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Original Research Paper

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STUDY OF TOXIN PRODUCTION IN PATIENTS WITH CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHOEA

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ABSTRACT

Introduction: *Clostridium difficile* infection (CDI) is defined as the presence of diarrheal symptoms and either a stool test result positive for *C. difficile* toxins or detection of toxigenic *C. difficile*, or colonoscopic findings demonstrating pseudomembranous colitis. *C.difficile* produces toxin A (enterotoxin) and B (cytotoxin), under favorable conditions. Both toxins severely affect GIT. The relationship between the amount of toxins in the feces and the severity of symptoms has been found.

Aim & Objectives : To detect *C.difficile* toxin production in stool samples with laboratory confirmed CDI and to correlate the presence of toxin with disease severity

Material & Methods: A prospective laboratory based study done in a tertiary care Medical college and allied hospitals in Gwalior (MP) in a duration of 11 months, which included stool samples of 118 patients with laboratory confirmed CDI. Toxin levels in stool samples were detected and correlated with the clinical condition.

Toxin A & B study were performed on stool sample with ELFA (Enzyme Linked Fluorescent Assay) technology (VIDAS instruments).

Results and Discussion: 118 patients with laboratory confirmed CDI. Toxin levels in stool samples were detected in 08 patients. The toxin positivity rate was 6.79%. All of these hospitalized patients had severe diarrhea and history of administration of broad spectrum antibiotics

Conclusion: Significant toxin load in the fecal samples may be associated with the significant deterioration of the general condition of the patient.

KEYWORDS : C.difficile, Toxin A, Toxin B, CDI

INTRODUCTION

C. difficile is recognized as one of the most important pathogens in hospital and community healthcare settings, with a steadily rising global incidence of infection and concordant increase in mortality [1,2,3].*Clostridium difficile* is an anaerobic, gram-positive spore-forming bacillus. It has the potential to produce a spectrum of clinical illness, ranging from asymptomatic colonization to pseudomembranous colitis with severe diarrhea [4] The *Clostridium difficile* is transmitted via feco-oral route. It is mostly acquired from the hospital environment, by touching the inanimate objects or surfaces contaminated with faeces (spores of *C. difficile*). In adults, colonization affects about 3 % of the population. This number increases considerably during long hospital stays.[5]

Asymptomatic *C. difficile* colonization is defined as a positive stool culture for *C. difficile* in the absence of diarrhea.[1]*C. difficile* can colonize the epithelial lining of the mucosa in the digestive tract, and the problems caused by the presence of bacteria are due to several different toxins it produces.[5] The first stage in asymptomatic *C. difficile* colonization is the ingestion of *C. difficile* spores. The spores survive the gastric acid and germinate into vegetative cells in the anaerobic environment of the colon.[1,6]he prevalence of asymptomatic *C. difficile* colonization varies depending on a number of host, pathogen, and environmental factors. [1]The number of colonized patients is higher than symptomatic CDI cases among hospital patients, particularly when disease is endemic. [1]

Symptomatic *Clostridium difficile* infection (CDI) defined as the presence of diarrheal symptoms and either a stool test result positive for *C. difficile* toxins or detection of toxigenic *C. difficile*, or colonoscopic findings demonstrating pseudomembranous colitis.[1]*C.difficile* produces toxin A (enterotoxin) and B (cytotoxin), under favorable conditions. [7] Both toxins also affect the strength of the intercellular bonds.[5] Toxin A leads to an increased secretion of fluid within the digestive tract, Mucosal inflammation and structural damage. Toxin B is in most cases responsible for the major problems associated with infection. It is estimated that it has approximately 10 times more impact on the gastrointestinal mucosa than toxin A.[8]

The relationship between the amount of toxins in the feces and the severity of symptoms has been demonstrated. Significant increases in toxins in the fecal load are associated with the significant deterioration of the general condition of the patient [5][9].

MATERIAL & METHODS

After approval of Institutional scientific and ethical committee, this study was conducted in the Department of Microbiology, Gajra Raja Medical College, Gwalior and the Duration of Study was 11 months. Study design : Prospective laboratory based study done on stool samples of patients admitted in various wards in G.R. Medical College, Gwalior and associated J A Group of Hospital Gwalior (MP).

INCLUSION CRITERIA: Patients admitted in various wards of the J.A. group of hospital for more than 48 hours. Hospitalized patient with complaint of diarrhea. Only the CDI confirmed diarrhea patients were taken for toxin detection.

EXCLUSION CRITERIA: Patients who are admitted for less than 48 hours and who have no complaint of diarrhea, patients who are not willing to participate in the study. Diarrhoea other than CDI .Children less than 2 years of age were also excluded.

Stool Sample Collection was done as per standard protocols after taking due consent from patient. Toxin A & B study were performed on stool sample with ELFA (Enzyme Linked Fluorescent Assay) technology (VIDAS instruments) at ACER diagnostic lab.

TABLE- 1. Isolation of *C. difficile* laboratory and detection of toxins

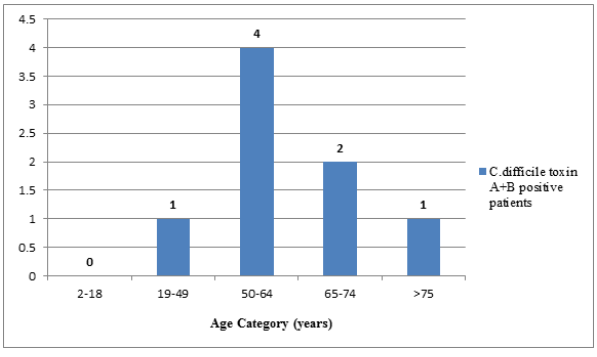
	Detection of bacteria		Detection of toxins		
Method	GDH	Culture	EIA	CTA	Toxigenic culture
Use	GDH Enzyme detection	Strain isolation, Susceptibility testing typing	Toxin A&B detection	Toxin B detection	Strain isolation, Toxin detection
Time-to-result	15 min - 2 hrs	2-4 days	15 min - 2 hrs	1-2 days	1-2 days
Main features	Sensitive, Manual, Automated, Rapid	Sensitive, Manual, Low price Excellent NPV*	Specific, standardized, Manual, Automated, Rapid	Sensitive, Notstandardized,Time-consuming, Technical expertise Required	

Table 27.Age wise distribution of C. *difficile*Toxin A+B positive patients

Age Category (years)	C. *difficile*Toxin A+B positive patients	Severity of Diarrhoea
2-18	00	
19 – 49	1	Severe
50 – 64	4	Severe
65 – 74	2	Severe
>75	1	Severe
Total	08	

118 cases with diarrhoea were tested for toxin A +B out of which, 8 cases were positive for Toxin A+B production. Positivity was more in 50-64 years age groups.

Percentage of positivity of toxin assay was 6.79%.



Graph-1: Age wise distribution of *C. difficile*Toxin A+B positive patients

DISCUSSION

*C. difficile*has emerged as the most common cause of hospital acquired diarrhoea due to broad spectrum antimicrobial use *C. difficile*is considered as the most frequent etiological agent of nosocomial diarrhoea occurring in hospitalized patients, spreading easily through the environment, the hands of health care workers and subsequently to other patients, particularly in large hospitals.[10][11]

We found that 6.78% patients suffering with diarrheoa were positive for *C. difficile* toxin production while Vaishnavi, et.al (2009) reported 19.4 % positivity for *C. difficile* toxin in adult population those on antibiotic receiving and hospitalized[11] similarly R.Katyal et. al (Chandigarh 2002) reported that 25% sample for *C. difficile* toxin were positive and all patient were on antibiotics and only 72% were present complaint of diarrhoea, which was more as compare to our study.[12]

It was reported by Vashnavi C.et. al (2011) that Most promising drug for treatment of CDAD is Vancomycin, but due to emergence of hypervirulent strains of clostridium lime North American pulsed field type-I (NAP-I), there has being increase in severity of illness, deaths and increased rates of recurrence of infection, needing retreatment.[13] in our study no *C. difficile* positive patients were found those who were using Vancomycin antibiotic.

Numerous studies have been conducted to study the prevalence of *C. difficile* and found to be associated with decrease prevalence in our country. The reasons for this may be stringent surveillance and antibiotic policy in institutions and isolation of patients having *C. difficile* infection and regular awareness programmes also help in regulating their incidence. However, future studies are always needed to study the change in pattern of colonization or infection. *C. difficile* is considered as the most frequent etiological agent of nosocomial diarrhoea occurring in hospitalized patients, spreading easily through the environment, the hands of health care workers and subsequently to other patients, particularly in large hospitals.[10] *C. difficile* has emerged as the most common cause of hospital acquired diarrhoea due to broad spectrum antimicrobial use.[11]CDAD is the most common cause of hospital acquired diarrhea in developed countries, with an incidence of 0.1-2%.[13]

The relationship between the amount of toxins in the feces and the severity of symptoms has been demonstrated. Significant increases in toxins in the fecal load are associated with the significant deterioration of the general condition of the patient [5] [9].

CONCLUSION

In our study patients suffering with diarrheoa were 6.9% positive for *C. difficile* toxin production while Vaishnavi, et.al (2009) reported 19.4 % positivity for *C. difficile* toxin in adult population those on antibiotic receiving and hospitalized[11] similarly R.Katyal et. al (Chandigarh 2002) reported that 25% sample for *C. difficile* toxin were positive and all patient were on antibiotics and only 72% were present complaint of diarrhoea, which was more as compare to our study.[12] Significant toxin load in the fecal samples may be associated with the significant deterioration of the general condition of the patient.

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