Section A-Research paper

AN INVESTIGATION OF THE CAUSES OF HOSPITAL-ACQUIRED DIARRHEA IN CHILDREN FROM A TERTIARY CARE FACILITY IN SOUTH INDIA

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ABSTRACT

Introduction: Hospital-acquired diarrhea, which affects roughly 2-32% of patients hospitalized to pediatric wards, is a serious risk, particularly for young patients. In underdeveloped nations, diarrhea affects children under the age of three on average three times per year. Diarrhea adds to the lack of important nourishment, which is essential for a child's growth, on each and every episode. The primary goal is to investigate the causes and prevalence of pediatric hospital acquired diarrhea.

Materials and techniques: A prospective clinical study was piloted at a tertiary care center at south India. Children of <15 years admitted to the medical reasons and had no complain of diarrhoea at the admission but later developed diarrhoea, were included to the study. Each patient's complete medical history was recorded, together with any laboratory tests. Stool samples were analysed for the presence of viral, bacterial, parasitic, and fungal organisms was determined using a number of diagnostic laboratory procedures. Toxins produced by Clostridium difficile were detected using an ELISA, and the human rotavirus antigen was found using a latex agglutination test. There was no statistical significance for the distribution of the micro-organisms and the age of the children (p=.671).

Results: Of the 121 hospitalized children between the ages of 1 and 15 all had diarrhoea for at least three days after the admission for other medical conditions. No significant variance observed between the two sexes (p=.785). Majority of the subjects were from the age groups below 8 years. There was a statistical variance between the subjects for age distribution (p=.001). Enteropathogenic Escherichia coli was in over one fourth of the included subjects (25.61%).

Conclusions: In this investigation, infectious agents, especially bacteria, caused more hospital-acquired diarrhea cases than non-infectious ones. This study proposes routine general stool inspection, stool culture/sensitivity for microbial infection for pathogens like

E.coli, and Cl. difficile detection by ELISA for all patients to diagnose hospital-acquired diarrhea. Healthcare programs must target hospital workers and community.

Key words: Diarrhoea, Hospital Acquired Infections, Children, Nosocomial, Escherichia Coli

INTRODUCTION

Acute diarrhea with at least three loose stools for more than 12 hours, nausea, or a fever of more than 38 degrees Celsius, and no apparent non-infectious etiology is considered hospital-acquired diarrhea.[1] In India, hospital-acquired diarrhea is a serious health concern. According to recent data from Maharashtra and Rajasthan, over 80% of hospitalized children have diarrhea.[2-4] So, it's crucial to assess the aetiology of pediatric hospital-acquired diarrhea, especially the microbiological profile. The majority of research on hospital-acquired diarrhea has been done in Western nations, with an emphasis on finding Clostridium difficile in stool samples.[5-7] Low- and middle-income countries are expected to have diverse bacterial profiles for hospital acquired diarrhea. There is a dearth of recent research on the microbiological profile of hospital acquired pediatric diarrhoea in LMIC settings despite the fact that it is rather common. Hospital-acquired diarrhea, which affects roughly 2-32% of patients hospitalized to pediatric wards, is a serious risk, particularly for children.[7,8] Diarrheal sickness is the most prevalent illness that kills people and causes serious illness worldwide, primarily in developing nations and especially in cases of children. According to the WHO, there are 1.7 billion instances of diarrheal sickness each year, and 525,000 children under the age of five die from them worldwide.[9] In underdeveloped nations, diarrhea affects children under the age of three on average three times per year. Every time there is diarrhea, there is a deficiency in important nutrition, which is essential for a child's growth. Continuing use of broad-spectrum antibiotics can disrupt normal colonic flora in industrialized nations, which can lead to Clostridium difficile colonization and be a prevalent cause of newly developing diarrhea in hospitalized patients.[10,11] Patients who stay in hospitals run the risk of picking up diseases from infected patients, hospital equipment that is contaminated, or healthcare personnel as carriers. At low-income hospitals, infection control is frequently laxer, which increases the risk. Age, length of hospitalization, immunological condition, nutritional status, and exposure to gastrointestinal procedures including endoscopy and nasogastric intubation are host-related risk factors for hospital-acquired diarrhea, according to research from developed and developing nations.[12-15] In hospitals and nursing homes, the prevention and spreading of hospital-acquired diarrhea caused by tainted food is of primary importance due to frequently dangerous methods of food handling, preparation, and storage being used by inexperienced workers. Additionally, preventing outbreaks of waterborne infectious diarrhea is a persistent problem due to insufficient resources and hazardous drinking water quality. The major goal is to describe the proportion of distribution of common pathogens in order to understand the etiology and prevalence of hospital-acquired diarrhea in children.

MATERIAL AND METHODS

Study design

A prospective clinical study was piloted at the pediatric department and the microbiology department, at a tertiary care center at south India. The ethical clearance was obtained for the study. Guardians of the included children were explained of the study's design in their local language and only after they understood the consent was taken.

Subjects

In total, 121 children of <15 years admitted to the medical reasons and had no complain of diarrhoea at the admission but later developed diarrhoea, were included to the study. The study excluded children who were given purgatives, those who underwent enema, gastrointestinal operations, or endoscopies, as well as those who had known underlying chronic gastrointestinal illnesses such celiac disease, inflammatory bowel disease, or chronic pancreatitis.

Methodology

The "*Centers for Disease Control and Prevention (CDC)"* definition of hospital diarrhea was used for the diagnosis of hospital acquired diarrhea in the hospitalized patients.[16] Each patient's complete medical history was recorded, together with any laboratory tests. Stool samples were taken from the cases and put in a wide-mouth container as part of the sample collection and study protocols. Based on past evidence that revealed the role of Escherichia coli, Shigella, Salmonella, Rotavirus, Cryptosporidium, and C. difficile in the cause of hospital-acquired diarrhea the bacteria included for testing of stool samples in the current study.[1,2] The presence of viral, bacterial, parasitic, and fungal organisms was determined using a number of diagnostic laboratory procedures. Human rotavirus antigen was detected using a latex agglutination test, while Clostridium difficile toxins were detected using an *"enzyme-linked immunosorbent assay (ELISA)".* Finding ova and cysts: A portion of the sample was examined under a microscope while the feces was being examined in saline to check for the presence of pus cells, red blood cells, ova, and cysts. To find Cryptosporidium oocysts and other coccidian parasites, Kinyoun's acid-fast staining was used. The oocysts of Cryptosporidium were found to be round to oval, 4-6 m in diameter, and to have a tiny vacuole-like structure. By studying a saline/iodine wet mount of a stool sample under magnifications of 10 and 40, fecal ova and cysts of other pathogens were recognized based on their shape.

C. difficile toxin detection: Using the Premier Toxins A and B kit, the second portion of the stool was kept at 20°C for C. difficile toxin detection (Meridian Bioscience, Inc., USA). For the detection of C. difficile toxin A and toxin B, an enzyme immunoassay is used. Monoclonal and polyclonal antibodies that are specific to toxins were used to coat breakaway microwells. Horseradish peroxidase (HRP)-conjugated antitoxin A and B polyclonal antibodies were applied to microwells along with diluted samples. A substrate/chromogen (peroxide and tetramethylbenzidine) was applied to the wells after the washing steps.

According to the manufacturer's instructions, a microplate reader (Molecular Devices, CA, USA) was used for visual evaluation and spectrophotometric measurement.

Rotavirus detection: According to the manufacturer's guidance, the EpiTuub faecal rotavirus antigen detection quick diagnostic test kit (Epitope Diagnostics, USA) has a sensitivity of 97.1% and a specificity of 98.5%. It uses solid-phase specific rotavirus antibodies as well as dye-conjugated monoclonal antibodies directed against group A rotavirus antigen VP6. To remove rotavirus antigen from the specimen's stool, an extraction solution was applied first. After that, the test strip was inserted into the sampler. The specific antibodies located on the membrane caught the colored particles as they were carried by the extracted sample as it moved through the chamber and onto the test strip. Depending on how much virus was present in the sample, various colored lines appeared. These lines were read using the manufacturer's comparator after 5 min of room temperature incubation.

Shigella, Salmonella, and E. coli were found after processing the third portion of the feces for bacterial culture. Using a battery of media (MacConkey agar, xylose lysine deoxycholate agar, and Selenite-F broth), enteric pathogens were cultured from stool samples and identified using standard laboratory procedures10. Traditional biochemical tests for E. coli were used to identify lactose-fermenting colonies from MacConkey agar. Shigella and Salmonellae spp. were recognized by the fact that their colonies did not ferment lactose, which prevented MacConkey agar from changing color.

Patient care: In accordance with the standard protocols for the treatment of acute diarrhea, these patients were handled with oral rehydration solution and zinc supplementation. Throughout the hospital stay, the clinical course of the diarrhoea was observed, and patients were released once the underlying sickness and the diarrhoea had subsided (2 loose stools in 48 hours). A 72-hour (telephone) post-discharge follow-up was conducted to check for symptom recurrence.

Sample size: In a previous investigation,[1,2] one of the pathogens (rotavirus, C. difficile, or diarrheagenic E. coli) was found in 66% of cases of nosocomial diarrhea in children. The number of children with hospital-acquired diarrhea in whom any of these pathogens could be detected with a 95% confidence level and a 10% absolute precision was estimated to be 87, which is an adequate sample size. A total of 121 kids with hospital-acquired diarrhea were enrolled in order to cover any sample losses that might occur during storage and processing.

Statistical analysis

Data were entered into a Microsoft Excel worksheet, and IBM SPSS Statistics for Windows Version 23.0 was used to conduct the statistical analyses (IBM Corp., Armonk, NY, USA) Results from were presented as percentages and was analysed using the chisaquara test with the significance at lower than 95%.

RESULTS

A total of 121 subjects were included in the study. Of these total 121 male children were 62(51.23%) slightly greater than that of the female children. However there was no significant variance observed between the two sexes (p=.785).when the age was considered the majority of the subjects were from the age groups below 8 years. There was a statistical variance between the subjects for age distribution (p=.001) **Table 1**

Table 1: Gender and age distribution

Gender	**N**	**(%)**	**P**
Male	62	51.23	Chi-squared: 0.0744
Female	59	48.6	DF: 1
Total	121	100	P = 0.7851
Age distribution			
1-4	41	33.88	Chi-squared: 16.2432
4-8	35	28.92	DF: 3
8-12	20	16.52	**P = 0.0010**
12-16	15	12.39	
Total	121	100	

When the pathogen distribution examined it was noted that Enteropathogenic Escherichia coli was in over one fourth of the included subjects (25.61%). All the other pathogens were almost similarly distributed with the next common infections with Clostridium difficile, Entamoeba histolytica. There was no statistical significance for the distribution of the micro-organisms and the age of the children (p=.671). **Table 2**

Table 2: Distribution of the various pathogens compared to that of age

Infectious agent	**AGE**					**%**	**P**
	1-4	**4-8**	**8-12**	**12-16**	**total**		**0.6719**
Candida albicans	3	1	0	0	3	2.4	
Clostridium difficile	5	4	4	1	14	11.5	
Cryptosporidium parvum	3	2	3	1	9	7.4	
Entamoeba histolytica	8	4	4	2	18	14.8	
Enteropathogenic Escherichia coli	10	10	6	5	31	25.61	
Giardia lamblia	1	0	1	1	3	2.4	
Klebsiella oxytoca	2	2	2	2	8	6.6	
Proteus mirabilis	2	2	2	2	8	6.6	
Pseudomonas aeruginosa	3	2	3	1	9	7.4	

Rotavirus	5	3	3	1	12	9.9	
Salmonella enteritidis	3	1	0	0	4	3.3	
Shigella flexneri	1	0	1	0	2	2.4	
Total	46	31	29	16	121	100	

DISCUSSION

Hospital-acquired diarrhea has been linked to a number of different variables. The supplies of food and drink during hospitalization and interaction with contaminated insects are examples of external variables. Internal variables include enema, endoscopy, and placement of a nasogastric tube.[1] Hospital-acquired acute diarrhea is identified by its site and onset time after 72 hours. The 3-day cut-off removes community-acquired bacteria that remain latent in the body without clinical signs.[16] According to a study from northern Brazil, hospital acquired diarrhea affects children at a rate of about 40%, which is lower than this study's finding because it documents a higher prevalence.[17] In contrast, a study from India found that hospitalized children under the age of 36 months had a 20% prevalence of the condition.[2,18] This disparity in occurrence may be due to a variety of factors, including local endemic bacteria, hospital cleanliness standards, and hygiene habits. When patients are admitted to the hospital and remain there for a longer amount of time without receiving normal infection control, the likelihood that they may come into contact with possible pathogens in hospital settings and develop new cases of diarrhea may increase. Also, a risk factor for patients is the fact that many caregivers in hospitals experienced diarrhea while providing care to ill patients.[19,20] This study demonstrated a higher incidence of cases in children under the age of five, and this prevalence was consistent with research from Saudi Arabia and Iraq.[21,22] Several studies conducted in India have revealed that infants have a significant frequency of diarrhea.[1] Several investigations revealed that the gastrointestinal system occurs more frequently in general pediatric service.[23] This study's findings, which are similar to those of Jagrwal et al.,[2] who found that ~20% of instances of infectious diarrhea were caused by Enteropathogenic Escherichia coli, suggest that rotavirus is the most prevalent viral agent responsible for infectious diarrhea in children. Clostridium difficile was reported as 21% in a study from Turkey by Oguz F et al.,[24] which is comparable to this study. No clinical evidence of Vibrio cholerae-caused cholera or diarrhea with food poisoning caused by Staphylococcus aureus was detected during the study period. Other bacteria that are occasionally involved with hospital acquired diarrhea in children, such as Campylobacter jejuna and Yersinia enterocolitica, were also not discovered. This is similar to the finding of Jagrwal et al.[2]

Previous use of antibiotic medication may be linked to conditions such C. difficile infection (CDI), fungal overgrowth syndromes, notably Candida, and antibiotic-associated diarrhea (AAD). AAD and bacterial overgrowth typically go away when medicines are stopped, while CDI requires focused therapy. The persistence of resistant strains of gut microorganisms

under selection pressure with horizontal transfer of resistance genes has also been linked to long-term usage of broad-spectrum antibiotics.[25-29]

Strength and limitations

Current study's primary strength was its prospective design, in which patients were admitted to wards before experiencing diarrhoea. 7.4% of the cases had cryptosporidium found by microscopy. The samples might have shown higher detection rates had the Cryptosporidium antigen been tested. The 12 positive rotavirus cases did not undergo molecular typing to determine the pathogenic virus type. Moreover, no tests were performed to check for additional viruses including astroviruses, caliciviruses, noroviruses, or adenoviruses. Another drawback was the lack of a control group for stool test results, which would have allowed us to establish a link between diarrhea and the presence of the necessary organism in the stool. It was also difficult to assess the gut microbiome's response to medications since stool samples from hospital-acquired diarrhea cases were not analyzed before antibiotic administration. Last but not least, despite the fact that this study offers specifics on the aetiological agents of hospital-acquired diarrhea in children, the physician may not find much use for this knowledge without clinical details analysis.

CONCLUSION

Overall, the study's findings indicate that the aetiology of diarrhea during hospitalization is comparable to that of studies done on people who had diarrhea from a community source. Therefore, in similar hospital settings, similar interventions ought to be applicable for the management and prevention of hospital-acquired diarrhea. In impoverished nations like India, diarrhea in children is a serious issue. This study also showed that hospital-acquired diarrhea. This study proposes routine general stool inspection, stool culture/sensitivity for microbial infection for pathogens like E.coli, and Cl. difficile detection by ELISA for all patients to diagnose hospital-acquired diarrhea. As a result, healthcare strategies and campaigns need to concentrate on a particular hospital personnel and community region.

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