

Original Article

# A study to assess the prevalence and risk factors for *Clostridioides difficile* infection in patients with inflammatory bowel disease in a tertiary care hospital in Northern India

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## ABSTRACT

**Objectives:** The prevalence of *Clostridioides difficile* infection (CDI) is on rise among patients with inflammatory bowel disease (IBD). This study sought to describe the prevalence and risk factors of CDI in patients with IBD as compared to non-IBD controls.

**Materials and Methods:** This was a prospective study conducted at a Department of Microbiology in collaboration with a Department of Gastroenterology. The patients with IBD and controls without IBD presenting with diarrhea were included in the study. The screening test for *C. difficile* infection was done by glutamate dehydrogenase (GDH) assay and toxin detection by enzyme-linked immunoassay (ELISA). Anaerobic culture for *C. difficile* was done on a selective cycloserine cefoxitin fructose agar and polymerase chain reaction (PCR) was done for Toxin A (TcdA) and Toxin B (TcdB) gene detection. *C. difficile* infection was confirmed if GDH and toxin ELISA or PCR were positive.

**Statistical Analysis:** Data were analyzed with the Statistical Package for the Social Sciences version 20.0. The numerical variables were presented by means and standard deviations. Comparison of continuous variables was done using Student's t-test. Categorical variables were analyzed by Chi square test.  $P < 0.05$  was considered to be statistically significant.

**Results:** A total of 160 cases and 112 age- and gender-matched control were included in IBD group and non-IBD group, respectively. Only one culture was positive, 12 and six were positive for GDH ELISA and TcdA and TcdB ELISA, respectively, and 7 were positive by PCR for toxin genes. The factors found significantly associated with CDI were proton-pump inhibitors use ( $P = 0.001$ ), levofloxacin ( $P = 0.001$ ), and azathioprine ( $P = 0.042$ ). Using PCR as a reference method for *C. difficile* toxin detection, the sensitivity, and specificity of GDH ELISA and ELISA for toxins were 100%, 96.8% and 85.7%, and 100%, respectively.

**Conclusions:** The prevalence of CDI among patients with IBD has been found to be low, that is (only 4.4%) in this study population.

**Keywords:** *Clostridioides difficile*, Inflammatory bowel disease, Prevalence

## INTRODUCTION

Inflammatory bowel disease (IBD) is an entity that includes two distinct conditions, ulcerative colitis (UC) and Crohn's disease (CD). The main characteristics of these two conditions are the chronic relapsing inflammation of the gut in individuals who are genetically predisposed, and exposed to defined environmental risk factors.<sup>[1,2]</sup> According to the European Crohn's and Colitis

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Foundation, all patients with IBD who are on corticosteroids, immunomodulators, and biological agents should be considered immunocompromised and at increased risk for opportunistic infections.<sup>[3]</sup> In North America and Europe, over the past several decades, the prevalence of *Clostridioides difficile* infection (CDI) in IBD patients has increased rapidly.<sup>[4]</sup> The clinical presentation of CDI varies widely and ranges from an asymptomatic carriage to fulminant colitis with toxic megacolon. However, the global epidemiology remains undetermined due to insufficient data from underdeveloped countries. In a retrospective study conducted by Vaishnavi *et al.*, from a tertiary care hospital in India, 19% of patients with IBD were positive for *C. difficile* toxin.<sup>[5]</sup> This study aims at enhancing awareness and improving the knowledge of CDI in IBD patients, which are important elements to optimize patient outcomes to implement preventive or early diagnostic and therapeutic strategies.

## MATERIALS AND METHODS

This study was conducted in the Department of Microbiology in association with the Department of Gastroenterology from November 2017 to September 2019. Stool samples from 160 IBD cases, classified as UC and CD, were processed, diagnosed by clinical and endoscopic findings as per the World Gastroenterology Organization Global Guidelines on IBD.<sup>[6]</sup> The severity of UC was classified into mild, moderate, and severe using the Truelove and Witt's criteria.<sup>[7,8]</sup> In this study, 112 patients without IBD but presenting with diarrhea were enrolled as controls. The demographic, clinical, and laboratory details of the patients were collected in a predesigned questionnaire. Anaerobic culture was put up on selective Cycloserine Cefoxitin Fructose Agar (CCFA) after alcohol shock and screening for glutamate dehydrogenase (GDH) was done by Meridian Bioscience Premier GDH kit while toxin detection was done by A/B by Meridian Bioscience Premier toxin A and B kit. For molecular detection, polymerase chain reaction (PCR) was done for TcdA and TcdB gene detection. For Toxin A (601bp) Forward Primer: YT28 - 5'CGATGATAAGGCAACTTCAGTGGTA 3' Reverse Primer: YT29 - 5' GAGTAAGATTCCTCCTGCTCCATTCAA 3'. For Toxin B (399 bp) Forward Primer: YT17 - 5' GGTGGAGCTGCTTCATTGGAGAG 3' Reverse Primer: YT18 - 5' GTGTAACCTACTTTCATAACACCA 3'. The reaction mixture contained 1x buffer (10 M Tris-HCL, 50 mM MKCL, 1.5mM MgCl<sub>2</sub>), 200 pmol of each deoxynucleoside triphosphate (MBI, ferments), 20 pmol of Toxin A and B primers, and 1.25 U of Taq polymerase (Bangalore gene, India). The template was denatured for 5 min at 94°C, and DNA was amplified for 30 cycles consisting of 1 min at 94°C, 1 min at 52°C, and 7 min at 72°C. The DNA fragments of *C. difficile* amplified by PCR were identified by agarose gel electrophoresis.<sup>[9]</sup> *C. difficile* infection diagnosis was made as per the American College of Gastroenterology guidelines.<sup>[10]</sup>

## Statistical methods

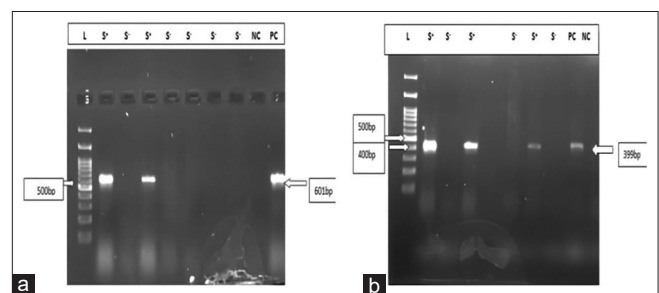
The data were analyzed with the Statistical Package for the Social Sciences version 20.0. The numerical variables were presented as by means and standard deviations. Comparison of continuous variables was done using Student's *t*-test. Categorical variables were analyzed by Chi-square test.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

A total of 160 IBD patients were included in this study, out of which 24 had CD and 136 had UC. 45% patients were females, and 55% were males. The mean age of IBD patients was  $37.19 \pm 16$  years. In this study, 7 out of 160 IBD patients and none of the non-IBD group had CDI confirmed by PCR ( $P = 0.04$ ). The prevalence of CDI in IBD patients in this study was 4.4%. Predominant clinical symptoms present in IBD patients were diarrhea (98.75%), followed by blood in stool in 94.4%, fever in 8.12%, abdominal pain in 11.9%, and vomiting in 2.5%. Out of these, fever was significantly associated with CDI as compared to patients without CDI ( $P = 0.001$ ).

In this study, CDI in IBD patients was detected by culture on selective CCFA media, GDH enzyme-linked immunoassay (ELISA), Toxin ELISA, and PCR. Out of 160 patients, only 1 (0.6%) culture was positive, 12 were positive for GDH by ELISA, 6 were positive for TcdA and TcdB by ELISA, and 7 were positive by PCR for toxin gene [Figure 1 a and b]. Among the control group, culture and PCR were negative in all, ELISA was positive in 9 (8%) patients. The sensitivity, specificity, positive predictive value, and negative predictive value of various tests are shown in Table 1.

The risk factors, including use of medications such as 5 amino salicylic acid (5-ASA), levofloxacin, cephalosporins, proton-pump inhibitors (PPIs), metronidazole, infliximab, and steroids were studied. Out of 7 toxin-positive cases



**Figure 1:** (a) Gel doc picture showing toxin A gene; Lane 1- Ladder, Lane 2- sample, Lane 3- sample, Lane 4- sample, Lane 5- sample, Lane 6- sample, Lane 7- sample, Lane 8-sample, Lane 9- negative control, and Lane 10- positive control. (b) Gel doc picture showing toxin B gene; Lane1- Ladder, Lane 2- sample, Lane 3- sample, Lane 4- sample, Lane 5- sample, Lane 6- positive control, Lane 7- sample, Lane 8- positive control, and Lane 9- negative control.

by PCR, 5 were on azathioprine, 6 on mesalamine, 5 on levofloxacin, 2 on cephalosporin, 1 on infliximab, 5 on steroids, 5 on PPI, and none was on metronidazole. The factors found significantly associated with CDI were PPI use ( $P = 0.001$ ), levofloxacin ( $P = 0.001$ ), and azathioprine ( $P = 0.042$ ), as shown in Table 2.

Clinical outcomes of patients with CDI: Out of 7 CDI patients, only 1 was outpatient, and 6 were inpatients. The maximum duration of hospitalization among patients with CDI was 14 days, with a mean duration of 7.83 days. In this study, among the total GDH-positive cases, 25% were UC in remission, 25% had UC relapse, 33% had severe UC, and 17% were CD patients [Table 3]. All toxin-positive cases presented as IBD flare, and none was in remission. All CDI patients received oral vancomycin as treatment and these patients subsequently recovered.

### Comparison of IBD versus non-IBD group

The CDI prevalence was significantly increased in IBD group compared to non-IBD group, ( $P < 0.05$ ). Symptoms including fever, abdominal pain, and blood in stool were found to be significantly more prevalent in IBD than non-IBD group ( $P < 0.001$ ). A significant higher usage of antibiotics was observed in non-IBD group as compared to IBD group ( $P < 0.001$ ). However, the use of levofloxacin and CDI was

found to be significant only in IBD group ( $P < 0.001$ ). Table 4 shows the comparison between IBD and non-IBD group.

### DISCUSSION

To the best of our knowledge, this is the largest prospective study on *C. difficile* infection in patients with IBD in India. There are many studies on *C. difficile* infection in hospitalized patients, but very few studies have been conducted emphasizing CDI in IBD patients. We found the prevalence of CDI in IBD patients 4.4%, which is less as compared to other studies. There are very few studies from India on *C. difficile* infections in IBD patients. Kochhar *et al.*, found that *C. difficile* toxin was present in 6 of 25 (24%) of patients with active colitis. Balamurugan *et al.* found that *C. difficile* was detected in 34 of 37 patients with UC.<sup>[11,12]</sup> These studies included a small number of patients. Another study by Vaishnavi *et al.*, found a prevalence of 19% by toxin detection among patients with IBD, but it was a retrospective

**Table 1:** Sensitivity, specificity, NPV, and PPV of testing methods for *C. difficile*.

Testing methods	Sensitivity	Specificity	PPV	NPV	TAT (hours)
GDH	100	96.8	58.3	100	2
Toxin	85.7	100	100	99.3	2
Culture	14.28	100	100	96.2%	48
PCR	Reference method for detection of <i>C. difficile</i> toxin				48

NPV: Negative predictive value, PPV: Positive predictive value, *C. difficile*: *Clostridioides difficile*, GDH: Glutamate dehydrogenase, PCR: Polymerase chain reaction, TAT: Turn around time

**Table 2:** Medication usage in IBD patients.

Medication use	IBD with CDI	IBD without CDI	P-value
Levofloxacin	5	7	0.001
Cephalosporin	2	12	0.116
5-ASA	6	135	0.959
Steroids	5	93	0.721
Infliximab	1	3	0.168
PPI	5	18	0.001
Azathioprine	4	31	0.042
Metronidazole	0	8	0.535

IBD: Inflammatory bowel disease, CDI: *Clostridioides difficile* infection, 5-ASA: 5 amino salicylic acid, PPI: Proton-pump inhibitors

**Table 3:** Positive results of culture, GDH, toxin ELISA, PCR, and patients' clinical profile.

S. No.	GDH	Toxin	Culture	PCR	Clinical profile
1.	Positive	Positive	Negative	Toxin B	Severe UC
2.	Positive	Negative	Negative	Negative	Crohns
3.	Positive	Negative	Negative	Negative	UC in remission
4.	Positive	Negative	Negative	Negative	UC in remission
5.	Positive	Negative	Negative	Negative	Crohns
6.	Positive	Negative	Negative	Negative	UC in remission
7.	Positive	Positive	Positive	Toxin B	Severe UC
8.	Positive	Positive	Negative	Toxin A	Severe UC
9.	Positive	Positive	Negative	Toxin B	UC relapse
10.	Positive	Positive	Negative	Toxin A	UC relapse
11.	Positive	Negative	Negative	Toxin B	Severe UC
12.	Positive	Positive	Negative	Toxin A	UC relapse

GDH: Glutamate dehydrogenase, PCR: Polymerase chain reaction, UC: Ulcerative colitis, ELISA: Enzyme-linked immunoassay

**Table 4:** Comparison of IBD and non-IBD group.

Variables	IBD (n=160)	Non-IBD (n=112)	P-value
Female: Male	72:88	43:69	0.042
Age year, (mean±SD)	37.19±16	41±18.89	0.089
<i>C. difficile</i> positive	7	0	0.045
Watery diarrhea	158	102	0.523
Abdominal pain	62	19	<0.001
Fever	78	14	<0.001
Blood in stool	152	7	<0.001
Antibiotic usage	34	37	0.008

*C. difficile*: *Clostridioides difficile*, IBD: Inflammatory bowel disease, SD: Standard deviation

study, and only ELISA for toxins was done for diagnosis.<sup>[5]</sup> The reason for the low prevalence rates observed in our study might be that we have used PCR as a confirmatory method, which clearly ruled out false positive test results.<sup>[13]</sup>

The results of our study are in accordance with the study by Masclee *et al.*, which reported toxigenic *C. difficile* in 3.6% IBD patients.<sup>[14]</sup> In another study by Gillespie *et al.*, from the USA, the incidence of CDI among IBD patients was 6.7% among 654 IBD patients, which is closer to our study.<sup>[15]</sup> Another case-control study by Li *et al.* 2018 reported 7.41% of CDI cases among IBD patients.<sup>[16]</sup>

In our study, all the CDI cases had UC, but none had CD. Risk factors that were found to be significantly associated with the development of CDI in IBD patients in this study were use of PPI ( $P = 0.001$ ), fluoroquinolone ( $P = 0.001$ ), and azathioprine ( $P = 0.042$ ). However, there was no significant relationship between the use of infliximab, 5-ASA, and steroids. Janarthanan *et al.*, showed a significant relationship between CDI and PPI therapy, which is consistent with our study; while in contrast, Bossuyt *et al.*, did not find any association between PPI use and CDI in IBD patients.<sup>[17,18]</sup> In our study, significant association between azathioprine use and CDI in IBD, which is in accordance with the study by Issa *et al.*, which reported a greater than a two-fold increased risk of CDI with maintenance immunomodulator azathioprine.<sup>[19]</sup> Marwick *et al.*, showed fluoroquinolones, which are widely used in IBD as a risk factor for CDI similar to findings in our study.<sup>[20]</sup> Schneeweiss *et al.* reported that the risk of CDI tripled with corticosteroid initiation among the IBD patients, while no such association was found with the initiation of immunomodulators or biologics (infliximab).<sup>[21]</sup> Similarly, there is no study from India that had compared the sensitivity and specificity of different testing methods for CDI. In this study, we found that GDH has high sensitivity so it can be used for exclusion of *C. difficile* infection or carriage. This is in accordance with a study by Swindells *et al.*,<sup>[22]</sup> which showed toxin detection by ELISA methods had lower sensitivity. Moreover, the most appropriate diagnostic results are obtained with PCR following combined antigen and toxin detection.<sup>[22]</sup>

The strength of this study is a prospective design in which consecutive patients were enrolled. Most of the previous studies have used retrospectively collected cases. A multistep algorithm is used for diagnostic approaches of *C. difficile* infection in symptomatic patients. However, one of the limitations of the study is the toxigenic culture, which is considered as gold standard for toxin detection, was not done as it is not suitable for routine diagnosis since it is a time-consuming and labor-intensive method. In our study, PCR is taken as a reference method for detection of toxin. In our study, diagnosis of *C. difficile* infection was made by laboratory diagnosis of toxigenic strain confirmed by PCR along with clinical symptoms. All patients with *C. difficile*

infection were relieved of the symptoms by oral vancomycin. In addition, we performed PCR in all cases, which is considered as the highly sensitive method for confirmation of *C. difficile* infection, whereas the previous studies have diagnosed CDI using toxin ELISA, which has a low sensitivity. Therefore, in this study, the overall prevalence of CDI in patients with IBD was relatively more precise.

## CONCLUSIONS

An IBD flare and CDI have similar presentations; therefore, an early diagnosis is important. In this study, GDH ELISA had high sensitivity and low specificity. GDH ELISA has low cost, thus making it appropriate for a screening test for CDI. More studies are required to determine whether the patients with negative GDH and toxin enzyme immunoassay results and positive for toxin PCR results should be treated or not. However, this prospective study provides significant data on the prevalence of CDI in IBD in Indian patients, associated risk factors, and sensitivity and specificity of different testing methods.

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## Ethical approval

The Institutional Review Board and Ethics clearance was obtained (No. 2016-134-EMP-EXP).

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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## Conflicts of interest

Dr. Chinmoy Sahu is on the Editorial Board of the Journal.

## Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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