SUPPLEMENT ISSUE

ARTICLE

PREVALENCE AND RISK FACTORS OF *CLOSTRIDIUM DIFFICILE* - ASSOCIATED DIARRHEA IN HOSPITALIZED PATIENTS IN ZAHEDAN

Somayeh Jahani[1], Mohamad Ali Mohaghegh[2], Shahram Shahraki Zahedani[1], Mehdi Azami[3], Masoud Salehi[1*]

[1]*Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, IRAN*

[2]*Department of Medical Basic Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, IRAN*

[3]*Department of Veterinary, Islamic Azad University, Shahrekord Branch, Shahrekord, IRAN*

ABSTRACT

Background: *Clostridium difficile is a frequently identified cause of nosocomial gastrointestinal disease. It has been proved to be a causative agent in antibiotic-associated diarrhea.* ***Aims:*** *This study was aimed to determine the prevalence and risk factors of Clostridium difficile-associated diarrhea (CDAD) in hospitalized patients with nosocomial diarrhea in Zahedan, Iran.* ***Methods:*** *In this study from June to December 2014, a total of 100 stool samples of patients with nosocomial antibiotic associated diarrhea that were admitted in to the intensive care units (ICUs) (50), surgery (20) and internal medicine wards (30) in Emam Ali hospital, Zahedan, Iran were collected. All stool samples were analyzed by cytotoxicity assay and enzyme immune assay for detection and conformation of toxins.* ***Results:*** *The mean ± standard deviation of age was 40.4 ± 11.8 and 75 (61.5%) of patients were male. Nine (7.4%) cases of nosocomial diarrhea were diagnosed as CDAD that all isolates were toxigenic. Five of 65 organs receive transplant patients and 4/41 hospitalized patients in ICUs ward were developed CDAD. None of samples that obtained from surgery ward infected with C. difficile. Ceftazidime and Ampicilline-Sulbactam were the most common antimicrobial drugs used. Multivariate analysis showed that use of diapers, antibiotic and immunosuppressive therapies were significantly associated with CDAD (P < 0.05).* ***Conclusions:*** *Hospital transmission of C. difficile commonly occurred, supporting infection-appropriate measures directed toward the reduction of CDAD.*

KEY WORDS
Antibiotic associated diarrhea,Clostridium difficile, nosocomial diarrhea

INTRODUCTION

Nosocomial diarrhea in an important recognize cause of morbidity, mortality and cost for hospital in the developed and developing countries.[1] *Clostridium difficile* is a major cause of antibiotic associated diarrhea.[2] The incidence of infection with this organism is increasing in hospitals world-wide, consequent to the widespread use of broad-spectrum antibiotics.[3]
Hospital-acquired *C. difficile* disease is associated with not only antimicrobial use, but also advanced age, laxative use, proton pomp inhibitors, antineoplastic chemotherapeutic agents use, renal insufficiency and gastrointestinal surgery or procedures [4-6] are important.

Over the past several years, numerous reports have been published regarding *C. difficile* infection in immunocompromised patients and hospitalized patients.[7-13] Only scanty data is available on the prevalence of *C. difficile* as a cause of diarrhea in Iran. The objective of our study was to examine the importance and risk factors for *Clostridium difficile* associated diarrhea (CDAD) in hospitalized patients, in Zahedan, Iran.

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MATERIALS AND METHODS

In this study from June to December 2014, a total of 100 stool specimens of patients with nosocomial antibiotic associated diarrhea that were admitted in to the ICUs (n=50), surgery (n=20) and internal medicine (n=30) wards in Emam Ali hospital related to Zahedan University of Medical Sciences were collected. Specimens were processed immediately (the day of receiving samples) or stored at −20°C until they were tested. Nosocomial diarrhea defined if the onset of the enteric illness occurred ≥72h after admission of the patients to the hospital. Inclusion criteria were antimicrobial therapy, long stay hospitalization, and loose or liquid stool. Exclusion criteria were patient without diarrhea, hospitalized patients without antibiotic therapy and children under 2 years of age.

Demographic and basic medical information were collected from the medical records. Bacterial cytotoxicity was assayed on Vero (African green monkey kidney) tissue culture monolayers. A filter-sterilized 1:10 dilution of faces was used to oculate Vero cell monolayers. Tissue cultures were examined for cytopathic effect at 24 and 48h. The enzyme-linked immunosorbent assay (ELISA) (Wampole kit, TECHLAB Inc., The Netherlands), was used for detection and conformation of *C. difficile* toxine A and B. The procedure was carried out according to manufacture instruction.

***Corresponding Author**
Email:
shahestan@gmail.com
Tel.:+98-9151430561

Statistical analysis

SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used to analysis data. The study variables were compared between the patients who have CDAD and remaining patients who have nosocomial diarrhea but were negative for fecal *C. difficile* toxins. Student *t*-test and Chi-square test were performed to compare continuous and categorical variables, respectively. For analysis of risk factors for development of CDAD, logistic analysis regression was used. A $P < 0.05$ was deemed as statistically significant.

RESULTS

A total of 100 patients were admitted to ICUs, surgery and Internal medicine wards in Emam Ali hospital during the period from June to December 2014. During this time, 12 (12%) patients acquired CDAD that 8 isolates were toxigenic by cytotoxic assay and ELISA. The demographics and outcomes of these patients are shown in [Table 1]. The mean ±standard deviation of age was 45.1 ± 11.8. Seventy five (65%) Of patients were male.

The median interval between admission and diarrhea onset and CDAD diagnosis were 5 (5-9) and 7 (4-11) respectively. There was no significance difference with respect to age, sex, number of days between admission and CDAD diagnosis and clinical features and risk factors except malignancy, immunosuppressive therapy, antibiotic therapy, chemotherapy and use of diapers [Table 1]. The majority of patients (50%) were ICUs ward.

In univariate analysis of risk factors, immunosuppressive therapy, antibiotic therapy, chemotherapy and use of diapers were significantly associated with the development of CDAD [Table 2]. In the multivariate model assessing factors associated with CDAD, use of diapers, antibiotic therapy and immunosuppressive therapy remained significant risk factors [Table 3].

DISCUSSION

C. difficile that can be found in most of peoples without causing symptoms, but in some people it can cause a severe colitis. Predisposing actors include antibiotic therapy. The *C. difficile* toxin damages the fragile lining of the bowel causing loose watery bowel movements.[14]
The organism is usually acquired from the hospital as environmental contamination is common and health care workers may carry it in their hands, or on contaminated instruments.[15]

Prevalence of CDAD in adult inpatients from Saudi Arabia, Iran, India, chile, and Argantina have ranged from 4.6% to 22% respectively.[16-20] Three studies in Iranian hospitalized patients developing acute diarrhea showed prevalence rates of 5.3%, 6.7% and 21% respectively.[13,15,21] We found a prevalence rate of 12% in hospitalize patients. This amount was reported 4.9% for Turkish patients with diarrhea.[22]

CDAD has been reported to more common in women and older patients.[23] Among patients positive for *C. difficile* toxin, fever, cramping abdominal pain and diarrhea have been reported to be more common.[24,25] The mean age of patients in our study was 45.1±11.8 and there were more men (61.5%). Other studies have reported varying male-female ratios.[26] In this study, there was no difference in the occurrence of fever and abdominal pain between CDAD positive and CDAD negative group. Several risk factors associated with patients acquired CDAD have been reported including advanced age (>65 years), length of hospital stay, colonization pressure (the duration of time and number of infections contacts that a susceptible patients is exposure it), antibiotic therapy, immunosuppressive therapy, malignancy and vancomycin resistant enterococci [27]. Few studies [28,29] have evaluated the relative risk of CDAD in hospitalized patients who received antibiotic therapy. In our study, Ceftazidime was the most common antibiotic used in patients with CDAD and followed by Ampicillin-Sulbactam. Similar to other reports.[18,27] the third generation of Cephalosporins was the most common antibiotic used. The precise mechanism is unknown, but the most accepted hypothesis is that antibiotics alter the resident flora of the colon, leading the colonization of *C. difficile* with production of its toxins. Recently, outbreaks in the United States and Canada have been strongly related to the use of fluoroquinolones.[30] A prospective study published in 1974 showed that 20% of patients who received clindamycin developed CDAD.[31]

We don't find use of acid-suppression drugs to be significant risk factor for CDAD. 84% and 38% of our patients received acid suppressed medication such as proton pump inhibitor and antihistamine, respectively. Controversy remains about the role of antisecretory medication and CDAD development. Studies of ICU acquired CDAD have thus far failed to show any significant association[32,33] but, other researcher reported that use of proton pump inhibitor doubled the risk of CDAD in patients who received antibiotic treatment while in the hospital.[34] The normal acidity of the stomach is an important host defense against enteric infections[35] and its imbalance from the use of proton pump inhibitor increases the susceptibility of hospitalized patients to colonization and subsequent infection with *C. difficile*.

The important factor related to the development of CDAD in this study was fecal incontinence and the use of diapers. This factor has not been described in other adult settings. Only in a recent study in Peru, 72.7% of patients who infectious with *C. difficile* used of diapers that this was significantly associated with CDAD negative group.[18] A previous study showed an increased incidence of nosocomial diarrhea n a pediatric ward among diapered children and when multiple patients were housed together in the same room.[36] Laxative use has also been linked to CDAD in hospitalized patients, but the strength of this association remains unclear. One study has suggested constipation in the critically ill requiring laxative treatment is a true risk factor[32] while another group inferred that diarrhea could be occurring in asymptomatic carriers as results of laxatives use rather than as result of CDAD.[37] In our study 3(33.3%) infectious patients received lactulose prior to CDAD diagnosis.

We found a 12% (5 patients of 50 hospitalized patients in ICUs) of CDAD in ICUs hospitalized patients. There may be several explanations for the increased susceptibility of CDAD among this patient, including the increased use of antibiotics, immunosuppression, recent surgical procedure and longer and more frequent hospital admission.

The limitation of our study should be acknowledged. First, we performed a descriptive cross-sectional study; second, we only evaluated patients during the hospitalization. Thus, we cannot comment on long-term follow-up, including relapse of diarrhea and mortality. Third, this study was limited only to one center. The small number of infections and limitation to one center make generalization to other centers in Iran difficult.

In conclusion, our study provides evidence that CDAD is an important health problem in our hospital. It will be necessary to evaluate the epidemiology and to implement measure to control nosocomial spread. Use of rapid and sensitive techniques for laboratory diagnosis, a change in antibiotic policy, tight restriction of unnecessary antibiotic use, especially broad-spectrum ones and implementation of standard infection control measure is necessary to reduce morbidity to CDAD infections in hospitalized patients.

CONFLICT OF INTEREST

There is no conflict of interest.

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Table 1: Demographic and clinical data of patients with nosocomial diarrhea

Variable	Patients with nosocomial diarrhea			*P* value
	total	*C.difficile* positive	*C.difficile* negative	
Total patients	100(100)	12(12)	88 (88)	—
Male sex	65(65)	4(33.4)	61(69.4)	0.42
Age (years) (mean[SD])	45.1(11.8)	46.1(12.5)	47.2(13.0)	0.82
Median number of days between admission and CDAD Dx	7(5-11)	6(5-9)	7(4-11)	0.33
Clinical feature and risk factors				
Smoking	53(53)	10(83.3)	43(48.9)	0.41
Alcohol	4(4)	2(16.7)	2(2.3)	0.89
Fever	55(55)	7(58.4)	48(54.5)	0.10
Abdominal pain	43(43)	5(41.7)	38(43.2)	0.87
Hematochezia	11(11)	2(16.6)	9(10.3)	0.28
Inflammatory bowel disease	31(31)	6(50)	25(28.5)	0.73
Previous gastrointestinal surgery	3(3)	1(8.4)	2(2.3)	0.87
Malignancy	5(5)	3(25)	2(2.2)	0.01
Organ transplant	3(3)	2(16.6)	1(1.2)	0.27
Antibiotic therapy				
Use of one antibiotic	11(11)	5(41.7)	6(6.8)	0.047
Use of two antibiotics	54(54)	10(83.3)	44 (50)	0.021
Use of three or more antibiotics	35(35)	8(66.6)	27(30.6)	0.038
Immunosuppressive therapy	81(81)	11(91.6)	70(79.5)	0.018
Proton pomp inhibitor	84(84)	7(58.4)	74(84.1)	0.95
Antihistamines	38(38)	5(41.7)	33(37.5)	0.44
Chemotherapy	5(5)	3(25)	2(2.3)	0.015
Enteral feeding	30(30)	3 (25)	27(30.6)	0.24
Use of diapers	26(26)	7(58.4)	19(21.5)	0.005
Laxativsus use Laxative suse	32(32)	3(25)	29(32.9)	0.71

CI= confidence interval, CDAD= C. difficile-associated diarrhea, C. difficile= Clostridium difficile

Table 2: Result of univariate analysis of risk factors for development CDAD

Factor	Adjusted odds ratio (95% CI)	P value
Age	1.12(0.85-1.08)	0.098
Sex	1.34(0.87-3.65)	0.076
Malignancy	1.73(0.61-5.30)	0.250
Immunosuppressivetherapy	2.30(1.23-4.75)	0.013
Chemotherapy	2.45(0.25-3.77)	0.653
Useofoneantibiotic	2.56(1.21-3.50)	0.023
Useoftwoantibiotics	2.43(1.15-3.81)	0.021
Useofthreeormoreantibiotics	3.65(1.80-4.32)	0.000

CI= confidence interval, CDAD= C. difficile-associated diarrhea, C. difficile= Clostridium difficile

Table 3: Result of multivariate analysis of risk factors for development CDAD

Factor	Adjusted odds ratio (95% CI)	P value
Immunosuppressive therapy	2.12 (1.54-2.89)	0.023
Use of one antibiotic	2.22 (1.23-3.43)	0.045
Use of two antibiotics	2.20 (1.11-4.08)	0.012
Use of three or more antibiotics	3.07 (1.76-5.11)	0.003
Use of diapers	3.65 (1.23-7.97)	0.005

CI= confidence interval, CDAD= C. difficile-associated diarrhea, C. difficile= Clostridium difficile