Short Report

Clostridium difficile infections in HIV-positive patients with diarrhoea

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ABSTRACT

Background. Patients with HIV/AIDS are at a high risk of being infected with toxin-producing strains of *Clostridium difficile* (*C. difficile)* because of frequent hospitalization, exposure to antibiotics and antibiotic prophylaxis for opportunistic infections. There are little data from India on the prevalence of *C. difficile* infection in such patients.

Methods. We assessed the occurrence of *C. difficile* infections in HIV-positive patients with diarrhoea by looking for the presence of its toxin as well as by culturing. Enzyme immunoassay (EIA, Premier toxins A and B; Meridian Diagnostic Inc.) was used to detect toxin from 237 fresh stool samples collected from HIV-positive patients with diarrhoea. Culture was done on cycloserine–cefoxitin–fructose agar and brain–heart infusion agar.

Results. *C. difficile* was found in 12 of 237 (5.1%, 95% CI 2.64%–8.68%) HIV-positive patients with diarrhoea (9 patients were positive by EIA and 3 by culture). The presence of *C. difficile* in patients who had received antiretroviral therapy (7/66 [10.6%]) was significantly higher ($p < 0.016$) compared with those who had not (5/171 [3%]). Of the 12 patients positive for *C. difficile*, 7 were on antiretroviral therapy for a mean (SD) of 34.4 months with mean CD4+ count of 186 (98.81) cells/cmm and 5 patients were anti-retroviral-naïve with mean CD4+ count of 181 (68.7) cells/cmm. All the 12 patients were on antibiotics for previous 2 months and 4 of 12 had been hospitalized in the previous 30 days.

Conclusion. *C. difficile* infections occurred more frequently in patients who had received antiretroviral therapy. Our study population had a lower frequency of *C. difficile* infections compared to previous studies.

Natl Med J India 2014;27:138–40

INTRODUCTION

Patients with HIV/AIDS are at a high risk of developing opportunistic infections during their lifetime. Among them, diarrhoea is a major cause of morbidity[1] and is an independent marker of poor prognosis.[2] Recent reports indicate that diarrhoea occurs in 40%–80% of HIV infected patients who are treatment-naïve for highly active antiretroviral therapy (HAART) in developed countries and in about 90% of such patients in developing countries.[3,4] Patients with HIV/AIDS are at a high risk of infection with toxin-producing strains of *C. difficile* because of frequent hospitalization and exposure to antibiotics. According to previous studies, prevalence of *C. difficile*-associated diarrhoea in HIV-positive patients varies from 10% to 34%,[5,6] while its prevalence among HIV-negative patients varies from 4% to 12.1%.[6,7] However, there are limited data from India regarding *C. difficile*- associated diarrhoea (CdAD) among patients with HIV/AIDS.[8–10]

METHODS

We did a hospital-based, cross-sectional study to estimate the prevalence of *C. difficile* diarrhoea and determine associated risk factors among HIV-infected patients attending the antiretroviral therapy (ART) clinic at the All India Institute of Medical Sciences (AIIMS).A total of 237 fresh stool samples were collected from 267 screened HIV-positive patients with diarrhoea (30 patients were excluded:1 was HIV-2-positive and 29 patients were unwilling to participate). HIV-1-positive patients above 18 years of age with diarrhoea (defined as more than three episodes of loose watery stools per day)were included in the study. *C. difficile* was isolated using stool culture on cycloserine–cefoxitin–fructose agar (CCFA) and brain–heart infusion agar (BHIA) and identification of the organisms was done by standard Gram-stain and biochemical methods as described in previous studies.[5] This was also augmented by ELISA (Premier toxins A and B; Meridian Diagnostic Inc.) to detect *C. difficile* toxins A and B in stool samples. A patient was considered to have tested positive for *C. difficile* if the cultures grew *C. difficile* or if the ELISA for stool cytotoxin assay was positive. The CD4 count and viral load were done in all patients to assess the HIV status of the study participants. Stool was also tested for *Salmonella, Shigella* and enteroparasites; other pathogens were not tested due to resource constraints.

The study population was a convenience sample and included patients visiting the ART clinic during the study period (2007–11). The primary analysis was comparison of patients with diarrhoea who were *C. difficile*-positive and *C. difficile*-negative. The data are presented as proportions (with 95% CI) for qualitative variables and mean (standard deviation [SD]) for quantitative variables. Bivariate analysis of the quantitative variables was done using *t*-test, that of qualitative variables using chi-square test and that of ordinal variables using Mann–Whitney U test. Information and stool samples were collected from patients after obtaining informed consent and the study protocol was approved by the Institutional Ethics Committee of AIIMS.

RESULTS

A total of 237 fresh stool samples were analysed for *C. difficile* from HIV-positive patients who had diarrhoea. The maximum number, 220/237 (92.8%) of patients, were recruited from the outpatients while 17 (7.2%) were inpatients. The age range of the patients was 14–84 years and most (75.5%) were men (Table I). The mean (SD) CD4+ T cell counts were lower in *C. difficile*-

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positive patients (183.69 [79.83] cells/cmm) compared with *C. difficile*-negative (234.51 [188.94] cells/cmm) patients, but this difference was not statistically significant. The HIV viral load in *C. difficile*-positive patients (219 963 [260 803] copies) was higher compared with that in the *C. difficile*-negative group (156 272 [206 221] copies) but not statistically significant. There was also no significant difference in the duration of diarrhoea and pattern of use of antibiotics between patients with *C. difficile* and those without.

TABLE I. Comparison of clinical characteristics in both study groups

Variable	HIV-positive patients with diarrhoea (ART-naïve and ART-treated)		p value
	C. difficile-positive (*n*=12) *n* (%)	*C. difficile*-negative (*n*=225) *n* (%)	
Mean (SD) age	44.3 (8.99)	35.4 (9.19)	<0.01
Mean (SD) body mass index (kg/m^2)	17.1 (3.20)	17.9 (2.65)	0.33
Male: Female ratio	11:1	168: 57	0.34
Opportunistic infection present	9 (75)	79 (33.3)	0.01
History of tuberculosis	5 (42)	53 (24)	0.15
Other parasitic infections in stool: *Isospora belli*	4 (33.3)	26 (12)	0.05
Route of HIV transmission (heterosexual)	11 (92)	199 (81.4)	0.99

Of the 237 patients, 12 tested positive (5.1%; 95% CI 2.6%–8.7%) for *C. difficile* infection. Of these 12, 9 were positive for the toxin but had negative cultures, 3 tested negative for the cytotoxin but had a positive culture, but none tested positive for both. Seven of 12 patients were on HAART therapy for a mean of 34.4 months with a mean (SD) CD4+ count of 186 (98.81) cells/cmm and 5 patients were ART-naïve with a mean (SD) CD4+ count of 181 (68.7) cells/cmm. All 12 patients were on antibiotics before the onset of diarrhoea. Five of these had tuberculosis as an opportunistic infection and 1 had CMV retinitis. We also observed a statistically significant (5/171 [3.0%] *v*. 7/66 [10.6%]; p<0.01) difference in the presence of *C. difficile* in ART-naïve and ART-treated patients, respectively.

The other pathogenic organisms isolated from the study group included *Isospora belli* in 27/237 (11.4%) and *Shigella* in 1 patient. No *Salmonella* species were isolated from any stool samples. The mean duration of illness was 7 days (range 1–60 days) and the mean stool frequency was five episodes per day. A total of 195/237 (82.3%) patients were on multiple antibiotics. Of the 195 patients who were on antibiotics, 12 (6.2%) had *C. difficile*-associated diarrhoea (CdAD). Most of the patients had diarrhoea during treatment or within 15 days of starting antibiotics (Table II).

TABLE II. Factors associated with *C. difficile* diarrhoea in HIV-positive patients

Variable	HIV-positive patients with diarrhoea (ART-naïve and ART-treated)		p value
	C. difficile-positive (*n*=12) *n* (%)	*C. difficile*-negative (*n*=225) *n* (%)	
Patients admitted to hospital in past 3 months	4 (33.3)	13 (6)	<0.01
Hospital stay >10 days	2 (17)	5 (2.2)	0.04
Patients on antitubercular therapy	5 (42)	53 (24)	0.16
Patients receiving co-trimoxazole	8 (67)	104 (46.2)	0.27
Patients on quinolones	9 (75)	128 (57)	0.25
Patients on metronidazole	5 (42)	95 (42.2)	0.97
No history of antibiotic use	0	42 (19)	—

DISCUSSION

C. difficile was detected in 5.1% of our study population, which is lower than the 58.8% reported by Wongwanich *et al.*[11] and 10% by Uppal *et al.*[6] However, there are conflicting reports of the prevalence of *C. difficile* diarrhoea in HIV-positive patients in the pre-HAART and post-HAART era. Anastasi and Capilli[12] reported that *C. difficile* is still the most common infectious cause of diarrhoea in HIV patients. Although Tacconelli *et al.*[13] found that while the incidence of CdAD in HIV patients has decreased in the HAART era in western countries, it is still higher than that in patients without HIV infection.

We found a statistically significant difference in the prevalence of *C. difficile* in ART-naïve and ART-treated patients. ART-treated patients are more prone to *C. difficile* infection and this difference in positivity in either group may be due to the use of ART, as well as the use of prophylactic antibiotics for opportunistic infections in addition to CD4+ counts <200 cells/cmm indicating immunological failure in 5 of the 7 patients put them at higher risk in the ART-treated group. This risk was absent in ART-naïve subjects except low CD4 cell count.

C. difficile was present in a significantly higher proportion among the older age group and among men (p=0.001) and is similar to the findings of another study.[14] This could be because of low immunity in the elderly and a higher risk of exposure in men.

The prior use of antibiotics is an important risk factor for infection with *C. difficile*. We found that patients affected with CdAD were taking more antibiotics for longer durations though there was no statistically significant difference. The common antibiotics associated with CdAD were clindamycin, penicillin and cephalosporins. However, antibiotics associated with diarrhoea in our study (quinolones, antitubercular drugs and co-trimoxazole) are traditionally not associated with a high risk for CdAD.[15] This finding may be due to an association with tuberculosis or more intensive empirical use of antibiotics in a sicker group of patients.

We observed that patients with more advanced HIV disease (those with low CD4+ counts) were more frequently infected with *C. difficile* though this was not statistically significant. Also, patients positive for *C. difficile* had more opportunistic infections; a finding consistent with previous studies.[16] This is also consistent with the hypothesis that an advanced HIV infection leads to an immunocompromised host, who has more frequent hospitalization and more antibiotic use, all of which are risk factors for *C. difficile* infections.

For the diagnosis of *C. difficile* infections, the standard methods include stool culture and ELISA for *C. difficile* toxins (A and B). Stool culture requires a longer time and cannot differentiate the toxigenic from the non-toxigenic strains. Toxin detection is a rapid and specific (toxin B) method for the diagnosis of *C. difficile* infection.[17] Stool culture positivity in the absence of detection of toxin may be due to non-toxigenic strains or less

production of toxin.[18] The prior use of antibiotics (metronidazole) may be responsible for negative cultures in toxin-positive patients. *C. difficile* toxin EIA is used for the diagnosis of CdAD[19] but we used ELISA for toxin A and B and culture for diagnosis of *C. difficile* infection.

As observed in a previous study in the same laboratory, on a patient population of the same hospital, the prevalence of *C. difficile* in HIV-negative patients with diarrhoea was 7.2% (63/876). In our study, the prevalence of *C. difficile* in HIV-positive patients with diarrhoea was 5.1%. This lower rate of prevalence could be because most of our patients had already started empirical antibiotics and a lower percentage of our study population was hospitalized. Also, the prevalence of CdAD in HIV-negative patients is variable.[6,8] This variation in prevalence could also be due to a variation in the population and/or geographical area.

The lower rate of enteric bacterial pathogens detected is consistent with the result of studies by Uppal *et al*.[6] and Becker *et al*.[20] The prevalence of enteric bacterial pathogens is low at our hospital and may be due to use of antibiotics before or during the hospital visit.

Conclusion

The prevalence of *C. difficile* is variable. Traditional risk factors for *C. difficile* are common, and are more prominent in patients with advanced HIV disease. Any hospitalized HIV-infected patient with diarrhoea should have stool samples tested for *C. difficile* toxin and culture and should be treated early empirically, if clinical symptoms are consistent with CdAD to prevent complications associated with it.

ACKNOWLEDGEMENTS

We are grateful to the Indian Council for Medical Research (ICMR) and National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India for funding this project.

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