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ORIGINAL ARTICLE

Risk Factors for Clostridium Difficile Infection in a Tertiary Care Hospital

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ABSTRACT

Introduction: Clostridium difficile infections have had significant morbidity and mortality in the last decade leading to high healthcare costs. Our prospective case-control study from October 2013 to May 2015 in a tertiary care hospital in rural India aimed to evaluate the risk factors, treatment, outcome, and complications of Clostridium difficile infections in hospitalized patients. **Materials and Methods:** The study involved a total of 183 patients, of which 61 were cases, and 122 were controls. Data was analyzed using multivariate logistic regression. **Results:** Antibiotic intake in the past four weeks (p=0.003), hypoalbuminemia (p=0.001) and duration of hospital stay before the onset of diarrhea (p<0.001) were proven to have significant risk. We subdivided cases into severe and non-severe cases, and we found that complications were statistically higher in severe cases (OR= 1.685, <0.001). **Conclusion:** Identifying severe cases and administering timely and appropriate treatment is prudent.

INTRODUCTION

Clostridium difficile is a strictly anaerobic, Gram-positive, spore-forming bacterium. Clinical manifestations of human *C. difficile* infections are due to toxins (A and B) produced by pathogenic strains of *C. difficile* (1).

In some hospitals, Methicillin-resistant *Staphylococcus aureus has been surpassed by* hospital-acquired *Clostridium difficile* infection (CDI) as the leading source of healthcare-associated infection, and because of its significant morbidity and mortality, CDI has been of concern over the last decade (2).

Antibiotic use which disrupts the microbiota increases the risk of CDI. Other factors associated with CDI include recent hospitalizations, longer hospitalizations, use of proton pump inhibitors, use of multiple antibiotics, longer duration of antibiotics, chemotherapy, older age, chronic kidney disease and use of feeding tubes (3). We performed this study to determine risk factors associated with CDI among patients with hospital-acquired diarrhea and with poor prognosis in a tertiary care hospital.

MATERIALS AND METHODS

This study was conducted in Kasturba Hospital, a tertiary care hospital in Manipal, Karnataka, India from October 2013 to May 2015. The study applied a mixed study design where a prospective case-control study design was used for the primary objective and a cohort study design for the secondary objective. The case to control ratio in the study was taken as 1:2. Ethical approval was sought from the Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee.

This study included patients ≥18 years of age, who developed diarrhea (defined as having ≥3 unformed stools with Bristol Score type 7) after a minimum of 48 hours of hospital stay and who were tested for *Clostridium difficile* by rapid test and/or ELISA and/or stool culture. Patients with laxative intake in the last 48 hours were excluded from the study. ELISA Rapid test kit used in this study was C diff QuikChek Complete, manufactured at TechLab Inc., Blacksburg, VA, USA, that detects both *C. difficile* GDH antigen and toxins A and B via rapid membrane enzyme immunoassay.

Amongst patients who developed diarrhea after 48 hours of hospital stay, those who tested positive for CDI were considered cases while those who tested negative for CDI were considered controls. All patients were followed up till discharge or death.

The severity score-derived from the study conducted by Zar et al, and determined by fever (>38.3 °C), leukocytosis (>15.0x10$^{9/L}$), Acute Kidney Injury (AKI), stool frequency

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(>6/day), hypoalbuminemia (serum albumin<25g/L) each given one point and presence of radiological signs, given 2 points- was of prognostic value in predicting the occurrence of complications. Patients having a severity score between 0-2 were classified as non-severe cases, whereas those with scores >2 were severe cases (Table1) (4).

The data obtained was analyzed using statistical package for the social sciences (SPSS) version 20. All independent variables with values <0.05 in the univariate analysis were included in stepwise multiple logistic regression to identify factors associated with CDI among patients with hospital-acquired diarrhoea. In the multivariate model, independent variables were eliminated from the highest to the lowest value but remained in the model if their value was <0.05. The Odds ratios (ORs) were estimated along with the 95% confidence intervals (CIs).

RESULTS

This study involved a total of 183 patients, of which 61 were cases, and 122 were controls. The mean age was 49.77 in cases and 40.53 in controls. Further demographic characteristics of the respondents are given in Table 2.

Ninety-four controls (77.04%) and 10 cases (16.39%) had a hospital stay prior to the onset of diarrhea of less than one week. Twenty-four controls (19.67%) and 40 cases (65.57%) had a hospital stay between one and three weeks (p<0.001). Four controls (3.27%) and 11 cases (18.03%) had been admitted in the hospital for ≥ three weeks (p<0.001). 27 cases (44.3%) underwent recent surgery, as compared to 27 controls (22.1%) (p=0.002).

Table 1. Markers of severity

Factors	Points
WBC count >15×10^9/L	1 point
Hypoalbuminemia (<25g/L)	1 point
Acute rise of creatinine levels	1 point
Stool count >6/day	1 point
Body temperature >38.3°C	1 point
Severe grade of radiological signs	2 points

WBC: White Blood Cell

The Charlson comorbidity index interquartile range was 3 for cases and 2 for controls.

The median number of days of gastric suppression therapy in cases was 14 and was 4 in controls. Other risk factors for CDI are given in Table 3.

Multivariate logistic regression (Table 4) showed that history of antibiotic intake in the past four weeks (p=0.003), hypoalbuminemia (p=0.001) and duration of hospital stay (p<0.001) were significant risk factors. Gastric acid suppression therapy was also proven to be a significant risk with a p-value of 0.002.

Leukocytosis was a significant marker for severity (p<0.005); 68.9% of cases had leukocytosis as compared to 39.3 % controls. (Table 5)

Of the 61 cases, 21 had a fever while 56 of the 122 controls had a fever. AKI was present in 15 out of 61 cases, and 19 out of 122 of controls had AKI. Neither p-value was significant.

Amongst the 61 patients with *C. difficile* infection, antibiotics were withdrawn in 37 of them. A single antibiotic was used to treat 18 of them, 16 were treated only with metronidazole and 2 with only vancomycin; 43 were treated with both vancomycin and metronidazole.

Hyponatremia (Na^+<135 mmol/L) and hypokalemia (K^+< 3.5 mmol/L) were some of the observed electrolyte imbalances. Further analyses indicated that 55.7% developed hyponatremia and 42.6% developed hypokalemia amongst the cases whereas in controls, only 24.6% had hyponatremia and 13.9% developed hypokalemia. Amongst the 61 cases, 60.7% developed complications (p<0.001); 55.7% developed electrolyte imbalances (p<0.005) and 24.6% developed AKI. Controls, on the other hand, had a 25.4% rate of complications; 24.6% had electrolyte imbalances, and 15.6% had AKI.

Of the cases 86.9% improved, 4.9% had recurrences, and 8.2% expired. Amongst controls, 98.4% improved and 1.6% expired.

Subanalysis

The cases were divided into two groups for analysis; severe and non-severe. There was no significant difference between severe and non- severe cases regarding relative risk

Table 2. Patient characteristics

Characteristics	Case % (n/N)	Control % (n/N)
Age (mean age in years)	49.77	40.53
Female sex	44.3 (27/61)	34.4 (42/122)
The median duration of hospital stay before onset of diarrhoea (IQR)	14 (8-19)	4 (4-8)
Number of antibiotics used before the onset of diarrhea		
0	1.6 (1/61)	37.7 (46/122)
1	21.3 (13/61)	19.7 (24/122)
2	21.3 (13/61)	17.2 (21/122)
3	19.7 (12/61)	13.1 (16/122)
4	23 (14/61)	11.5 (14/122)
5 or more	13.1 (8/61)	0.8 (1/122)

for antibiotic intake (p=0.693). 16.7 % of non-severe cases were above the age of 65 years, as compared to 10.5% of severe cases. Severity of disease in cases did not show any co-relation with duration of the hospital stay [relative risk: <1 week- 1.131 (p=0.582); 1-3 weeks- 0.873 (p=NA); >3 weeks- 1.036 (p=0.674)]. 52.6 % severe cases had prior surgical procedure as against 40.5% of non-severe cases but was not found to statistically significant (p=0.415).

The majority (78.9 %) of severe cases were treated with both antibiotics compared to 66.6 % of non-severe cases. Metronidazole as a single agent was used in 30.9% of non-severe cases. Complications were statistically more significant in severe cases (p<0.001). Up to 15.8 % of severe cases succumbed to the disease against 4.8% of non-severe cases (p=0.145). Recurrence was seen in 5.3% and 4.8% of cases with severe and non-severe infections, respectively (p=0.681).

Table 3. Other risk factors for CDI

Risk factor	Significance
Antibiotic intake in last 4 weeks	0.003
Cephalosporin	0.862
Amikacin	0.931
Metronidazole	0.591
Co-trimoxazole	0.171
Penicillin	0.223
Prior surgical procedure	0.983
Hypoalbuminemia	0.001
Duration of hospital Stay	<0.001
Charlson comorbidity index	0.589
Gastric acid suppression	0.002

DISCUSSION

This observational study carried out in a tertiary care hospital in Karnataka had 61 cases and 122 controls.

Increasing age increases the risk as well as the severity of CDI (5, 6). Yang et al demonstrated age greater than 70 years (adjusted odds ratio [OR], 1.76; 95% confidence interval [CI], 1.12 to 2.75; p=0.01), to be an independent risk factor for CDI (7). However, in this study, age was not a risk factor for the development of CDI nor a marker of severity of disease, as outbreaks probably occurred in wards with relatively younger age groups and only a few patients were aged above 65 years.

Although some studies, including Yang et al., have suggested that female sex may be a predictor, it is difficult to explain the relationship between sex and CDI (7, 8, 9).

Almost all antibiotics, including metronidazole and vancomycin, can predispose to *C. difficile*. According to Khanafer et al., prior to CDI, 38 patients (95.0%) were exposed to antibiotics, and 12 (30%) received at least four antibiotics. Fluoroquinolones, 3rd generation Cephalosporins, and Co-amoxiclav were prescribed most frequently (65%, 55%, 40% and 37.5%, respectively) (10). Our study demonstrated use of antibiotics such as Cephalosporins (OR= 4.329, 95%CI, p<0.001), Amikacin (OR= 2.794, 95%

Table 4. Risk factors and their significance through multivariate logistic regression

Risk factors	Cases (n=61) (%)	Controls (n=122) (%)	*P* value	CI
Age>65 years	09 (14.8)	12 (9.8)	0.228	0.667 (0.297-1.495)
Details of antibiotics received				
Penicillin	13 (21.7)	25 (32.9)	0.521	1.051 (1.922-7.140)
Cephalosporins	51 (85)	66 (86.8)	<0.001	4.329 (2.012-9.306)
Amikacin	27 (45)	27 (35.5)	0.002	2.794 (1.442-5.416)
Trimethoprim/Sulphamethoxazole	18 (30)	12 (15.8)	0.001	3.837 (1.705-8.635)
Metronidazole	32 (53.3)	28 (36.8)	<0.001	3.104 (1.922-7.140)

Table 5. Markers of severity in cases and controls

Markers	Case % (n/N)	Control % (n/N)	Or value 95% CI	*p*-value
Fever (>38.3°*C*)	34.4 (21/61)	45.9 (56/122)	0.619 (0.327-1.170)	0.092
Leukocytosis (15×10^9/L)	68.9 (42/61)	39.3 (48/122)	3.408 (1.775-6.543)	<0.001
Hypoalbuminemia (<25g/L)	36.1 (22/61)	10.6 (13/122)	0.211 (0.097-0.459)	<0.001
AKI	24.6 (15/61)	15.6 (19/122)	1.767 (0.825-3.782)	0.138
Frequency of stools >5/day	36.1 (22/61)	8.2 (10/122)	6.317 (2.750-14.512)	<0.001

AKI: Acute Kidney Injury

CI, p=0.002), Metronidazole (OR= 3.704, 95% CI, p<0.001) and Cotrimoxazole (OR= 3.837, 95% CI, p= 0.001) had significant association. Total antibiotic intake was found to be statistically significant after multivariate logistic regression (p=0.003), but none of the individual antibiotics showed statistical significance. This observation might have been because combination antibiotics were used during the hospital stay.

Increased length of hospital stay prior to the onset of diarrhoea was found to be statistically significant with the development of CDI in our study (p<0.005), which was in accordance with previous observational studies as demonstrated by Freeman et al. and Morrison et al. (11, 12). This could be due to multiple reasons: prolonged exposure to spore-bearing bacilli, use of antibiotics, use of PPIs and other comorbidities (11, 12). As previously mentioned, increased risk of CDI was seen with surgical procedures. Among patients with preoperative prophylaxis (PAP), the risk of CDI was 14.9 cases per 1000 surgical procedures (5). According to McCarter et al., regardless of the surgical procedure, for pretreated patients, the risk of CDI was 4.2 times higher than that of the control group (13). Our study also showed an increased risk of CDI in patients who had undergone a surgical procedure, which was statistically significant (OR=2.794, 95% CI, p=0.002).

Dial *et al.* estimated an adjusted risk ratio for CDI with current use of gastric acid suppression as 2.9 (95% CI: 2.4-3.4) (14). Recurrent C. difficile colitis risk is also increased with PPI therapy. However, Henrich et al. found no association between gastric acid suppression and severe CDI and attributed this to the acid resistance of *C. difficile* spores (15). Patients on PPIs are 4.17 times more likely to develop CDI compared to patients, not on PPIs (16). Based on analysis of gastric contents of patients on PPIs, Jump found that vegetative *C. difficile* survival increased at a pH of >5 (17). We compared the median number of days for the exposure to gastric acid suppression before the onset of diarrhoea. The cases had 14 days (IQR [Interquartile Range] = 8-19) while controls had 4 days (IQR= 4-8) which was statistically significant (OR= 2.105, p<0.005).

In this study, proton pump inhibitor therapy could not be assessed as a risk factor for CDI as both cases and controls were given PPIs. PPIs are associated with increased risk of CDI most likely because it decreases the barrier to colonization by vegetative forms of *C. difficile* (14, 18).

Charlson Co-morbidity index was calculated in recent CDI studies since previous observational studies found co-morbidities like diabetes, cancer, HIV to be independent risk factors. Kurti et al, showed significant association with Charlson co-morbidity index (p= 0.004) (19). In this study, we observed a similar picture, with the index being 3 (IQR= 2-4) in cases while in controls it was 2 (IQR= 2-3) (OR= 1.504, 95% CI, p<0.001).

Amongst the risk factors, after initial univariate analysis, antibiotic intake (total, cephalosporins, amikacin, metronidazole and cotrimoxazole), duration of hospital stays before the onset of diarrhoea, prior surgeries, Charlson co-morbidity index and gastric acid suppression were found to be statistically significant. Following a multivariate analysis of the significant risk factors total antibiotic intake (p=0.003), duration of hospital stays (p<0.001) and gastric acid suppression (p=0.002) were significant.

In our study we compared cases to controls in accordance with Zar et al., we found a statistically significant association between leukocytosis (OR= 3.408, 95% CI, p<0.005) and frequency of stools (OR=6.317, 95% CI, p<0.005). Hence, patients with diarrhoea during hospital stay should carry a high suspicion in the presence of the above markers. If possible, the provoking antibiotics should be discontinued to facilitate regeneration of the normal gut microflora. An antibiotic with activity against *C. difficile* should be started. Initial therapies based on the severity of disease include metronidazole for mild-moderate disease, vancomycin for severe disease or a combination of the two for severe-complicated disease (20). In a recent study by Khanafer et al., metronidazole was administered as a single agent to 25 patients, vancomycin to 2 (5%), 2 CDI medications to 8 (20%) and no antimicrobials against CDI to 5 (10).

In this study, withdrawal of the inciting antibiotic was done in 60.7% (37/61) patients, while combination of metronidazole and vancomycin was given to 70.5% (43/61), metronidazole alone to 26.2% (16/61) and vancomycin alone to 3.3% (2/61).

There has been a significant rise in severe cases, colectomies, and deaths related to CDI [21]. In our study, complications (OR=4.526, p <0.001) and electrolyte imbalance (OR= 3.826, p<0.001) were found to be significantly more in cases when compared to controls. Further, it was seen that 86.9 % (53/61) patients improved clinically and recurrence was observed in only 4.9% (3/61) while 0.08% (5/61) cases expired of which three died primarily of complications related to CDI. Identifying patients who are at high risk for severe CDI early during infection may direct therapy and help improve outcomes. A severity score such as one developed by Zar et al., greatly increases the strength of detection of complications in CDI.

Further studies with larger sample sizes should use such scores to analyze their benefits and practicality. We subdivided cases into severe and non-severe cases using the severity score parameters as previously described and analyzed it for relative risk for each risk factor. In the previous studies, such categorization between cases based on severe and non- severe cases was only made for deciding the treatment options (22).

There was no difference in treatment strategies between the severe and non- severe group. This study involved 31.1% (19/61) severe and 68.9% (42/61) non-severe CDI, and there was no statistically significant difference for all the risk factors previously discussed between severe and non-severe groups. Also, there was no difference in the treatment offered to either group. Patients in the severe group had significantly high complication rates (OR= 1.685, p<0.001). Amongst patients with severe disease, 15 (78.9%) recovered while 38 (90.4%) with non-severe disease improved. Recurrence was seen in one (5.3%) of the severe cases and two (4.8%) of the non-severe cases, while three (15.8%) severe cases died as against two (4.8%) non-severe cases.

Some of the limitations of this study include the fact that the study was related to the single-center observation-

al design. Also, selection bias is a possibility because the selection of controls is often challenging. Further, age and sex matching could not be done as both were pre-identified individual predictors of CDI.

CONCLUSION

In conclusion, *Clostridium difficile* infection is an important cause of morbidity and mortality and should be suspected in all cases of hospital-acquired diarrhea. Some of the risk factors for CDI include antibiotic intake, gastric acid suppression, hypoalbuminemia and prolonged hospital stay. Complications are frequent in severe cases and hence the need for identifying these cases for timely institution of appropriate treatment. Lastly, inpatient hospital outbreaks of CDI should be prevented by proper isolation of the index case, contact precautions, and appropriate hand washing techniques.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in this study.

CONFLICT OF INTEREST

None

ETHICAL STANDARDS

Ethical approval was sought from the Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee.

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