

Clostridium Difficile Associated Diarrhea in Pediatric Oncology Patients Receiving Chemotherapy

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ABSTRACT

Background: Nowadays, patients with cancer receive more intensive chemotherapeutic regimens together with broad spectrum antibiotics during periods of profound immunosuppression. Thus, cancer patients are susceptible to infection with clostridium difficile but the role of this pathogen in pediatric oncology patients is poorly defined.

Patients and methods: The prevalence of *C. difficile* infection in pediatric oncology patients with diarrhea following chemotherapy was prospectively monitored, by testing for the presence of its A toxin, at National Cancer Institute, Cairo University.

Results: *C. difficile* toxin A was detected in fifteen (14.4%) of 104 diarrhea episodes following chemotherapy. Hospitalization, the use of antibiotics and relapsing disease were found to be significantly associated with this pathogen. Two outbreaks were observed. Severe enterocolitis with dehydration was significantly associated with *C. difficile* than with other causes of diarrhea. Case fatality rate directly attributable to *C. difficile* infection was 20%.

Conclusion: *C. difficile* is an important cause of diarrhea following intensive chemotherapeutic protocols and is associated with prolonged hospital stay and antibiotic use. Moreover, *C. difficile* have shown a strong association with morbidity and increased mortality and should be searched for as a cause of diarrhea in hospitalized pediatric cancer patients receiving empirical antibiotics.

Key Words: *Diarrhea - Clostridia difficile - Pediatric oncology.*

INTRODUCTION

C. difficile is a spore-forming toxigenic bacterium [19]. It is an opportunistic pathogen that causes nosocomial diarrhea and colitis after the normal gastrointestinal flora has been eliminated or drastically altered, most typically by antibiotics and also by chemotherapy [3]. *C. difficile* is responsible for 15-25% of cases of antibiotic-associated diarrhea (AAD) and for

virtually all cases of antibiotic-associated pseudomembranous colitis (PMC). This anaerobic bacterium has been identified as the leading cause of nosocomial diarrhea in adults and can be responsible for large outbreaks [2]. Risk factors for *C. difficile*-associated diarrhea include antimicrobial therapy, older age (> 65 years), antineoplastic therapy and length of hospital stay. Other interventions with high risk associations are enemas, nasogastric tubes, gastrointestinal surgery and antiperistaltic drugs [2,4]. Prospective studies have shown that nosocomial transmission of *C. difficile* is frequent but diarrhea is infrequent and often remains asymptomatic. Patients can be contaminated from environmental surfaces, shared instrumentation, hospital personnel hands and infected roommates. Once an outbreak starts, *C. difficile* may spread rapidly throughout the hospital environments where spores may persist for months [2]. The contribution of *C. difficile* to the occurrence of diarrhea in pediatric cancer patients on chemotherapy has been reported in a small number of studies [4,8,17]. This prospective study has been conducted to evaluate the contribution of *C. difficile* in pediatric oncology patients with diarrhea while on chemotherapy in order to ascertain its incidence, risk factors and implication on outcome.

PATIENTS AND METHODS

We prospectively studied pediatric oncology patients receiving chemotherapy at National Cancer Institute, Cairo University, who developed diarrhea during the period from October 1999 to July 2000. Diarrhea was defined as an

abnormal increase in stool liquidity and more than 3 bowel movements per day. One hundred and four episodes of diarrhea were recorded during this period. The patients were subjected to thorough history taking and clinical examination as concerns type of malignancy and state of disease whether new, relapsed or in a complete remission, GIT invasion by malignancy and presence of associated infections especially respiratory tract infections. In addition, clinical examination was extended to include associated manifestations accompanying diarrhea as fever, vomiting, abdominal colic and dehydration. During the diarrhea episode, the following data were recorded: stool frequency and duration of diarrhea. Complete blood counts were obtained.

Diagnosis of nosocomial infection was confirmed after ruling out community based infections present at admission and if patient developed diarrhea after 4 days of symptom free hospital stay. Presence of fever and a complete blood picture were recorded at day 1 of diarrhea. Stool specimens were obtained at the onset of diarrhea and specimens were studied as regards consistency, color and presence of mucus. Evaluation of case as regards abdominal infiltration of the disease and presence of hepatosplenomegaly was done clinically and by means of imaging.

Response to treatment was considered satisfactory if cessation of diarrhea occurred in ≤ 1 week period. Follow up of cases was done as regards occurrence of dehydration, duration of diarrhea and the outcome of the patient whether alive or dead. If death occurred, assessment of the most probable cause of death was carried out.

Microbiological evaluation: Stool specimens were cultured for the presence of pathogenic bacteria and fungi. Clostridia difficile toxin was determined in stool by an Immuno-card® Toxin A enzyme immunoassay obtained from Meridian Diagnostics, Inc., USA [10]. Stool specimens were investigated for the presence of cryptosporidium by modified acid-fast stains and other parasites by standard laboratory methods. Diarrhea was defined as infectious in cases of recovery of a known enteric pathogen.

Statistical methods: Univariate p values were calculated from Chi-square tables and from Mann Whitney test using SPSS version 10.0. Multiple logistic regression models were

fitted for both risk factor analysis of *C. difficile* infection and for predicting successful outcomes with different pathogenic organisms.

RESULTS

The study involved a total of 104 diarrhea episodes experienced by pediatric cancer patients under myelo-ablative therapy. The duration of diarrhea lasted 3-30 days (mean 11.4 ± 7.0) with the number of motions ranging between 5-17 per day (mean 6.5 ± 2.7).

Risk factors and characteristics of diarrhea in C. difficile infected patients: Fifteen patients (14.4%) developed *C. difficile* infection during a 10 month period of prospective evaluation of pediatric oncology patients who developed diarrhea at the NCI, Cairo University. The clinical data, characteristics of diarrhea and outcome of *C. difficile* infected patients in comparison to other causes of diarrhea are summarized in Table (1). The *C. difficile* infected cases had an age range of 1.5-18 years and a 1.5 male to female ratio. The underlying disease was ALL, AML, NHL and solid tumors in 7,3,3, & 2 of the patients, respectively. All *C. difficile* infections were nosocomial-acquired at a median of 21 (14-37) days of hospital stay versus 17 (7-30) days in cases with other causes of diarrhea, $p = 0.04$. Two thirds of patients with *C. difficile* had received intravenous antibiotics for more than a week prior to the occurrence of diarrhea versus 10% in those without infection ($p < 0.001$). Fever ≥ 38.5 was recorded in all patients with *C. difficile* infection. Diarrhea was worse with *C. difficile* infection as evidenced by a significant number of bowel motions 10 (4-12) versus 5 (3-17) per day and by lasting more in infected (median 10 versus 8 days) than in the other group. As a consequence, dehydration was more significantly evident in *C. difficile* patients and necessitated iv-fluid replacement. *C. difficile* infection was isolated in 7 cases and combined with other infections in 8 cases.

Response and outcome: All patients received metronidazole as the first line of treatment with the addition of vancomycin in 3 cases. Poor response to therapy was encountered in most of *C. difficile* infected patients, 11/15. *C. difficile* was associated with an unfavorable outcome in 9 of the patients ($p < 0.001$). Relapsing disease was the most probable cause of poor outcome in 6 of the patients; whereas, *C.*

difficile infection being the direct cause of death in 3 (20.0%).

Outbreaks and recurrence: Clustering of cases was observed in 2 incidents. Three patients were diagnosed during a 5 day period in one ward; whereas, an outbreak was recorded in 4 cases in a 1 week period in the same ward in 2 consecutive months. Six *C. difficile* patients had a recurrence of diarrhea.

Other causes of diarrhea: Bacteria (strains known to be pathogenic) and fungi were isolated by culture in 51 and 27 of the patients, respectively. Cryptosporidium oocysts were detected in 10 patients of the study group by modified acid fast stain. Multiple pathogens were detected in 32 patients. No cause of diarrhea was found in 30 patients.

Statistical analysis: On univariate analysis,

risk factors for acquiring *C. difficile* infection were found to be increased length of hospital stay, prolonged use of antibiotics, relapsing disease and abdominal involvement by the original disease. On multivariate analysis, state of underlying disease was precluded to be an independent predictor of *C. difficile* infection (Table 2) *C. difficile* associated diarrhea was characterized by more number of bowel motions, prolonged diarrhea and dehydration. Different pathogenic organisms found in our pediatric patients were submitted to logistic regression analysis as predictor variables with mortality as an outcome. Successful outcome of diarrhea in pediatric oncology patients was less likely with *C. difficile* infection (OR = 0.04, 95% CI = 0.01-0.19), fungal (OR = 0.20, 95% CI = 0.05-0.74) and bacterial infections (OR = 0.20, 95% CI = 0.05-0.79). The results of statistical studies are illustrated in Table (3).

Table (1): Patients with and without *C. difficile* infection.

	With <i>C. difficile</i>	Without <i>C. difficile</i>	<i>p</i> value
Number of patients	15 (14.4%)	89 (85.6%)	
Age in years (range)	5 (1.5-18)	7 (1-18)	0.17
Male/female ratio	9/6	57/32	0.76
<i>Diagnoses:</i>			
Hematologic	13 (86.7%)	68 (76.4%)	
Solid	2 (13.3%)	21 (23.6%)	0.38
<i>State of disease:</i>			
Relapsed	8 (53.3%)	19 (21.3%)	
New/CR	7 (46.7%)	70 (78.7%)	0.009
<i>Chemotherapy type:</i>			
No	0	1 (4.5%)	
AraC	4 (26.7%)	20 (22.5%)	0.70
MTx	8 (53.3%)	39 (43.8%)	
VP16	3 (20%)	26 (29.2%)	
Nosocomial infection	15 (100%)	43 (48.3%)	< 0.001
LOS in days (range)	21 (14-37)	17 (7-30)	0.04
Antibiotics ≥ a week	10 (66.7%)	9 (10.1%)	< 0.001
GIT involvement by original disease	7 (46.7%)	13 (14.6%)	0.004
Number of motion/day (range)	10 (4-12)	5 (3-17)	0.03
Duration of diarrhea in days	10 (7-30)	8 (3-30)	0.03
Dehydration	12 (80%)	37 (41.6%)	0.007
Recurrence of diarrhea	6 (40%)	43 (48.3%)	0.55
ANC < 500	9 (60%)	59 (66.3%)	0.88
Good response to ttt	4 (26.7%)	44 (49.4%)	0.1
Overall mortality	9 (60%)	12 (13.5%)	< 0.001

Table (2): Multivariate logistic regression analysis for risk factors found to predispose to *C. difficile* infection on univariate analysis.

Variable	B	S.E.	p-value	OR	95% CI for OR	
Antibiotics \geq a week	3.3465	0.9742	0.0006	28.40	4.2086	191.69
Relapsed state	0.8432	0.6128	0.1689	2.32	0.6991	7.72
LOS	0.1657	0.0739	0.0249	1.18	1.0211	1.36
GIT involvement by original dis	1.9217	0.9619	0.0457	6.83	1.0371	45.02
Constant	-10.3375	3.3346	0.0019			

B = Regression coefficient. CI = Confidence interval.
 SE = Standard error. Ab = Antibiotics.
 OR = Odds ratio. LOS = Length of hospital stay.

Table (3): Multivariate logistic regression analysis for successful outcome of diarrhea using different infections as predictors of disease.

Organism	B	S.E.	p-value	OR	95% CI for OR
Cryptosporidia	-0.6839	0.8034	0.3946	0.5046	(0.10-2.44)
<i>C. difficile</i>	-3.3220	0.8511	0.0001	0.0361	(0.007-0.19)
Fungi	-1.6108	0.6653	0.0155	0.1997	(0.054-0.736)
Pathogenic bacteria	-1.6101	0.6981	0.0211	0.1999	(0.051-0.785)
Constant	3.5970	1.1843	0.0024		

DISCUSSION

Over the last decade, the possibility of administering more intensive chemotherapy, the introduction of new techniques such as allogeneic hematopoietic cell transplantation and autologous stem cell infusion, the application of growth factors, the implantation of central venous catheters and progress in diagnostic techniques and in antimicrobial chemotherapy are associated with the improved prognosis of patients with malignancies [16]. The achievement of prolonged survival during periods of profound immunosuppression has also rendered cancer patients susceptible to a broad array of potential pathogens causing infections [11]. Gastroenteric manifestations following newer protocols of chemotherapy are becoming more common.

C. difficile causes essentially all cases of pseudomembranous colitis and about 25% of antibiotic associated diarrhea [14]. Cancer patients often receive broad spectrum antibiotics in addition to antineoplastic chemotherapy. Both treatments are known to predispose oncology patients to colonization and infection with *C. difficile*. Generalized abdominal distension and diarrhea characterize classic pseudomembranous colitis caused by *C. difficile* in neutropenic cancer patients. If left untreated, mortality rate ranges from 50 to 100% because of compli-

cations such as bowel perforation and septic shock [11].

In our study, *C. difficile* infection was detected clinically and microbiologically in 14.4% pediatric cancer patients receiving chemotherapy and complaining of diarrhea. These results were found to agree with the results of a recent study on the importance of *C. difficile* associated diarrhea (Cdad) in patients with neutropenia who have hematologic malignancies. In the latter study, Cdad occurred in 7.0% of 875 courses of myelosuppressive chemotherapy and it was concluded that *C. difficile* infection was not rare and should be suspected whenever a hospitalized patient with neutropenia develops diarrhea [12]. Furthermore, the prevalence of *C. difficile* infection in a pediatric oncology ward was found to be 13% in a one year study [17]. In the latter study, patients with longer duration of hospital stay and those with lymphoid malignancies were at the highest risk for this type of infection. The diagnostic category at greatest risk were those most intensively treated, with protracted neutropenia and prolonged antibiotic exposure. Unlike the previous reports, it was concluded by Burgner and his colleagues [8] that *C. difficile* did not appear to be an important pathogen in pediatric oncology patients as toxigenic *C. difficile* may form part of the normal flora in young children.

Risk factors for the occurrence of this infection in our patients were found to be prolonged hospitalization, abdominal involvement of the original disease and intake of iv antibiotics for ≥ 1 week prior to diarrhea. Hospitalization was crucial for the acquisition of *C. difficile* associated diarrhea as all patients whose diarrhea was caused by *C. difficile* in our group of patients were hospitalized prior to the occurrence of diarrhea. Not only hospitalization but length of hospital stay did superimpose a risk on oncology patients with Cdad. These results are in agreement with other studies reporting *C. difficile* to be an important cause of childhood nosocomial diarrhea and to occur more frequently in cases given combined antibiotic treatment than in those given single antibiotic treatment [15]. In addition, hospitalization was an independent predictive factor of *C. difficile* colitis in oncology patients by multivariate analysis in a retrospective study done in a hematologic malignancy and bone marrow transplant unit [13]. Neutropenia, in spite of being recorded in 60% of cases with *C. difficile* infection, still this was not statistically different than the other patients with other causes of diarrhea. This could be due to that neutropenia occurred in most of our patients following anti-neoplastic chemotherapy and it was the determinant factor for the occurrence of infection in general.

Fever was a common manifestation in *C. difficile* associated diarrhea in this study. Severe enterocolitis associated with dehydration was encountered in 12/15 cases infected with *C. difficile*. Diarrhea was worse with *C. difficile* infection as evidenced by a significant number of bowel motions 10 (4-12) versus 5 (3-17) per day and by lasting more in infected (median 10 versus 8 days) than in the other group. As a consequence, dehydration was more significantly evident in *C. difficile* patients and necessitated iv-fluid replacement in addition to increased morbidity associated with *C. difficile* infection, mortality was also significantly related to *C. difficile* infection than with other causes of diarrhea. Nine of the 15 cases infected with *C. difficile* died while they had diarrhea. However, in 3 patients was infection considered to be the primary cause of death as death of the other patients was attributed to relapsing or recurrent malignant disease more than infectious causes.

Clostridium difficile infection was significantly more encountered in relapsing or recur-

rent malignant disease in our group of patients. This could be explained by the profound immunosuppression occurring with disease recurrence. It was previously shown that inadequate immune response predisposed to severe and recurrent *C. difficile* infection [19]. In a study carried out on allogeneic stem cell transplant patients, *C. difficile* infection was significantly associated with graft versus host disease (GVHD) grade 3-4 and it was concluded that the occurrence of *C. difficile* could be a surrogate marker for an impaired immune system as evidenced by increased viral infections and an adverse outcome [9]. Thus, whether *C. difficile* has a direct impact on the worsening of the immune system or a severely compromised immune system permits such infection is currently unclear.

Two outbreaks of Cdad were observed in this prospective study as 2 clusters of cases occurred in less than a week period each at the same ward. Both groups of infections were encountered in 2 consecutive months, pointing to the communicable capability of this pathogen. *C. difficile* was previously regarded as a communicable disease in pediatric oncology patients [8]. In the latter study, 21 cases of *C. difficile* occurred on a pediatric oncology unit in one year; of which 11 cases were clustered in 2 months period. Recently, the occurrence of clusters with distinct strains of *C. difficile* was confirmed by genotyping for toxins A & B of strains isolated from patients with Cdad [18]. The communicable nature of *C. difficile* was further supported by analysis of patients' isolates during a nosocomial outbreak of Cdad and the finding that this outbreak was due to transmission of a single strain of *C. difficile* [1].

C. difficile associated disease develops from the production of two toxins, A and B, which are also referred to as the enterotoxin and cytotoxin, respectively. There is some evidence that both toxins act synergistically during the onset and development of the disease [5]. Most toxigenic strains produce both toxin A and B, non-toxic strains, which are also non pathogenic, do not carry the toxin genes [7]. Toxin A binds to specific carbohydrates on the mucosa of colon and causes extensive damage, whereas toxin B binds to cells that are not surrounded by a carbohydrate and thus is toxic for cells exposed by the action of toxin B [14]. In addition, it was previously thought that strains of toxin A negative, toxin B positive *C. difficile* were not

associated with clinically significant disease [1]. Furthermore toxin A was found to be a more potent cytotoxin for tissue culture lines [14]. So, it is evident from the previous data that toxin A is more important in the pathogenesis of Cdad and therefore we searched for toxin A rather than toxin B.

Isolation of *C. difficile* is not sufficient for diagnosis because more than 20% of isolates do not produce toxins and therefore, are not pathogenic. Additional testing by tissue culture or enzyme immunoassay must be performed to demonstrate that the isolates can produce toxin [14]. Nowadays, toxin detection from stool is a prerequisite for *C. difficile* associated diarrhea and colitis [2]. Thus, in our study, we have depended on toxin detection by an enzyme immunoassay method rather than direct detection of the organism by culture for the diagnosis of *C. difficile* as a cause of diarrhea in pediatric oncology patients.

In conclusion, we report that with the intensive chemotherapeutic protocols used in pediatric cancer patients, diarrhea is occurring with increased frequency and that *C. difficile* is an important cause of diarrhea in these patients. In addition, Cdad is associated with increased morbidity and mortality in our patients. Investigations to detect *C. difficile* toxins should be carried out in children with cancer who have diarrhea, especially with the use of empirical antibiotic regimens together with hospitalization.

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