Original Research Article

Study of diagnostic methods for detection of clostridium difficile by glutamate dehydrogenase and toxin A/B combination for rapid diagnosis of antibiotic associated causes of diarrhea in pediatric age group

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Abstract

Background: Although the reports from India regarding the prevalence of C. difficile in children are scanty, the actual estimated picture of CDI in our country could be more because the detection of C. difficile requires tedious and costly anaerobic techniques. In the present study we aimed to evaluate diagnostic methods for detection of Clostridium difficile by glutamate dehydrogenase and toxin A/B combination for rapid diagnosis of antibiotic associated causes of diarrhea in pediatric age group. **Material and Methods:** Present study was single-center, descriptive observational cross-sectional study, conducted in pediatric age group patients (< 13years), hospitalized for diarrhea and receiving antibiotics for more than 5 days, underwent detection of GDH and toxin A/ B by rapid kit-based test. **Results:** Out of 120 cases, majority of the cases were in age group of 7- 9 years (31.67 %) and male to female ratio was 1.93:1. 76.67 % cases had fever; 78.33 % cases developed diarrhea; 42.5 % cases complained of pain in abdomen. 24.16% of the cases were GDH positive, 15.83% males and 8.33% females; 11.66% of the cases were toxin positive, 5.83% males and 5.83% females; 22.5% of the cases were culture positive, 14.16% males and 8.33% females. The toxin positivity rate amongst the GDH positives was 48.27%; 14 cases out of the total 29 cases of GDH positives; 36.84% in males and 70% in females; statistically significant difference between GDH positivity and toxin positivity. The toxin positivity rate amongst the culture positives was 51.85%; 14 cases out of the total 27 cases of culture positives; 41.17% in males and 70% in females; statistically significant difference between culture positivity and toxin positivity. **Conclusion:** Rapid test provide quick easy and cost effective means of accurately diagnosing CDI. The use of such test to screen both GDH and toxin A/B will allow the laboratory to detect more samples without having to test these specimens to more expensive and time-consuming test like cultures, EIA and PCR.
Keywords: Clostridium difficile, GDH, toxin A/B, rapid diagnostic tests

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INTRODUCTION

Clostridium difficile, an anaerobic gram positive spore[1] forming motile bacilli,[2] is responsible for benign, self-limited diarrhea,[3] frequently develops in hospitalized patients who are treated with antibiotic therapy.[4] It is one of the leading causes of hospital acquired infections.[2,5] The severity of the disease and the pathological finding highly varies from asymptomatic carrier to mild diarrhea to colitis depending on whether the patient has PMC (pseudomembranous colitis), AAC (Antibiotic associated

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colitis), AAD (antibiotic associated diarrhea) or is simply colonized with C. difficile or is an asymptomatic carrier.[6] Often the diarrhea subsides as the causative antibiotic is stopped or discontinued, while in other patients, the intestinal symptoms maybe more aggressive and the diarrhea persists. These patients can have antibiotic associated colitis for which 60 to 70% C. difficile is the responsible pathogen. Although, the reports from India regarding the prevalence of C. difficile in children are scanty,[7,8,9] the actual estimated picture of CDI in our country could be less because the detection of C. difficile requires tedious and costly anaerobic techniques,[5] and there are limited studies to estimate the burden of hospital acquired C. difficile infection in pediatric patients with diarrhea on antibiotic therapy.[9,10] In present study, we aimed to evaluate diagnostic methods for detection of Clostridium difficile by glutamate dehydrogenase and toxin A/B combination for rapid diagnosis of antibiotic associated causes of diarrhea in pediatric age group.

MATERIAL AND METHODS

Present study was single-center, descriptive observational cross-sectional study, conducted in Department of Microbiology, at Lokmanya Tilak Municipal Medical College & General Hospital, Sion Mumbai, India. Study duration was of 1 year. The study was initiated after obtaining approval from the institutional ethics committee.

Inclusion criteria: Pediatric age group patients (< 13years), hospitalized for diarrhea and receiving antibiotics for more than 5 days

Exclusion criteria: Immunocompromised; Abdominal tuberculosis; Diagnosed cases of inflammatory bowel diseases and other causes of diarrhea; Diarrhea associated with infective cause; Hospitalised and receiving antibiotics for less than 5 days having diarrhea.

Consent was taken from the parents/ guardians of patients to participate in the study after explaining the protocol to the relatives in the language that they best understood. A detailed history including the demographic profile, presenting complaints, past, co-morbidities and treatment was elicited for each patient and duly recorded in the case record form. Stool samples were collected in clean dry leak proof wide mouth container without disinfectants or detergent residue. Stool samples that could not be cultured within two hours of collection were sent in a transport medium and refrigerated immediately at 4^0C for a maximum of 48 hours. All samples were immediately transported to the laboratory and processed within two hours after receiving. The color and consistency of samples described whether they were semisolid or liquid. Also wet mount, Iodine mount and modified Gram's stain were performed on stool specimen. Stool samples were cultured in anaerobic workstation on Cycloseriene cefoxitin fructose agar (CCFA). Any growth observed on the CCFA was identified with smear stained by Hucker's staining procedure. On CCFA, circular, yellow, fimbriated colonies of 4mm size or larger, Gram-positive bacilli with subterminal oval spores having horse stable odour was presumptively identified as C. difficile. Confirmation of organism was done by standard biochemical tests. Detection of GDH and Toxin A/ B by rapid kit-based test for detection of GDH and toxins. Data was collected and compiled using Microsoft Excel, analyzed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

RESULTS

A total of 120 stool samples were processed for culture and rapid test for detection of GDH and Toxin A/B. Out of 120 cases, majority of the cases were in age group of 7- 9 years (31.67 %) followed by 10-12 years (30%); mean age was 7.21 years. In present study 65.83 % were males and 34.17 % were females; Male to female ratio was 1.93:1; There was male preponderance in most age group.

Table 1: Age and Gender distribution of the study population

Age in years	Male	Female	Total
1-3	17 (14.17 %)	5 (4.17 %)	22 (18.33 %)
4-6	14 (11.67 %)	7 (5.83 %)	21 (17.50 %)
7-9	22 (18.33 %)	16 (13.33 %)	38 (31.67 %)
10-12	26 (21.67 %)	10 (8.33 %)	36 (30.00 %)
13-16	0	3 (2.50 %)	3 (2.50 %)
TOTAL	**79 (65.83 %)**	**41 (34.17 %)**	**120**

In present study, 76.67 % cases had fever; 78.33 % cases developed diarrhea; 42.5 % cases complained of pain in abdomen.

Table 2: Symptoms

	Symptom Present	Percentage
Fever	92	76.67
Diarrhea	94	78.33
Pain in Abdomen	51	42.50

24.17 % of the cases were GDH positive; 15.83% males and 8.33% females; 11.66% of the cases were toxin positive; 5.83% males and 5.83% females and 22.5% of the cases were culture positive; 14.16% males and 8.33% females;

Table 3: Glutamate dehydrogenase (GDH), Toxin A/B and Culture results

	MALE	FEMALE	Total (n=120)
GDH POSITIVE	19 (15.83 %)	10 (8.33 %)	29 (24.17 %)
GDH NEGATIVE	60 (50 %)	31 (25.83 %)	91 (75.83 %)
TOXIN POSITIVE	7 (5.83 %)	7 (5.83 %)	14 (11.67 %)
TOXIN NEGATIVE	72 (60.00%)	34 (28.34 %)	106 (88.33%)
Culture POSITIVE	17 (14.17 %)	10 (8.33 %)	27 (22.50%)
Culture NEGATIVE	62 (51.67%)	31 (25.83 %)	93 (77.50%)

The toxin positivity rate amongst the GDH positives was 48.27%; 14 cases out of the total 29 cases of GDH positives; 36.84% in males and 70% in females; Statistically significant difference between GDH positivity and Toxin positivity. (P value<<0.0001)

Table 4: GDH positivity and Toxin positivity

	GDH POSITIVE	GDH NEGATIVE	P value*	Significance
TOXIN POSITIVE	14	00	<0.0001	Significant
TOXIN NEGATIVE	15	91		

The toxin positivity rate amongst the culture positives was 51.85%; 14 cases out of the total 27 cases of culture positives; 41.17% in males and 70% in females; Statistically significant difference between Culture positivity and Toxin positivity. (P value < 0.0001)

Table 5: Toxin positivity and Culture positivity

	Culture Positive	Culture Negative	P value*	Significance
TOXIN POSITIVE	14	0	< 0.0001	significant
TOXIN NEGATIVE	13	93		

Mean of duration of first appearance of symptom was 7.25 ± 3.04 days in GDH positive cases and 5.55 ± 1.84 days in GDH negative was seen and difference was not statistically significant (P=0.090). Mean of duration of first appearance of symptom was 9 ± 3.22 days in toxin positive cases and 5.55 ± 1.8 days in toxin negative was seen and difference was statistically significant (P=0.046). Mean of duration of first appearance of symptom was 7.25 ± 3.05 days in culture positive cases and 5.55 ± 1.84 days in culture negative was seen and difference was not statistically significant (P=0.090).

Table 6: GDH positivity and mean duration of first appearance of symptom (in days)

	Mean duration ± SD	P value*	Significance
GDH POSITIVE	7.25 ± 3.04	0.090	Not significant
GDH NEGATIVE	5.55 ± 1.84		
TOXIN POSITIVE	9 ± 3.22	0.046	Significant
TOXIN NEGATIVE	5.55 ± 1.80		
Culture POSITIVE	7.25 ± 3.05	0.090	Not significant
Culture NEGATIVE	5.55 ± 1.84		

DISCUSSION

Clostridium difficile is a common and important pathogen causing symptomatic diarrhea in pediatric patient on antibiotic therapy.[11,12] Illness associated with this organism ranges from mild self-limiting diarrhea to severe abdominal cramps, tenderness, distension, fever, leukocytosis, hemorrhagic colitis, toxic megacolon, perforating peritonitis, sepsis, shock and death.[12] Toxin A and Toxin B are reported to be the two main toxins responsible for the pathogenesis of CDI.[13,14] A major hindrance for the diagnosis of Clostridium difficile infection is demonstration of toxin production. The reason being slow turnaround time for isolation of the organism from stool specimen and also it is not a clinically useful diagnostic test and testing of stool specimen from asymptomatic patients will not yield any result. Rapid diagnostic tests for detection of toxin A/B for Clostridium difficile are emerging extensively to provide rapid diagnosis of antibiotic associated diarrhea.[13] In an Indian study done by Gogate A. *et al.*[8] in 250 children, maximum

number of cases were seen in the age group of 5- 8 years. This observation is similar to the present study. In a study done by Dutta P. *et al.*[15] in 111 hospitalized pediatric patients, maximum number of cases were from 1- 2 years. As per study conducted by Brown KA. *et al.*[16], there was increase in ward- level antibiotic exposure which was associated with increase in C difficile incidence as compared to intensive care units. Similar findings were noted in present study. In study by Sachu A. *et al.*[17] GDH was confirmed in 23.8% (157/660) of the study population. This was corroborated to the present study, while Dmitrieva N. *et al.*[18] noted GDH positivity rate as 38.6% (449/1164). Overall 11.66% of the cases were toxin positive (5.33% males and 5.33% females) in the present study. Also, when the patients were analyzed symptomatically, 14.89% of the patients who were having diarrhea were toxin positive (14/94). Sachu A. *et al.*[17] noted male positivity rate for toxin as 65.6% and female toxin positivity was 34.4%. This overall incidence was almost in concordance with the present study. In the study by Dmitrieva N. *et al.*[18] for the prevalence of Clostridium difficile- associated diarrhea in hospitalised patients, the overall toxin positivity rate was 21.7%. This was a bit higher than the present study. In the present study, the toxin positivity rate amongst the GDH positives was 48.27% (14 cases out of the total 29 cases of GDH positives; 36.84% in males and 70% in females). Similar study done by Sachu A.*et al.*[17] toxin positivity rate was 36. 9% (58/157) of the GDH positives, almost similar to the present study. Dmitrieva N. *et al.*[18] concluded the toxin positive rate amongst GDH positives as 56.35%, which was almost in concordance with to the present study. In the current study, 22.5% of the cases were culture positive (14.16% males and 8.33% females). Also in the culture positive group, 51.86% were toxin positive and 48.14% were toxin negative. Symptoms in the study group: In the present study 78.33% cases developed diarrhea, 76.67% cases had fever and 42.5% cases complained of pain in abdomen. Crews J. *et al.*[19] in his study on clinical characteristics of Clostridium difficile infection in children, concluded that fever was present in 37.6% children and abdominal pain in 45.9 % children. In addition, there were also the symptoms of vomiting (28. 4%), blood in stools (24. 8%). These were not found /included in the present study. The duration of the first appearance of the symptom such as diarrhea, fever and pain in abdomen was compared as per GDH positivity, toxin positivity, culture positivity and antibiotic therapy (monotherapy vs polytherapy). Yuhashi K. *et al.*[20] studied the clinical significance of toxin negativity in glutamate dehydrogenase- positive patients, and found a significant difference in mean diarrhea duration between the 39 patients in the positive stool group and in 29 patients with toxin- negative stool specimens (10.6 days vs 5.6 days; P=0.0179); which was similar to the present study. Ma H. *et al.*[21] evaluated whether combined administration of antibiotics increases the incidence of antibiotic-associated diarrhea in critically ill patients. They found that the mean duration of intensive care unit admission was longer among patients with AAD compared with patients without AAD (19.70 ± 12.16 vs 12.29 ± 8.06 days, P<0.001), with no significant difference in intensive care unit- related mortality rates. In the present study, the mean duration of hospital admission was longer among patients with AAD compared with patients without AAD In the study conducted by Nylund. C *et al.*[22] on Clostridium difficile infection in hospitalized children in the United States, the median length of hospital stay remained steady for CDI patients over the four time periods (from 1997 to 2006), at 5 to 6 days (P=0.09). This was much less than the present study. In this era of rapid change in C. difficile epidemiology, national surveillance is crucial to monitor the incidence, identify populations at risk, and characterize the molecular epidemiology of strains causing CDI. The use of such algorithm rapid tests would save institutional costs, curtail unnecessary isolation days, reduce the nosocomial transmission of disease, and increase the quality of care for patients hereby decreasing the morbidity and mortality in hospitalized pediatric population.

CONCLUSION

Rapid test provide quick easy and cost effective means of accurately diagnosing CDI. The use of such test to screen both GDH and toxin A/B will allow the laboratory to detect more samples without having to test these specimens to more expensive and time consuming test like cultures, EIA and PCR.

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