

Inflammatory Bowel Disease and *Clostridium difficile* Infection: A Report from a Tertiary Care Center of North India

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Abstract

Clostridium difficile infection (CDI) is reported to be frequently associated with inflammatory bowel disease (IBD). Because of the common characteristics of the clinical appearance of CDI and IBD the diagnosis and treatment of the same becomes complicated. We analyzed the prevalence of CDI in patients with IBD along with their demographic and clinical profile from our meticulously maintained laboratory records. During analysis, the IBD patients (n=721; M:F=1.10:1) comprised of test group and an equal number of gender-matched patients with no indication of IBD was included as non-IBD controls. The demographic and clinical data as well as fecal *C. difficile* toxin status of all the patients were retrieved from laboratory records. The number of *C. difficile* positivity was more in the non-IBD group (20%) compared to IBD patients (16%) with non-significant difference ($p < 0.064$). The patients in non-IBD group were relatively older ($p < 0.001$) compared to those in IBD group. The duration of diarrhea in the IBD group was significantly longer ($p < 0.001$) compared to the non-IBD group, but no significant difference ($p > 0.063$) in the frequency of diarrhea between the two groups was observed. Blood in stool and abdominal pain symptoms were found to be significantly higher ($p < 0.001$) among the IBD group whereas watery diarrhea and fever were significantly linked ($p < 0.001$) with non-IBD group. Significant improvement was seen in most of the parameters during the time of follow-up. Though the prevalence of CDI in both IBD and non-IBD groups were almost comparable, clinical symptom and age of presentation varied in them.

Keywords: Abdominal pain; *Clostridium difficile*; Diarrhea; Follow-up studies; Humans; Inflammatory bowel disease; Prevalence

Introduction

Clostridium difficile is notorious in causing infectious colitis usually in the elderly or hospitalized patients, or those with exposure to antibiotics [1]. However, recently there is a significant rise in *C. difficile* infection (CDI) incidence in the general population involving younger age group, and those patients who have undergone transplant or are immunocompromised [2]. An increase in the community prevalence of CDI is also being reported [3,4].

Inflammatory bowel diseases (IBD) broadly comprise of ulcerative colitis (UC) and Crohn's disease (CD) and such patients have been identified to be at increased risk of acquiring CDI in relation to the general population [5,6] with greater proportion associated with hospitalization, surgery, as well as mortality when compared to non-IBD patients with CDI [7,8]. The incidence of CDI among IBD patients has shown an increase of 2-3 folds [9] particularly including younger age-group, community acquisition and lack of antibiotic exposure [10]. Patients with IBD also experience worse outcomes [11] and high risk of CDI recurrence [12]. However, the reason for increased carriage and CDI risk in IBD patients is not quite clear.

C. difficile infection in India is different in the context of it being less severe. Data available of CDI in IBD patients in India is sparse [13]. A significant interest regarding the association of CDI with IBD is growing amongst workers [14,15] warranting increased vigilance regarding CDI in IBD patients. We receive fecal samples from patients suspected for CDI from different wards of the hospital, along with pre-printed pro-forma duly filled up by the treating clinicians. This paper

pro-forma is specially designed to note down all information pertaining to patients suspected for CDI and is maintained in our laboratory where they are kept in order and serially bound in a collection each comprising of 200 consequent pro-forma for future analysis. From this meticulously maintained laboratory records, we retrospectively investigated the prevalence of CDI in patients with IBD and analysed their clinical and demographic profile in relation to gender-matched non-IBD patients reporting during the same study period.

Patients and Methods

This work was carried out in the Microbiology Division, Department of Gastroenterology, Post Graduate Institute of Medical Education and Research, Chandigarh, India, which is a 2100 bedded tertiary care teaching hospital where patients from different states of North India receive treatment. The period of data collection was from October 2009 to May 2016. Patients suspected of CDI from both in and out-patient services as a matter of routine care formed the basis of investigation. Baseline and follow-up information of patients were obtained from laboratory records. Informed consent could not be taken as this is a retrospective analysis. The Institute Research Ethics Committee which operates according to the Helsinki Declaration approved the investigation.

All consecutive patients excluding pregnant women and children under 2 years of age reporting to the tertiary care hospital during the study period were enrolled for analyses. Patients whose data could not be completely acquired were removed from the analysis. A total of 721 IBD patients (711 UC; 10 CD) with an acute flare of the disease were included in the study. Diagnosis of IBD was done by the clinicians

based on the patient's signs and symptoms and/or evaluation of endoscopic, histopathological and radiological information. An equal number of gender-matched patients (n=721) referred for CDI but without any known history of IBD was included in the non-IBD group. Thus a total of 1442 patients who primarily provided fecal samples for CDI were investigated. Fecal samples for CDI investigation was also provided by 177 UC patients and 48 non-IBD patients on subsequent visits. Though fecal samples were received on two or three occasions in the follow-up cases, only the baseline and the last follow-up samples were analyzed. In the present study the follow-up of patients both in the IBD and in the non-IBD groups were carried out for a period of six years.

All submitted fecal specimens received in sterile containers are processed immediately and *C. difficile* toxins A and B detected using ELISA kits. The laboratory records were reviewed for patient demographics, clinical presentation, medical history, etc. All prescriptions for antibiotics and other drugs were also taken into consideration.

Statistical analysis

The whole data were entered into excel master sheet and analyzed by using SPSS 22.0 (IBM Corporation and other(s), 1989, 2013). Continuous variables like age of the patients and duration and frequencies of diarrhea were compared using Student's t-test. Other categorical variables were analyzed using Chi-square test. Follow-up data were analyzed using McNemar-Bowker test separately for each of the clinical symptoms in both the groups.

Results

IBD group

Of the 721 IBD patients analyzed in the study, 393 were males and 328 were females (1.10:1). The age range of the IBD patients was 6 to 86 years (mean age 37.5 years). There were 343 (47.6%) hospitalized patients, 362 (50.2%) outpatients and 16 (2.2%) patients with hospitalization status unknown. Predominant clinical symptoms present in patients with IBD were watery diarrhea in 32.7%, abdominal pain in 50% and fever in 15.5%. Bloody diarrhea was found in 270

(37.4%) of the patients indicating active disease, whereas 451 (62.6%) patients were non-bleeding (inactive disease). Mucus was observed in only 30% of all the samples. Antibiotics were received by 35.9% patients, with 49% of them receiving multiple antibiotics. *C. difficile* toxin was positive in 16% of IBD patients. Usage of antibiotics was not significant (p=0.304) in relation to *C. difficile* positivity. Steroids were received by 13.2% of the patients and proton pump inhibitors (PPI) by 5.1%.

Non-IBD group

In the gender-matched, non-IBD patients (n=721) the age ranged from 3 to 90 years (mean age 45.1 years). There were 652 (90.4%) hospitalized patients, 52 (7.2%) outpatients and 17 (2.4%) patients with hospitalization status unknown. Predominant clinical symptoms observed were watery diarrhea in 99.7%, pain abdomen in 40% and fever in 43% of the patients in the non-IBD group. Blood in stool was present in only 5.7% though mucus was seen in 41.5% of all the samples. *C. difficile* toxin was positive in 19.8% of non-IBD patients. Usage of antibiotics was not significant (p=0.238) in relation to *C. difficile* positivity. Fifty three (7.3%) of the non-IBD patients used PPI.

Comparative analysis of IBD group versus non-IBD group

Table 1 depicts comparative analysis of various parameters in the IBD and the non-IBD groups. There was no significant difference (p value=1; Chi square=0) in the proportion of males and females among the IBD and the non-IBD groups because of gender-matched population included in the study. Age was found to be significantly different (p<0.001) between the IBD and the non-IBD groups with patients in the non-IBD group being relatively older (mean age 45.15 ± SD 16.7 years) as compared to the IBD group (mean age 37.59 ± SD 13.2 years). Though the number of *C. difficile* toxin positivity was more in the non-IBD group compared to the IBD group, there was no significant difference (p=0.064) between the two groups. On comparison of the frequency and duration of diarrhea between both the groups, the mean frequency was not found to be significantly different (p<0.05), but the mean duration was significantly longer (p<0.001) in the IBD patients (mean 225 days) in comparison to the non-IBD patients (mean 38 days).

Variables	IBD group n=721 (%)	Non-IBD group n=721 (%)	P values
Male:Female	393:328	393:328	1
Age (Mean ± SD)	37.53 ± 13.20	45.15 ± 16.72	<0.001
<i>C. difficile</i> positive	116 (16.09)	143 (19.83)	0.064
Clinical symptoms			
Watery diarrhea	236 (32.73)	719 (99.72)	<0.001
Abdominal pain	363 (50.35)	288 (39.94)	<0.001
Fever	112 (15.53)	311 (43.14)	<0.001
Diarrhea attributes			
Frequency/day Mean ± SD	6.56 ± 3.79	6.96 ± 4.02	0.063

Duration(days)	224.6	38	<0.001
Blood in stool	270 (37.45)	41 (5.68)	<0.001
Presence of mucus	218 (30.24)	299 (41.47)	<0.001
Antibiotics usage			
Nil	462 (64.08)	48 (6.66)	<0.001
Single	131 (18.17)	283 (39.25)	<0.001
Multiple	128 (17.75)	390 (54.09)	<0.001

Table 1: Comparative analysis of various parameters in the IBD and the non-IBD groups.

When the use of antibiotics amongst both the IBD and the non-IBD groups was analyzed, a significant higher association ($p < 0.001$) in the usage of antibiotics in non-IBD group compared to the IBD group was observed. However, the usage was non-significant (IBD $p = 0.304$; non-IBD $p = 0.238$) in relation to *C. difficile* positivity in both the groups. A significant difference ($p = 0.014$) in the usage of multiple antibiotics compared to single or no antibiotics in relation to *C.*

difficile positivity was also observed. Table 2 shows *C. difficile* toxin outcome in relation to antibiotic use in general and Table 3 shows *C. difficile* toxin status in relation to antibiotic use in IBD and non-IBD groups.

There was no significant difference ($p > 0.05$) in the use of PPI in the IBD group compared to the non-IBD group.

Antibiotics	<i>C. difficile</i> positive n (%)	<i>C. difficile</i> negative n (%)	Total
Nil	75 (28.96)	435 (36.77)	510 (35.37)
Single	72 (27.8)	342 (28.91)	414 (28.71)
Multiple	112 (43.24)	406 (34.32)	518 (35.92)
Total	259 (100)	1183 (100)	1442 (100)

Table 2: *C. difficile* toxin outcome in relation to antibiotic usage in general.

Antibiotics	IBD				Non-IBD			
	<i>C. difficile</i> positive n (%)	<i>C. difficile</i> negative n (%)	Total n (%)	P value	<i>C. difficile</i> positive n (%)	<i>C. difficile</i> negative n (%)	Total n (%)	P value
Nil	68 (58.62)	394 (65.12)	462 (64.08)	0.304	7 (4.9)	41 (7.09)	48 (6.66)	0.238
Single	22 (18.97)	109 (18.02)	131 (18.17)	0.304	50 (34.97)	233 (40.31)	283(39.25)	0.238
Multiple	26 (22.41)	102 (16.86)	128 (17.75)	0.304	86 (60.14)	304 (52.6)	390 (54.09)	0.238

Table 3: Antibiotics in relation to *C. difficile* toxin status in individual groups.

Follow-up groups

There were 177 follow-up patients in the IBD group (age range 16-68 years) and 48 in the non-IBD group (age range 4-76 years). Table 4 shows a comparative analysis of various parameters between the IBD and the non-IBD groups at follow-up. Among the follow-up groups, there was a significant difference ($p < 0.001$) between the mean duration of diarrhea in the IBD group as compared to the non-IBD group. In *C. difficile* positive group there was a significant difference ($p = 0.026$) in the mean duration of diarrhea between the IBD versus the non-IBD groups. Similarly among *C. difficile* negative group also there was a significant difference ($p < 0.001$) in the mean duration of diarrhea between both the groups. However, in both the IBD and the non-IBD groups upon individual analysis there was no significant difference

($p > 0.05$) in the mean duration of diarrhea between *C. difficile* positive versus *C. difficile* negative groups. In the IBD group there was a significant decrease ($p < 0.001$) in the number of watery diarrhea at the time of follow-up compared to the base line data. Fever was found to be significantly present ($p < 0.001$) in the IBD group at the time of primary reporting compared to that at the time of follow-up. Significant decrease ($p < 0.001$) in the number of *C. difficile* negative IBD patients with fever symptom at the time of follow-up was observed compared to that at the time of admission. Among the *C. difficile* negative IBD group patients there was a significant change ($p < 0.001$) in the number of antibiotics used at the time of preliminary reporting compared to that at the time of follow-up.

Variables	IBD n=177(%)	Non-IBD (n=48)	P values*
Male: Female	94:83	31:17:00	0.15
Age (Mean ± SD)	36.44 ± 11.90	41.38 ± 19.52	0.03*
<i>C. difficile</i> positive	34 (19.2)	12 (25.0)	0.38
Clinical symptoms			
Watery diarrhea	57 (32.2)	45 (93.7)	0.00*
Abdominal pain	77 (43.5)	16 (33.3)	0.2
Fever	26 (14.7)	18 (37.5)	0.00*
Diarrhea attributes			
Duration (days)	1-1095	Jan-60	<0.001
Blood in stool	45 (25.4)	7 (14.6)	0.11
Presence of mucus	48 (27.1)	16 (33.3)	0.39
Antibiotic usage	107 (60.4)	46 (95.8)	0.00*

Table 4: Follow-up patients: Comparative analysis of various parameters in the IBD and the non-IBD groups.

Discussion

The risk of *C. difficile* acquisition with IBD patients has increased in frequency and severity [10]. The carriage of toxin producing *C. difficile* has been more often found in IBD patients in comparison to the general population [16,17]. Evidence has been established that IBD patients have gut flora which is different from patients without IBD [18], predisposing to colonization with *C. difficile* and other pathogens. This is particularly true because of their innate immune deficiencies, use of antibiotics and immunosuppressives for a long period of time as well as frequent hospitalizations [19]. In the present study 95 (13.2%) IBD patients were on steroids and at least 212 (29%) were hospitalized for treatment. Uncommon features like recurrent bloody stools, younger age-group patients and no previous hospital contact may be found in IBD patients with CDI [20,21]. In the present study a very large number of patients in the IBD group had bloody stools compared to the non-IBD group and the patients were also significantly younger. However, *C. difficile* toxin positivity was almost similar in both the IBD and the non-IBD groups, contrary to expectations. Ott et al. [22] also reported low risk of CDI in hospitalized patients with IBD in a tertiary referral center in Germany. Low prevalence of *C. difficile* in IBD patients was also reported by Masclee et al. [23] indicating that *C. difficile* does not commonly elicit IBD flare-ups in The Netherlands. In an earlier preliminary investigation we also observed that there was insignificant risk of CDI in IBD cases [13] probably as the hyper virulent strains present in other geographical region has not been detected here so far [24]. Moreover patients in the non-IBD group comprised largely of hospitalized patients suspected of CDI because of diarrhea.

An episode of CDI is characterized by watery diarrhea with abdominal cramps and fever [13]. The similar presentation of abdominal pain and diarrhea, makes diagnosis of CDI in IBD patients difficult. A higher prevalence of asymptomatic CDI is seen in IBD. Watery diarrhea was present in less than one-third of the IBD patients

to that present in the non-IBD group. Fever was also similarly present in a little more than one-third of the IBD patients to that present in the non-IBD group. Abdominal pain was However, found to be more frequent in presentation in the IBD group probably because of the inflammatory nature of the disease. Presence of mucus in stool is also a distinguishing aspect of *C. difficile* diarrhea [25]. However, in our IBD patients, the number of stool samples with mucus was much less compared to that of the non-IBD group. The only explanation in this context could be that lesser mucus was produced as the patients were under treatment or because mucus was probably camouflaged due to the presence of blood in stool in many of the IBD patients.

Apart from the usually encountered risk factors for CDI [26] diverse studies have found that IBD patients are at higher risk of developing CDI with varying risk amongst diverse patient subsets. Libby et al. [27] reported that 76% of CDI in IBD patients signify community acquired infections in contrast to those without IBD where the majority portion of CDI are hospital acquired as corroborated by others workers [28,29]. In our study there were 436 IBD patients treated as outpatients and of these 66 (15%) were positive for CDI. Similarly a rate close to 60% of antibiotic exposure in CDI affected IBD patients has been reported [20] implying thereby that the reduced diversity of microbiota in the IBD patients may be a predisposing factor for *C. difficile* colonization [30]. However, up to 40% of the IBD patients do not have recognized antibiotic exposure preceding CDI presentation [31]. In the present study also there was a significantly lesser use of antibiotic in the IBD patients compared to the non-IBD group.

It has been reported that CDI can trigger flares in IBD patients and cause further deterioration of the disease [32,33] regardless of CDI treatment. There are several reports of the incidence of CDI in IBD patients establishing that these patients have between two and seven times higher incidence of CDI than those without IBD [6,20,34,35]. When a patient with IBD relapses, detection of *C. difficile* or its toxin in fecal samples confirms the part played by the organism in the inflammatory process of the colon. Hookman et al. [2] found that

fulminant colitis is more often reported at times of *C. difficile* outbreaks in IBD patients with increased mortality than in those with no primary IBD.

Patients with UC are more vulnerable to CDI and have added severe outcomes compared to CD patients [20-34]. In a retrospective study Rodemann et al. [6] reported in a tertiary care hospital that the CDI occurrence in CD doubled and that in UC tripled over a 7-year period (1998-2004). In the present investigation, there were 113 UC and 3 CD patients with CDI. Hughes et al. [36] reported that UC patients present with lower IgA levels to *C. difficile* toxins in comparison to CD patients, and that IBD patients with preceding CDI did not have any increased antitoxin IgG. The limitation of this study was that CD cases were very few and therefore, CDI outcomes in CD could not be properly assessed.

The limitation of this study is that it is a retrospective analysis. However, it may be reiterated that records are well maintained in duly filled up pro-forma specially designed to note down all information pertaining to patients suspected for CDI for probable future analysis. The strength of this study is that follow-up patients were available for both IBD and non-IBD groups taken from a similar setting. Significant improvement was seen in all the parameters upon treatment and follow-up. However, upon comparison of various parameters between IBD and non-IBD follow-up groups, it was observed that there was a highly significant difference in variables like age, watery diarrhea, fever and the use of antibiotics between both the groups. This differentiates and highlights the factors associated with clinical suspicion of both IBD and non-IBD cases.

Clinicians should be cautious about the chances of CDI in patients who have an exacerbation of IBD. At times the IBD flare cannot be differentiated from CDI requiring a high degree of clinical suspicion and vouching for early stool testing for toxin assay [37]. When CDI in IBD are established primarily within two days of hospital admission it suggests that a good number of the infection was acquired before admission. CDI should, therefore, be suspected in differential diagnosis for intractable IBD patients, because many such patients need not present with a history of antibiotic exposure or hospital admission and may largely be receiving outpatient treatment. Absence of antibiotic exposure should not decrease the suspicion of CDI [38] in IBD patients. Even when diarrhea is not present, presence of other clinical symptoms and laboratory findings indicate a potential infectious condition, ruling out *C. difficile* would be a practical measure. Careful surveillance for CDI among all IBD patients exhibiting a flare of the disease should be done for early identification and aggressive treatment for reducing the morbidity of CDI among them.

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