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Original Article

Clostridium Difficile is associated with High Mortality among Cirrhotics in India

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ABSTRACT

Background: To assess the prevalence and impact of *Clostridium difficile* infection (CDI) in hospitalized patients with cirrhosis in India.

Methods: In this prospective observational study from June 2015 to March 2016, all hospitalized patients with cirrhosis and acute diarrhea at the time of admission or during hospitalization were included. We studied hospitalized patients with cirrhosis without diarrhea during the same period to detect asymptomatic colonizers. Stool samples were tested for CDI, bacterial cultures, and parasite microscopy in patients with diarrhea.CDI was detected using a stool PCR test that detects the pathogenicity locus of toxigenic *Clostridium difficile* gene. We analysed the impact of CDI on hospital outcomes and also assessed the risk factors for acquiring CDI.

Result: Among 92 hospitalized cirrhotic patients with acute diarrhea [male: 74; median age: 50 (range 19 to 80) years; Child's class A: B: C: 8:41:43; median MELD score: 18 (range 6 to 44)], 6 (6.5%) had CDI by positive stool PCR. Use of antibiotics (100% CDI Vs 55.8% non-CDI, p= 0.04) and steroids (50% CDI vs 10.5% non-CDI, p =0.028) emerged as risk factors for CDI among cirrhosis patients. Two of the 6 patients (33.3%) with CDI as compared to 6/86 patients (7%) with no CDI died (p-value: 0.08).There were no asymptomatic colonizers amongst 35 hospitalized cirrhosis patients without diarrhea.Conclusions: C. difficile, although uncommon, was an important cause of mortality in cirrhosis patients hospitalized with diarrhea in India.Prior use of antibiotics or steroids were identified as risk factors for CDI.

KEYWORDS: Antibiotic-associateddiarrhea, Cirrhosis, *Clostridium difficile*.

Introduction

Clostridium difficile infection (CDI) is one among the established causes of healthcare-associated diarrhea. In 2008, the incidence of CDI varied from 2.75 (Korea) to 8.75 (USA) cases per 1000 hospital admissions[1,2]. In India, CDI is responsible for 1.5% (Community-acquired) to 8% (hospitalized patients) cases of acute diarrhea.[3] In the West, CDI mortality in non-cirrhotic hospitalized patients was 9.6%. Mortality in patients with cirrhosis with and without CDI was 13.8% and 8.3%, respectively.[4] CDI prevalence and its impact on outcomes in cirrhotic patients have not been studied in India so far. Therefore, this study aimed to evaluate the etiology of acute diarrhea, predictors of mortality, prevalence, risk factors, and clinical profile of CDI amongst hospitalized cirrhotic patients with acute diarrhea.

Methods

This prospective observational study was carried out between June 2015 and March 2016 on consecutive cirrhosis patients, aged over 18 years, admitted to the hepatology ward with acute diarrhea, present at admission or developed during the hospital stay.

Definitions

The symptomatology of *C. difficile* diarrhea has been described as mild to moderate or profuse debilitating diarrhea, sometimes with lower abdominal cramps or abdominal distension along with fever and other systemic symptoms in literature[5]. Given that liver cirrhosis patients are on lactulose, we defined acutediarrhea as ≥ 3 loose stools/day for <4 weeks duration if the patient did not receive lactulose and ≥ 6 loose stools/day if on lactulose therapy.

Cirrhosis was defined according to clinical, laboratory, radiological, and endoscopic parameters. Acute on chronic liver failure was defined as per the 2014 consensus recommendations of the Asian Pacific Association for the Study of the Liver[6].

We identified CDI as the cause of diarrhea if the positive rapid stool PCR test for *Clostridium difficile*[7,8]. CDI was further classified according to the time of onset of disease and history of hospitalisation[8]. Health-care facility-onset health-care facility associated (HO-HCFA) CDI was defined as the onset of symptoms three days after hospital admission. Community-onset health-care facility associated (CO-HCFA) CDI is defined as onset of symptoms within four weeks of discharge from a hospital. Community-associated (CA) CDI is defined as the onset of symptoms in the community or in the hospital within three days of admission and has not been hospitalized in the previous 12 weeks. We defined prior antibiotic/ proton pump inhibitors (PPI)/ steroids/ immunosuppressants use as any use of these drugs within the preceding three months of enrollment into the study[9]. Procedures such as enteric tube feeding, urinary catheterization, insertion of vascular lines, hemodialysis and chemotherapy ports, and surgeries, especially GI surgeries, were considered invasive procedures[10,11,12]. Infections other than diarrhea were also noted.

We defined adequate response to treatment as the resolution of diarrhea (stool frequency less than three times/day) and systemic signs (improvement in lower abdominal cramps and absence of fever and leukocytosis). Retesting of stool for *C. difficile*, after the course of antibiotics, to document clearance was not done[8].

Baseline clinical data: For all study participants, demographic data and clinical data such as age, gender, co-morbidities were collected at the time of admission. Etiology of liver disease, indication for hospitalization, complications of portal hypertension, and infections were noted. Child-Pugh class and MELD scores were calculated at baseline. Information regarding prior use of antibiotics, proton pump inhibitors, hospitalizations, current or previous use of steroids/ immunosuppressants, and invasive procedures were collected from all participants.

Laboratory Investigations for Clostridium Difficile

All patients with diarrhea underwent stool tests for culture, microscopic examination for routine parasites, and rapid test for *Clostridium difficile* (PCR test).

We performed a rapid stool PCR test (Illumigene, Meridian Bioscience, USA) using LAMP technology (loop-mediated, isothermal, DNA amplification test), according to the manufacturer's instructions.The test amplifies and detects a conserved 204-bp sequence of the

tcdA gene. This test has 98% sensitivity and specificity with a 99% negative predictive value and 92% positive predictive value. Prepared stool sample collected on an Illumigene sample brush was added to a sample preparation apparatus containing sample diluents. The sample was vortexed for 10 seconds which was then transferred to Illumigene extraction tube and heated in a heat block at 95ºC for ten minutes. Fifty microlitres of this heat-treated sample was transferred to the reaction buffer tube and vortexed for 10 seconds. The reaction buffer tube sample was then transferred to the test and control chamber of the Illumigene test device. The Illumigene assay contains an internal control of formalin-inactivated *Staphylococcus aureus* and its specific primers set in each vial provided.External quality controls supplied by the manufacturer include a positive control (a solution containing plasmidic DNA with *S. aureus* and *C. difficile* DNA insert) and negative control (a solution containing plasmidic DNA with S. aureus DNA insert), which was run for each new reagent batch[13,14].

This test device was inserted into the Illumipro-10 (Meridian Bioscience, Inc.), and an amplification reaction and detection was initiated. Turbidity in the test chamber compared to the control chamber implied a positive result. The test duration was approximately 40 minutes, and results were reported within one hour as positive, negative, or invalid. Samples with an invalid result were repeated. In addition, a subset of cirrhosis patients admitted during the study period for complaints other than diarrhea were recruited to assess for asymptomatic *C. difficile* colonization.

Treatment and Follow Up

All patients received standard management of CDI[8] and liver disease. Appropriate efforts were taken to discontinue inciting antibiotics whenever possible.Lactulose was stopped or with held in all these patients admitted with diarrhea as part of management. In addition, antibiotics for CDI were initiated, electrolyte imbalance was addressed, constipation was avoided, and an optimum number of soft stools was ensured in our encephalopathic patients along with the standard of care for other portal hypertension complications. Mild CDI was treated with oral metronidazole (400 mg thrice daily for 10 days). In patients who did not respond to oral metronidazole in 5 days, enteral vancomycin (125 mg every 6 hours) along with IV metronidazole (500 mg thrice daily) was given until response. In-hospital outcome was noted.

Statistical Analysis

Sample size was calculated by assuming 12% of patients with diarrhea having CDI[3,15,16]. A sample size of 100 would give us a precision of 0.065 for our prevalence estimate. Continuous variables were expressed as median (range), and discrete variableswere expressed as numbers(%). We compared identified CDI risk factors such as prior antibiotic, PPI, or steroid use, prior hospitalization, and invasive procedures between CDI and non-CDI sub-groups. We also compared outcomes such as duration, cost of hospital stay, and in-hospital mortality between these two sub-groups. Mann Whitney U test was used to assess continuous variable distribution between groups. Fisher's exact or Chi-square test were used for discrete variable distribution between the groups. A p-value< 0.05 was considered significant. SPSS version 15 was used for statistical analysis. Informed consent was taken from all the participants. The study was approved by the local ethics committee and Institutional Review Board (Min No. 9446 dated 05.06.2015).

Results

Baseline Characteristics

Baseline characteristics of hospitalized patients with cirrhosis and acute diarrhea (n=92) are depicted in **Table 1**.

Etiology of Acute Diarrhea

Of the 92 cases, 6 (6.5%) had CDI by positive stool PCR test (**Table 2**). Eighty-six (93.5%) had a negative stool PCR test for Clostridium and comprised the non-CDI subgroup.

Three (50%) patients had CO-HCFA CDI, two patients had HO-HCFA CDI, and one had CA CDI. None of the patients had recurrent CDI.

Aeromonas species (7 patients) were the commonest organism (*Aeromonas hydrophila*: 4, and one

Table 1: Predictors of mortality in hospitalised cirrhosis patients with acute diarrhoea.

Variables	Baseline characteristics of all patients (n=92)	Alive (n=84)	Died (n=8)	P value
Age in years	50(19-80)	49(19-80)	56(26-76)	0.32
Gender (M:F)	74:18	68:16	6:2	0.65
Child's StatusA:B:C	8:41:43	8:36:40	0:5:3	0.46
MELD score	18(6-44)	18(6-36)	17.5(9-44)	0.76
DCLD:ACLF	70:22	64:20	6:2	1.00
Co-morbidities	58(63.04%)	51(60.7%)	7(87.5%)	0.25
SBP	18(19.56%)	16(19%)	2(25%)	0.65
AKI	52(56.52%)	45(53.6%)	7(87.5%)	0.13
HE	29(31.52%)	22(26.5%)	7(87.5%)	0.001
GIbleed	23(25%)	21(25%)	2(25%)	1.00
HCC	4(4.34%)	4 (4.8%)	0(0%)	1.00
CDI	6(6.52%)	4 (4.8%)	2(25%)	0.08
Other Infections	37(40.2%)	31(37.8%)	6(75%)	0.06

MELD score: Model for end stage liver disease score, DCLD: Decompensated chronic liver disease, ACLF: Acute on chronic liver failure, SBP: Spontaneous bacterial peritonitis, AKI: Acute kidney injury, HE: Hepatic encephalopathy, GI bleed: Gastrointestinal bleed, HCC: Hepatocellular carcinoma, CDI- Clostridium difficile infection.

each of *A. caviae, A. veronii,* and *A. sabria*), followed by non 01/0139 *vibrio* in 4 patients. In addition, *Salmonella typhimurium* (3 patients), Giardiasis (3 patients), Strongyloidiasis (2 patients), and *Enterobius vermicularis* (1 patient) were noted. In the study group, 48 patients did not have identified etiology for diarrhea, and 18 patients had SBP.

Predictors of Mortality in Hospitalized Cirrhosis Patients with Acute Diarrhea

Eight (8.7%) patients died during hospitalization. Of the variables analyzed, hepatic encephalopathy (OR: 13.4, 95% CI: 2.7 to 65.4,p =0.001) was identified as a significant predictor of mortality (**Table 1**). In addition, CDI (p=0.084) and other infections (p=0.06) showed a trend towards predicting mortality. We did not perform a multivariate analysis as events were limited.

Clinical Details, Risk Factors, and Hospital Outcomes in CDI Patients

Clinical profile of CDI patients [females: 4; age: 49 years (21- 71 years), median (range); Child's class A:B:C: 0:4:2] is depicted in **Table 2**. Risk factors for CDI were prior hospitalization (3), prior use of antibiotics (6), prior use of steroids or immunosuppressants (3).

Four CDI patients responded to a single course of oral metronidazole. In contrast,two (33%) patients died due to persistent diarrhea and multiorgan failure despite being escalated to receive enteral vancomycin and IV Metronidazole.

Table 3 depicts the comparison of risk factors for CDI and clinical outcomes in patients with and without CDI. Prior use of antibiotics (p=0.04) and steroids/ immunosuppressants (p=0.028) was significantly higher in the CDI cases. In-hospital mortality (p=0.084), duration of hospital stay (p=0.16), and cost of hospitalization, including drug costs (p=0.13), were not significantly different between these two groups.

Asymptomatic colonizers:

None of the 35 hospitalized cirrhotic patients without diarrhea (male: 28; age in years: 46(20 to 62), median (range); Child's class: A:B:C 3:8:24), tested positive for stool *C. diff* PCR.

Discussion

In this single tertiary-center, prospective, observational

Table 2: Clinical details of CDI patients.

VARIABLES	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5	CASE 6
Age in years	45	53	26	21	71	62
Gender	Male	Female	Female	Female	Female	Male
Child's status	C	B	C	B	B	B
Priorantibiotics	Piperacillin-Tazobactum (7 days)	Meropenem (30 days)	Meropenem (20 days)	Septran, Ceftriaxone (60 days)	Septran (90 days, ongoing)	Norflox (90 days, ongoing)
Steroids (current/prioruse)	No	Yes: for Rheumatoid arthritis and Bronchial asthma	No	Yes: for Autoimmune hepatitis, Ulcerative colitis	No	Yes, Alcoholic hepatitis
lmmuno-suppressants	No	No	No	Yes: mycophenolate mofetil	No	No
Prior hospitalisation	No	Yes	No	No	Yes	Yes
Treatment given	Oral Metro-nidazole	Oral metronidazole	Enteral Vancomycin +IV metronidazole	Oral metronidazole	Oral Vancomycin +IV Metronidazole	Oral Metroni-dazole
Response to treatment	Yes	Yes	No (died)	Yes	No (died)	Yes

Table 3: Comparing CDI and Non-CDI subgroups of cases.

Variables	CDI (N=6)	Non-CDI (N=86)	P value
Risk factors			
1. Prior antibiotic use	6 (100%)	48 (55.8%)	0.040
2. Prior PPI use	5 (83.3%)	43 (50%)	0.206
3. Prior hospitalisation	4 (66.7%)	49 (57%)	1.000
4. Current/ prior use of steroids immunosuppressants	3 (50%)	9 (10.5%)	0.028
5. Invasive procedures	2 (33.3%)	30 (35.3%)	1.000
Outcomes			
1. Duration of hospitalisation in days	9.5 (7-24)	7 (2-87)	0.161
2. Cost of hospitalisation in 10^5 Indian rupees	$0.96x10^5$ ($0.4x10^5$ to $5.9x10^5$)	$0.49x10^5$ ($0.03x10^5$ to $38x10^5$)	0.132
3. In-hospital mortality	2 (33.3%)	6 (7%)	0.084

CDI: Clostridium difficile infection, NON-CDI: Patients with diarrhoea letiology other than clostridium difficile or unidentified diarrhoea/etiology, PPI: proton pump inhibitors.

study from India, CDI was an infrequent cause (6/94; 6.5%) of acute diarrhea in admitted cirrhotic patients. There was a trend, albeit statistically not significant, for the presence of CDI to adversely affect in-hospital outcomes. Current or prior use of antibiotics and immunosuppression were significant risk factors for having CDI. This study also shows that asymptomatic colonization by *C. difficile* is absent (0/ 35).

Indian studies have estimated CDI prevalence to be ~1.3%[3] in the community and 8%-11%[3,16] in hospitalized patients. Some recent studies, esp. from southern and western parts of the country, estimate a much lower prevalence (4%-5%) of CDI in hospitalized patients with diarrhea[17,18]. *Clostridium difficile* toxin was identified in only one of the 31 bone marrow transplantation patients[19]. Bajaj et al., in their prospective, multi-centric cohort study from the USA, of 207 cirrhotic patients with infections, estimated 12% prevalence of CDI[20]. To our knowledge, this is the first study from India on CDI prevalence and impact on outcomes in patients with cirrhosis.

One reason for this wide variation in prevalence rates across countries and within our own country could

be the lack of uniform definitions of CDI and diagnostic tests employed. Our pre-test definitions were according to the 2013 guidelines[8] by the American College of Gastroenterology. In our study, we utilized the Illumigene test by LAMP technology as a stand-alone test for CDI diagnosis. It is rapid and cheap as it does not require a thermal cycler or alternating temperature cycles or steps employed in traditional PCR techniques and is carried out at a constant temperature.Furthermore, as reports were available within an hour of sample collection, we could start specific antibiotics for CDI as early as possible and stop inciting antibiotics whenever feasible.

To avoid sampling bias, we enrolled consecutive cirrhotic patients admitted for management of various complications of portal hypertension and who also had diarrhea at the time of admission or developed diarrhea eventually during the hospital stay. As there are no existing definitions for diarrhea in patients with cirrhosis already on lactulose, we defined diarrhea as 6 or more loose stools in one day while on lactulose or 3 or more loose stools for two days despite discontinuing lactulose to avoid further sampling bias in these patients who are all on lactulose for prophylaxis or treatment of hepatic encephalopathy.

The most frequent type of CDI in USA was HO-HCFA CDI (42% to 89%)[8,21]. In our study, CO-HCFA type was the commonest (50%), while 2/6 cases (33.3%) were HO-HCFA and CA CDI occurred in one case (16.6%). CDI was treated according to 2013 ACG guidelines[8].

Mortality in cirrhotic patients is contributed by multiple factors such as age, co-morbidities, Child's status, and complications of portal hypertension. Two patients with CDI died during the study despite optimal management of CDI. We did not retest for *C. difficile* in stool to demonstrate clearance, but the symptoms of diarrhea persisted with treatment.

A meta-analysis[22] that included 19 studies, 8725 patients revealed that over 8% of hospitalized patients were asymptomatic colonizers of toxigenic *Clostridium difficile* and had over six times higher risk for subsequent CDI. On testing stool for *C. difficile* PCR in 35 cirrhotic in-patients without diarrhea, we did not identify any asymptomatic colonizer in our study.

Bajaj et al. in their case-control study used a nationwide in-patient sample (NIS of 2005) and a liver transplant center database to compare cirrhosis patients with CDI and cirrhosis patients without CDI, and observed a mortality rate of 13.8% vs. 8.2%, length of hospital stay of 14.4 days vs. 6.7 days and hospital charges in US dollars of $70,000vs. $30,000.[4] In the present study, there was a trend towards increased mortality in patients with CDI compared to other causes of diarrhea. However, the duration of hospital stay and cost of hospitalization were comparable between groups. Due to the limited number of events and patients, we could only do univariate analysis. Therefore, in this study, we could not determine whether CDI independently influences outcome in cirrhotic in-patients. A much larger multi-centric study is required to understand the impact on the overall outcome.

Previous studies have noted that the use of antibiotics and PPI are important risk factors to acquire CDI[4,23]. Our study noted prior antibiotic use and current/ prior use of steroids/ immunosuppressants in 100% and 50% of patients with CDI, respectively. This was significantly higher compared to patients without CDI. Prior PPI use (83.3% v/s 50%), on the other hand, was similar in both groups. Our study did not note the indication, dosage, and duration of these agents.

We can conclude from this single-center prospective study, although with a limited sample size, that *C. difficile* is an uncommon cause of acute diarrhea and is not an asymptomatic colonizer in cirrhotic in-patients from India. Use of antibiotics and immunosuppression are risk factors identified for CDI. Further larger multi-centric studies are required to confirm the impact of CDI on outcomes in these patients.

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