thus, the conversion of *C. difficile* status during IBD treatment can be a critical issue in PIBD patient. Based on previous studies, the status of *C. difficile* infection is routinely monitored for 1-2 years or at specific time-points such as time IBD is first diagnosed or at the time of an IBD flare-up [9,23,25]. However, no studies have reported the conversion of *C. difficile* status during the disease course of IBD yet. Therefore, our study followed up PIBD patients for a relatively long period of time. Thus, we could identify the conversion rate and the pattern of *C. difficile* infection. Despite the limitations secondary to the retrospective nature of our study, *C. difficile* colonization, which might have affected disease progression in PIBD patients, was observed in most patients over a 6-year observation period.

Although various risk factors associated with *C. difficile* infection have been reported, to date, it is unclear which factors other than IBD itself affect the development of *C. difficile* infection in IBD patients. Our present study showed that among several factors studied, antibiotic use was the only significant risk factor associated with the occurrence of *C. difficile* colonization during the course of PIBD in patients receiving treatment, and that the length of hospitalization was a significant risk factor associated with *C. difficile* infection during PIBD treatment. Longer the period of hospitalization and higher the severity of the disease, greater was the risk of *C. difficile* infection among patients in a hospital setting. Moreover, we observed that *C. difficile* infection in patients with IBD affects the clinical course of IBD and increases the length of hospitalization [26]. Therefore,

it is important in clinical practice to adopt systematized strategies to prevent the vicious cycle of recurrent *C. difficile* infection superimposed on the disease course in PIBD patients.

Usually, antibiotic use is considered as the primary risk factor associated with *C. difficile* infection. Previous studies have not reported a statistically significant effect of antibiotic usage on the development of *C. difficile* infection in IBD patients as was shown by our study [2,6,27]. However, our present study demonstrated that the use of antibiotics was clearly associated with *C. difficile* colonization during the disease course of PIBD. *C. difficile* infection occurs as a result of *C. difficile* colonization; therefore, antibiotic use should be avoided or minimized in patients with IBD to prevent *C. difficile* colonization and subsequent infection.

Limitations of our study: 1) ours was a single center study with a retrospective design. The number of patients recruited in this study was relatively small; thus, a few factors that are known to be risks factors for development of *C. difficile* infection did not show statistically significant result in this study. Further studies involving a larger number of patients are needed to conclusively establish results in this context. 2) The sensitivity of the toxin assay performed using the EIA method for the diagnosis of *C. difficile* infection is very low [19,23,24]. Therefore, a few patients with *C. difficile* infection might have been incorrectly classified as those with *C. difficile* colonization rather than an infection. Although a polymerase chain reaction test is a relatively accurate to detect *C. difficile*, it is an expensive and time-consuming method for routine use in clinical practice [28].

In conclusion, *C. difficile* colonization is prevalent at the time of initial diagnosis of PIBD, and *C. difficile* colonization and infection can occur frequently during the disease course of PIBD. Antibiotic usage and longer hospitalization were observed to be significant risks factors associated with for the conversion of *C. difficile* status in PIBD patients during the disease course. Therefore, early and accurate diagnosis of *C. difficile* infection in PIBD patients based on the risk factors suggested in the present study

may facilitate prompt and correct diagnosis and institution of appropriate treatment strategies in PIBD patients and thereby improve the prognosis of PIBD.

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