Incidence of Rotavirus and Efficacy of Enterococcus Faecium SF68 in Infantile Diarrhea

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Abstract

Objective: To know the incidence of diarrhea due to Rotavirus in infants and to evaluate the efficacy of probiotic E. faecium SF68 in acute infantile diarrhea.

Material and Methods: It was a randomized controlled clinical trial Reg. No 01/159/16 conducted in Children Hospital Lahore. 70 infants suffering from acute diarrhea were divided randomly into two groups. Infants of control group received routine treatment for diarrhea while in infants of experimental group also received probiotic Enterococcus faecium SF68 in addition to routine treatment for diarrhea. Stool samples of all infants were sent for Rotavirus testing.

Results: Stool samples of 19 out of 70 infants were positive for Rotavirus. Probiotic decreased frequency of diarrhea in infants of experimental group.

Conclusion: In this trial, 27% infants were suffering from acute Rotavirus gastroenteritis. Enterococcus faecium SF68 decreased the severity of diarrhea in infants.

Keywords: Infants, Rotavirus diarrhea, Probiotics, Enterococcus faecium Sf68.

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Introduction

Diarrhea is the significant health problem worldwide leading to hospitalization and death in infants and young children in third world countries. According to WHO, diarrhea is the fecal discharge having frequent and watery stool which occurs due to infection of small bowel resulting in loss of electrolytes and fluids.[1] Worldwide, 525,000 children below five years of age die because of diarrheal disease annually. This shows 8% of all deaths, so diarrhea is the important reason of mortality in young children.[2] Most cases of diarrhoeal diseases do not require laboratory investigation as they are diagnosed clinically. Presentation having acute watery and non-bloody diarrhoea shows a viral aetiology, whereas diarrhoea having blood in stool and high-grade fever shows bacterial infection.[3] Rotavirus and E. coli are one of the main causative agents of diarrhea in young children in developing countries. Globally, Rotavirus being main cause of diarrhea in infants leads to significant mortality in children below five years. In addition to E. coli and Rotavirus, other enteropathogens which cause diarrhea in young children include Campylobacter, Vibrio chloera, Salmonella and Shigella species[4]. Rotavirus was recorded to be responsible for about 215,000 deaths in children under five years of age in 2013. Due to high mortality in children, vaccination for Rotavirus is important public health strategy.[5] The focus for treatment of diarrhea is prevention as well as hydration therapy and to promote good nutritional status.

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.Children having mild dehydration should be given oral rehydration salts (ORS) and those who are not able to take fluids or children suffering from severe diarrhea should be given intravenous (IV) fluids. Children suffering from acute diarrhea should also be given zinc supplements.[6] Probiotics are microorganisms which are beneficial to the host by colonizing in the human body. They promote reproduction and growth of beneficial intestinal flora and provide immunity against pathogenic bacteria.[7] Enterococcus faecium SF68 is a probiotic initially launched in Austria and Switzerland. It is indicated to be used in acute gastroenteritis in adults as well as in children and for treatment of diarrhea associated with antibiotics. However, previous trials already done on SF68 were not of good standard having poor allocation concealment and even no blinding in few trials. Analysis on the role of SF68 in diarrhea especially in infants cannot be made.[8]

Materials and Methods

The study was done in Children Hospital Lahore from July 2016 to October 2016. The study was conducted according to the principles of World Medical Association. The trial was reviewed and approved by Ethical Committee of Children Hospital Lahore. After taking consent from the parents, infants were enrolled in the study. It was a randomized controlled Reg. No 01/159/16, single blinded, clinical trial and study followed Consort guidelines. The age of infants in the study was selected from six months to twelve months. The infants having acute diarrhea were enrolled in the trial. According to WHO, "Acute diarrhea is defined as the passage of three or more loose or liquid stools per day and not lasting more than 14 days." The infants suffering from typhoid fever, chronic disease or having bloody diarrhea were excluded from the study. In this study, non-probability purposive sampling was done. The sample size was calculated according to previous study[9]. In previous study, sample size was 32. We enrolled 35 infants in each group. The infants were divided in to two groups according to randomization technique. The random number list was generated by the computer. The randomization was done by a person who was not associated with the study. The person who did randomization also prepared the sealed envelopes which contained the management plan of the infants. The parents of infants received sealed envelopes in accordance with randomization. The infants were put in control and experimental groups after opening of envelopes, so management plan was concealed from researcher until beginning of intervention. After enrollment, stool sample was sent for microbiological testing of Rotavirus. Total 70 infants were enrolled in the study and each group had 35 infants. Infants of control group or group A were given routine or standard management for diarrhea. Standard treatment of diarrhea included ORS, Zn supplements and IV fluids in case of severe dehydration. Infants of experimental group or group B received probiotic E. faecium SF68 in addition to standard treatment for diarrhea. E. faecium SF68 was in capsule form. The contents of capsule were mixed with water for feeding. The probiotic was given twice daily for five days. Feeding was continued in infants of both groups with soft diet. Frequency of stool was recorded in both groups for five days. As Rotavirus is the important causative agent of diarrhea in infants, so stool samples were sent for testing of Rotavirus. Stool samples were stored at 2 to 24 8 C° in lab grade refrigerator. ProSpecT ROTA VIRUS KIT (OXOID) was used for detection of Rotavirus antigen. Sample was processed as follows:

1. Dilution of sample was done with 1 ml of diluent adding to 2-3 drops of liquid stool or pea size stool in case of semiliquid stool in a glass tube.
2. It was mixed well in gyromixer. Sample was kept for 10 min in test tube stand.
3. Sample was the centrifuged at 400 rpm for 5 min & Supernatant was taken.
4. Then we performed the Eliza test.

The data was processed by SPSS 20. Stool frequency was presented in mean and SD. Qualitative variables were shown as proportions and frequencies. For analyzing the significance between two groups ANOVA was used. For pairwise comparison among two groups, post hoc Tukey's test was applied.

Results

This trial was carried out in Children Hospital Lahore. All the infants enrolled in the study had moderate to severe dehydration and they required IV fluids. Clinical findings related to dehydration and some other important findings are shown in **Table-1**. Samples of stool were taken from all infants and sent for Rotavirus detection. Out of 70 samples, 19 were found to be positive for Rotavirus. The number of samples positive for Rotavirus in each group was shown in **Table-2**. Diarrheal frequency was noted by observing number of stools infants were passing in a day. It was observed for

Table 1: *Clinical findings in control group and experimental group*

Signs	Group A (control)	Group B (SF68)
Cold clammy skin n (%)	17 (49%)	12 (34%)
Dry mucous membrane n (%)	33 (94%)	34 (97%)
No tears n (%)	12 (34%)	7 (20%)
Pallor n (%)	21 (60%)	11 (31%)
Throat congestion n (%)	1 (2.9%)	0 (0%)
Oral thrush n (%)	1 (2.9%)	0 (0%)
Enlarged tonsilsn (%)	1 (2.9%)	0 (0%)

Table 2: *Number of stool samples positive for Rotavirus in group A and group B*

Groups	Stool for *Rotavirus*	
	Positive	Negative
Group A (control) n (%)	10 (29%)	25 (71%)
Group B (SF68) n (%)	9 (26%)	26 (74%)

period of five days. **Table-3** shows the mean number of stools ± SD of control group and experimental group. ANOVA showed diffe-rence between group means was significant from day 1 to day 5.

Discussion

The study was carried out in Children Hospital Lahore. The age group of infants in the study was from 6 months to 12 months. In infants, Rotavirus is an important causative agent of diarrhea having significant global impact on childhood hospitalization and death[10]. In Africa and Asia Rotavirus is responsible for 49% of all deaths due to diarrhea. The incidence of diarrhea due to Rotavirus is similar in poor and developed countries but the morbidities are higher in low-income versus rich population. This may occur due to lack of health facilities as well as presence of other conditions like malnutrition[11]. In this study, 70 infants were enrolled and randomly divided into two groups. Stool samples of all infants were sent for microbiological testing of Rotavirus. Out of 70 samples of stool, 19 were found to be positive for Rotavirus. In this trial 27% infants were suffering from Rotavirus. Stool samples of 10 infants of group A (29%) and 9 infants of group B (26%) were found to be Rotavirus positive. All the infants had moderate to severe dehydration and IV fluids were given to them. Rotavirus surveillance which was conducted during 2015-2016, analyzed 3446 children less than five years hospitalized due to diarrheal illness and found 802 (23.2%) positive on ELISA[12]. Previous study suggested that prevalence of Rotavirus decreased with increase in age. This is due to the fact that infants have higher exposure to contaminated materials as they put everything into their mouths.[5] Standard management of diarrhea was given to the infants of group A and infants of group B were also provided with probiotics in addition to standard management of diarrhea. Stool frequency was recorded in both groups for five days. Probiotics are supplements or food that contain beneficial microbes like yeast and bacteria which colonize the small intestine and provide health benefits. Whether the probiotic bacteria can be used in the management of intestinal disorders, the various researches in this regard are still going on.[13] Their use is encouraged to support healthy gastrointestinal system and to boost the immune system. Currently, the use of probiotics via different food products is in great demand. Probiotics are known as functional food. Functional food will be defined as food which resembles traditional food but they have known physiological benefits[14]. Previous studies explored therapeutic effect of probiotics on acute diarrhea as they helped in decreasing stool frequency and duration of diarrhea. However, good quality researches are still required in clinical settings to evaluate role of probitics[7]. Enterococcus faecium SF68 is a member of lactic acid bacteria (LAB), which is used as a probiotic in food supplements and pharmaceutical preparations in animals and humans[15]. Previous trials regarding use of E. faecium SF68 in humans are not sufficient. One study recently published showed beneficial effect of E. faecium SF68 in reducing the duration of diarrhea and length of hospital stay in infants[16]. In one trial E. faecium SF68 decreased the duration of diarrhea in non-Rotavirus cases in infants, however E. faecium SF68 probiotic did not significantly decrease duration of diarr-

Table 3: *Number of stools after treatment in control group and experimental group*

Groups	Day 1	Day 2	Day 3	Day 4	Day 5	p-value
Group A(control) n=35	10.89±2.94	9.17±2.93	7.46±3.16	6.26±3.00	4.63±2.50	
Group B(SF68) n=35	7.97±3.11	5.03±3.08	3.66±2.66	2.86±2.53	2.26±2.34	<0.001

hea in Rotavirus positive cases in infants[17].

In this clinical trial, infants of group B had reduced stool output than infants of group A having p-value less than 0.001 from day 1 to day 5. Stool frequency was reduced significantly in infants of group B. Infants in group B received E. faecium SF68 twice daily for five days. E. faecium SF68 capsule contains 75 million live micro-organisms and its contents were mixed with water for feeding the infants. In animal studies E. faecium SF68 promoted expression of pro-inflammatory cytokines like TNF-α, IL-17A, IL-22 and thus improved immune responses against pathogens. In one study, E. faecium SF68 increased production of NO by phagocytic cells of pigs thus it modulates innate immunity against pathogens[18]. It can be considered as a good probiotic due to its immunomodulatory effects.

Conclusion

In this randomized controlled trial, 27% of infants were suffering from Rotavirus gastroenteritis. Probiotic E. faecium SF68 reduced the severity of acute diarrhea in infants.

Conflict of interest : None

Funding Source: None

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Authors Contribution

FAK: Conceptualization of Project
ZI: Data Collection
FYB: Literature Search
NY: Statistical Analysis
FP: Drafting, Revision
STZ: Writing of Manuscript