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To Study the Prevalence of Clostridium Difficile in Cases of Antibiotic Associated Diarrhoea in a Tertiary Care Hospital in North India.

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ABSTRACT

Background: Clostridium difficile is a gram positive spore forming bacilli which can be normally present in human colon in some individuals. It can cause clostridium difficile infection which can lead to Clostridium difficile associated disease(CDAD) which is manifested by diarrhoea and in fulminant cases by pseudomembranous colitis and can lead to death. Disruption of normal intestinal flora by antimicrobials and lowering of immunity leads to its overgrowth and disease manifestations. Aims And Objectives: 1. To find the prevalence of clostridium difficile in stool samples of patients presenting with antibiotic associated diarrhoea. 2. To find the risk factors associated with the disease. **Methods:** The study was conducted from January 2017 to June 2018 on 131 stools samples of patients who developed diarrhoea after three days of starting antibiotics by ELISA based method for detection of Toxin A/B. **Results:** Out of 131 stool samples analysed, 6 samples (4.58%)were found to be positive for toxin A/B. Correlation between use of third generation cephalosporin and toxin positivity was found to be insignificant. Significant correlation was found between use of chemotherapeutic agents and toxin positivity. It was also found that advanced age was also significant risk factor for development of CDAD. **Conclusion:** The present study proves that Cdifficile should be kept in mind as an etiological agent in cases of antibiotic associated diarrhoea. Risk factors include advancing age, use of chemotherapeutic agents and antibiotic exposure. To prevent C difficile infection, unnecessary use of antibiotics should be stopped and screening of stools for Toxin analysis in cases of antibiotic associated diarrhoea should be done so that it can be diagnosed and treatment isstarted at the earliest.

Keywords: Clostridium difficile, CDAD, Antibiotic associated diarrhoea, Pseudomembranous enterocolitis.

INTRODUCTION

Clostridium difficile is an anaerobic gram positive motile, spore forming bacilli that can become establish in human colon in 2-5 % of adult normal population.[2] It causes clostiridiumdifficile infection (CDI) which is manifested by watery diarrhoea, fever, nausea, and abdominal pain. In severe cases it can lead to pseudomembranous colitis,toxic megacolon and perforation of colon.[2]

C. diffficile spreads by faeco-oral route by transmission of spores which are spread by hands of healthcareprofessionals2. The spores resist the acidity of stomach and germinate into the vegetative form in the small intestine. Disruption of normal gut flora by antimicrobials allows C. difficile to proliferate which causes spectrum of clinical manifestations that range from asymptomatic carriage to diarrhoea of varying severity to fulminant colitis and even death.[3]

Virulence of C. difficile is due to exotoxins, toxin A and B which are coded by pathogenicity locus. Toxin A and toxin B leads to disease through variety of cytotoxic mechanisms amongst which most notable are the loss of cytoskeletal structure leading to cell rounding and cell death.[4,5]

A number of risk factors have been found to be associated with CDI of which prolonged hospitalization has been found to be a significant risk factor. Exposure to antimicrobial agents such as clindamycin, cephalosporins, flouroquinolones are also a significant risk factor for development of CDI.[6] Other important risk factors are older age, underlying comorbid conditions, use of chemotherapeutic agents and use of acid suppression therapy.[7]

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The diagnosis of clostridium difficile infection should be suspected in all patients with nosocomial diarrhoea. Various methods for detection of clostridium difficile include detection of toxin A/B in stool by ELISA, cytotoxicity assays, detection of common Glutamate dehydrogenase antigen (GDH) by latex agglutination test. Stool culture for detection of organism is less useful because asymptomatic carriage of C.difficile strains that are non toxigenic is quite common. Nowadays molecular methods for diagnosis such as PCR are being used which have excellent sensitivity and specificity, but they are not routinely available.

The crucial step in management of CDAD is cessation of inciting agent such as antibiotic which is being given to the patient. The initial treatment of choice is oral metronidazole given for 10-14 days. The second line of management is orally given vancomycin8.Other supportive therapies such as correction of hydration and electrolyte abnormalities are also done in the management.

Incidence of clostridium difficile infection varies in different parts of the world, with some areas showing low prevalence and some showing higher prevalence.

Therefore this study was undertaken to find the incidence of CDI in our hospital and to find the risk factors which lead to CDI.

Aims and Objectives

1. To find the prevalence of Clostridium difficile in stool samples of patientspresenting with antibiotic associated diarrhoea.
2. To find the risk factors for clostridium difficile associated diarrhoea.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Government Medical College, Amritsar, comprising of patients aged more than 1 month, who were admitted in different wards of attatched hospitals from January 2017 to June 2018 for diseases unrelated to diarrhoea and who were receiving antibiotics and thereafter developed diarrhoea after three days of receiving antibiotics. Ethical clearance was taken from the hospital's ethical clearence committee.History, demographic details, exposure to class of antibiotics and any other risk factor was noted in the pre- designed proforma.

Inclusion Criteria

1. Patients with clinical signs and symptoms of antibiotic associated diarrhoea who were admitted in Government Medcial College, Amritsar.
2. Patients who developed diarrhoea after three days of receiving antibiotics.
3. In children the study group will be considered after neonatal period.

Exclusion Criteria

1. Patients already having diarrhoea on admission.
2. Patients who developed diarrhoea before three days of receiving antibiotics
3. Neonates (0-28 days) were not considered for study group.

The test procedure was done by the kit RIDASCREEN Clostridium difficile Toxin A/B supplied by R- Biopharm AG Germany and procured from OSB life sciences, New Delhi.

The stool samples collected for clostridiumdifficile analysis were stored at 2-8 degree Celsius and processed within 3 days. Multiple freezing and thawing was avoided. No preservative was added to the sample as preservatives, animal sera, metal ions, oxidising agents may interfere with clostridium difficile toxin A/B test. Statistical analysis was done by using SPSS version 17 software. P value <0.05 was considered as significant.

RESULTS

A total of 131 stool samples were analysed for clostridium difficile toxin from suspected cases of antibiotic associated diarrhoea. Out of the total suspected cases, 51.9% (68) were males and 48.10% (63) were females. Maximum number of stool samples received were from surgery (41.9%) and orthopaedics (22.1%) departments. Hypertension was the most common comorbidity found in 6.87% of patients. Maximum number ofpatients (38.9%) were in the age group of 20-40 years followed by age group of 40-60 years (31.2%). Mean age of patients from whom the stool samples were sent for analysis was 38.08 years.

Third generation cephalosporins were the most common group of antibiotics used in 80.15% of patients (105/131) from whom the stool samples were sent. Other significant groups of antibiotics used were aminoglycosides (45.8%) and amoxicillin(29%). 91.41 % of patients were receiving more than one antibiotic. Majority of patients (41.98%) developed diarrhoeabetween 7 to 10 days of starting antibiotics. 33.5% of patients developed diarrhoea between 5 to 7 days after starting antibiotics.

Out of total 131 patients screened for Clostridium difficile toxin A/B, six patients (4.58%) were found to be positive for Toxin A/B.In our study, out of total six patients whose stool samples were positive for toxin A/B, 2 were males (33.33%) while 4 were females(66.67%). The association of stool toxin positivity among different genders was found to be statistically not significant.

All the six patients in whom Toxin A / B ELISA was positive were more than 50 years of age. One (0.76%) patient was in the age group of 40-60 years while five (3.81%) patients were in the age group of more than 60 years. The result was found to be statistically significant.

In our study, all the positive cases were on multiple antibiotics and 83.33 % of positive cases were receiving third generation Cephalosporins. Although third generation Cephalosporin use was associated with C. difficile infection, the association was not found to be statistically significant.

In the study, out of six toxin positive cases, only one (16.66%) developed diarrhoea within seven days of starting antibiotics while five patients (83.33%) developed diarrhoea after seven days of starting antibiotic. The association was found to be statistically not significant.

In our study, out of six toxin positive cases, half of the toxin positive cases (50%) were on chemotherapeutic agents and the result was found to be statistically significant proving that use of chemotherapeutic agents is a significant risk factor for CDAD.All the toxin positive cases were on antacid medications, either PPI or H-2 blockers, but the association between PPI / H2 blocker use was found to be statistically not significant.

DISCUSSION

In our study prevalence of C.difficile was found to be 4.58% (6 positive cases out of 131). Similar results were found in the studies done by Gulnaz Bashir et al9 (4.32%), Rama Chaudhary et al,[14] (6%) and S. Kamble et al,[13] (6%). A little higher prevalence was found in the study done by Meenakshisinghet al,[11] (8.55%) and ShashidharVishwanath et al,[12] (8%). However ArunSachuet al,[10] and Justin et al,[15] found a higher prevalence of 8.8% and 10.8% respectively. A very high rate of toxin positivity (26.7%) was found in Egypt.[16] The lower prevalence of CDAD in India can be due to number of factors namely poor recognition of illness, lessawareness and no routine surveillance for Clostridium difficile illness. Majority of patients were on anti C. difficile treatment by the time investigation for detection of toxin was requested. Good immune response towards C. difficile and high fibre diet can also be the reasons for lower frequency of CDAD in India. Apart from these factors, absence of virulent NAP1 strain in India could also be the reason for less frequency of CDAD in India.[17]

In our study, the association of stool toxin positivity among different genders was found to be statistically not sigificant. This is in concordance with other studies conducted by Gulnaz Bashir et al,[9] Rama Chaudhary et al,[14] ArunSachu et al,[10] and Meenakshi Singh et al,[11] who also found no correlation between gender and toxin positivity.

It was proved in our study that advancing age is a significant risk factor for clostridium difficile associated disease (CDAD). Similar results were found in the study done by Gulnaz Bashir et al,[9] who found three patients out of seven had age group more than 45 years. Similarly Rama Chaudharyet al,[14] found that 33.33 percent of patients were in age group between 50 to 60 years.

Table 1: Clinical Profile Of Patients With Clostridium Difficile Toxin A/B Positivity

Name	Age/ Gender	Diagnosis	Day Of Diarrhoea	Antibiotics Used	Comorbidity	Acid Supression	Recent Surgery/ Procedure
Kartar Kaur	65 Female	Nhl With Cervical Lap	8	Amoxycillin Clavulanate Gentamycin	Hypertension	Ppi	Nil
Gurschit Singh	60 Male	Fracture Right Tibia	8	Cefoperazone Gentamycin	Nil	H2 Blocker	Lcp Fixation
Dalbir Kaur	67 Female	Cystolithiasis	9	Ceftazidime Gentamycin	Nil	Ppi	Cystolithotomy
Jagir Kaur	60 Female	Carinoma Anal Canal	7	Ceftazidime Amikacin	Dm Ht	H2 Blocker	Apr
Surinder Kaur	62 Female	Endometrial Cancer	10	Ceftazidime Amikacin	Nil	H2 Blocker	Hysterectomy
Tejinder Singh	54 Male	Cirrhosis	9	Cefotaxime Piperacillin	Portal Ht	Ppi	Nil

Table 2: Positivity Of Stool Samples For Clostridium Difficile Toxin A/B

Samples	Number	Percentage
Positive	6	4.58
Negative	125	95.42
Total	131	100

Table 3: Table Showing Correlation Between Different Parameters And Detection Of Clostridium Difficile Toxin A/B In Stool

	Toxin A/B In Stool		
Parameter	Positive	Negative	P Value
Day of diarrhoea > 7 days	5	55	0.06
Male gender	2	66	0.35
Use of 3rd generation cephalosporins	5	75	0.672
Use of Chemotherapeutic agents	3	11	0.001
Use of Acid suppression therapy	6	106	0.302
Age more than 40 years	6	51	0.001

In our study, out of six toxin positive cases, half of the toxin positive cases (50%) were on chemotherapeutic agents and the result was found to be statistically significant proving that use of chemotherapeutic agents is a significant risk factor for CDAD. The result is similar to other studies done by Gulnaz Bashir et al,[9] and ArunSachu et al.[10] While the association between PPI / H2 blocker use was found to be statistically insignificantand the findings were consistent with the findings of Gulnaz Bashir et al,[9] and Meenakshi Singh et al.[11]

CONCLUSION

Clostridium difficile is an important cause of antibiotic associated diarrhoea in hospitalised patients. Indiscriminate use of antibiotics and emergence of hypervirulent strains along with inadequate infection control measures in hospitals have led to increase in incidence of CDAD. The incidence of diarrhoea increases with advancing age of patients. Almost all antibiotics including third generation cephalosporins, aminoglycosides and flouroquinolones are associated with risk of clostridium difficile infection.

Stringent methods should be employed to prevent nosocomial spread of clostridium difficile infection. Antimicrobial stewardship programmes of hospitals should be strengthened so that unnecessary use of antibiotics should be avoided. Patients developing diarrhoea after starting antibiotics should be evaluated by ELISA based method for detecting Toxin A/B so that appropriate treatment can be initiated at the earliest.There is also urgent need to develop point of care (POCT) diagnostic method so that CDI can be diagnosed at the earliest.

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