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Efficacy and Safety of *Saccharomyces boulardii* in Acute Rotavirus Diarrhea: Double Blind Randomized Controlled Trial from a Developing Country

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ABSTRACT

Objective: To study the efficacy and safety of *Saccharomyces boulardii* (SB) in acute childhood rotavirus diarrhea.

Methods: Children (3 months to 5 years) with WHO-defined acute watery diarrhea and stool rotavirus positive ($n = 60$) were randomized into intervention ($n = 30$) and control ($n = 30$) groups. The intervention group received SB (500 mg/day) for 5 days.

Results: The median duration (hours) of diarrhea was significantly shorter in the intervention group (60 vs. 89; 95% CI: −41.2 to − 16.8). A significantly shorter duration of hospitalization (74 vs. 91; 95% CI: −33.46 to − 0.54) was also seen in the intervention group, but no significant difference was seen for fever and vomiting. There was also no difference between the two groups in the proportion of children requiring parenteral rehydration and persistence of diarrhea lasting beyond day 7. There was no report of any adverse events.

Conclusions: The present trial showed that SB is effective and safe in acute rotavirus diarrhea.

KEYWORDS: Reoviridae, acute gastroenteritis, probiotics, pediatric.

INTRODUCTION

Acute diarrhea kills more than 1.5 million children under 5 years of age every year globally, and is the second most common cause of death in this age-group [1]. Mostly acute diarrhea in children is of viral origin, and the commonest agent being rotavirus, worldwide [1]. Epidemiological studies have shown that nearly all children under five suffer at least one rotavirus infection, irrespective of the socio-economic status [2]. Globally, rotavirus causes approximately 600 000 deaths in children per year, around 80% of which occur in developing countries [1]. In India, annually, rotavirus causes an estimated 122 000–153 000 deaths, 457 000–884 000 hospitalizations and 2 million outpatient visits in children under five. India spends Rs. 2.0–3.4 billion (US$41–72 million) annually in medical costs to treat rotavirus diarrhea [3].

The mainstay of treatment of an acute rotavirus diarrhea episode includes oral rehydration therapy (ORT) and zinc [4]. ORT aims to prevent or reverse dehydration, and has no effect either on the

duration of diarrhea or on the stool output. Zinc is not universally effective in the treatment of acute diarrhea, and has been used mainly in developing country settings [5]. For this reason, various other medications (e.g., loperamide, diosmectite) have been studied in the treatment of acute childhood diarrhea, and the effects are neither consistent nor supported by the evidence [6, 7].

"Probiotics" are live microorganisms, which when administered in adequate amounts confer a health benefit on the host [8]. They have been studied in many pediatric diseases including acute childhood diarrhea [9, 10]. There are different probiotic strains available in the market, but the efficacy and safety of *Lactobacillus GG* and *Saccharomyces boulardii* has been consistently established in acute childhood diarrhea [11]. *Saccharomyces boulardii* (SB) is a non-pathogenic yeast that have demonstrated an anti-inflammatory, anti-microbial, enzymatic, metabolic and anti-toxin activity including trophic effect by enhancing the metabolic function of the gut mucosa [12]. Few studies from developed countries have demonstrated the therapeutic efficacy and safety of SB in acute rotavirus diarrhea [13–15]. However, there is no published study from developing country including India specifically assessing the therapeutic efficacy and safety of SB in acute rotavirus diarrhea. Hence, the present study aimed at evaluating SB as a possible therapeutic intervention in acute rotavirus diarrhea in Indian children.

MATERIALS AND METHODS

This double-blind, randomized controlled trial was conducted in the Department of Pediatrics at Tata Main Hospital, Jamshedpur, India, from November 2007 to March 2009. Children with acute diarrhea of $<$48 h duration, moderate to severe dehydration and aged between 3 months to 5 years were eligible for inclusion. Diarrhea was defined as passage of $\geq$3 unformed or loose stools in the last 24 h [4]. Children with severe malnutrition (weight for height $<$3SD of WHO growth chart), co-existing systemic illnesses and chronic diseases, taking probiotics including in the preceding one week, taking antibiotics for current episode of diarrhea and history of receiving rotavirus vaccine were excluded from the study.

Methodology

Acute diarrhea cases with positive stool rotavirus antigen test were included. They were randomized to receive either SB (intervention group) or no SB (control group). Sequence was generated by a person not directly involved in execution of the study. Allocation concealment was done using serially numbered sealed opaque envelopes. The study protocol was approved by the hospital ethical committee. Informed written consent was obtained from the parents of children enrolled in the study.

On admission to the study, data about clinical history, physical examination, nutritional status, hydration status (as per WHO guidelines), fever, oral tolerance and stools characteristics were recorded on a predesigned *pro forma*. Cases were managed as per the WHO guidelines for management of acute diarrhea [4]. After the patient clinically stabilized and maintained hydration, children were randomized into two groups: Intervention group received SB and control group received a similar product, both being administered twice daily for 5 days. Placebo and probiotic products had similar color and taste. SB is available in a lyophilized powdered form, and a single product (Econorm, Dr Reddy's Laboratories) in sachets of 250 mg was used throughout the study. Mothers were advised to mix it in 15 ml of normal drinking water and to give orally at one go or in small aliquots [to smaller babies] within 1 h of preparation. Mothers were advised not to mix the SB with warm or hot fluids and to avoid any warm/hot food for 2 h of giving the probiotics. The children remained in hospital till improvement in their clinical condition, and after discharge were followed till 7 days. They were monitored for number of loose stools, consistency of stool and time since last loose stool (every 4 hourly per 24 h). They were also monitored for adverse events (fever, vomiting, pain abdomen, need for admission and any other new symptom).

Rotavirus testing

Maintaining adequate sterilization, in two disposable vials, stool samples were collected from all cases. One vial was sent to microbiology lab for routine microscopy. The other sample was used for rotavirus antigen testing by Enzyme immunoassay (EIA). When a time lag was anticipated, the samples were stored at 2 to 4 °C, maximum up to 6 h. '*Premier Rotaclone*' EIA kit

(Meridian Biosciences, USA) was used. Procedure as described in manual for the test kit was followed meticulously. Readings were taken by observing color changes. The positive control was blue in color. Test results deeper than the positive control were considered positive. Negative control was colorless. Test results, which were colorless or fainter in comparison to the positive control, were taken as negative (Fig. 1).

Sample size

This was calculated using the data published by Grandy, *et al.* [13], who described the effect of SB in children with acute rotavirus diarrhea and found that diarrhea decreased from a median duration of 85 to 58 h; using 80% power, 0.05 (one-sided) significance, and assuming 24-h difference between the intervention and control groups, the sample size was calculated as 30 cases per group.

Outcome measures

Primary:

1. Duration (in h) of acute diarrhea

Secondary:

1. Duration (in h) of vomiting
2. Duration (in h) of fever
3. Duration (in h) of hospitalization
4. Proportion of children requiring parenteral rehydration
5. Proportion of children having diarrhea lasting beyond day 7
6. Any adverse effects

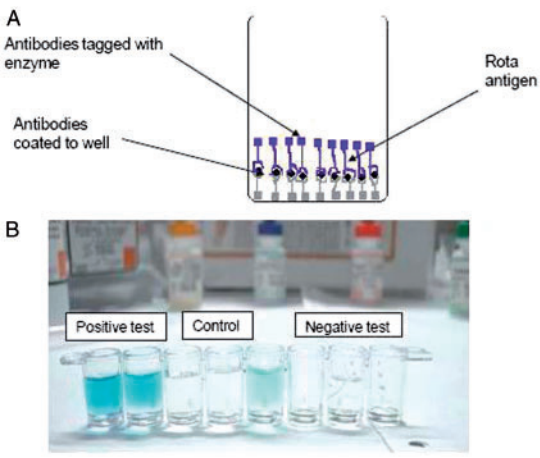

Fig. 1. Figure depicting rotavirus antigen testing by EIA in stool sample. A. Tagging of rotavirus antigen to antibodies coated to the well. B. Test results (dark one positive, light one negative).

Duration of acute diarrhea was defined as the time (in hours) from the first to the last abnormal (loose or liquid) stools preceding normal stool return. The duration was judged based on the number of stools per day and their consistency. Consistency was evaluated through a scoring system, as described by Guarino, *et al.* [16], and stool was graded as 1 (normal), 2 (loose), 3 (semiliquid) and 4 (liquid). This evaluation was done every 4 h till discharge. Duration of vomiting was measured in hours from admission to the last episode. Duration of fever was measured in hours from admission to the last episode. Duration of hospitalization was defined as the time (in hours) from admission to discharge. The discharge decision was taken by the attending physician after taking into account of the clinical condition of the child based on the stool consistency and number per day, vomiting or fever. Number of children requiring parenteral rehydration was those who required parentral rehydration after initial stabilization and randomization. Change in body weight (in gm) was calculated from admission and discharge weight. Adverse effects of the probiotic strain (if any) were also studied.

Statistical analysis

All the data were entered into the Microsoft excel sheet. The data were analyzed using SPSS software (version 20.0 Chicago, IL, USA). Statistical tests used for comparison included Chi-Square and the Mann–Whitney U. The difference between the two medians and calculation of 95% CI (confidence interval) was done by the method proposed by Bonett and Price [17]. Because many of the continuous variables were not normally distributed, when a Mann–Whitney U was used to compare the groups, the medians and interquartile ranges (IQR) were presented. Intention to treat analysis was used for the primary outcome. *P*-value <0.05 was taken as significant.

RESULTS

Out of 115 children, 60 were found eligible and enrolled in the study, 58 (96.6%) completed the 7 days follow-up (Fig. 2). One child discontinued the intervention after discharge on day 3 in the intervention group, and two children did not come for follow-up in the control group. The baseline characteristics in the two groups were comparable in age, sex and anthropometry. Demographic status and characteristics of acute diarrhea between the two groups was comparable (Table 1).

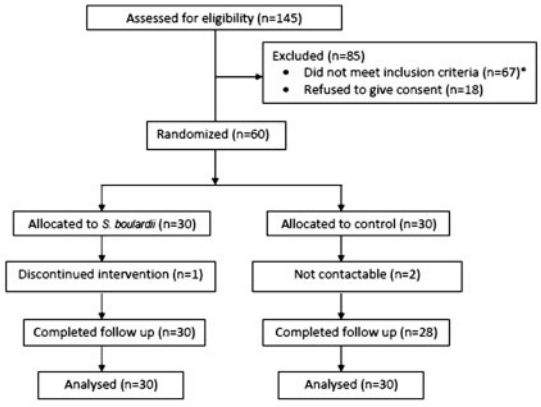

Fig. 2. Flow of participants in the study.

Primary outcome measures

In the *SB* group, the median duration of diarrhea was significantly lesser by about 29 h [95% CI: −41.2 to −16.8] compared to the control group (Table 2).

Secondary outcome measures

Effect on duration of hospitalization: In the *SB* group, the median duration of diarrhea was significantly lesser by about 17 h [−33.46 to −0.54] compared to the control group (Table 2).

Effect on fever: In the *SB* group, the median duration of diarrhea was lesser by about 11 h [−23.04 to 1.04] compared to the control group, but the difference was not significant (Table 2).

Effect on vomiting: In the *SB* group, the median duration of diarrhea was lesser by about 7 h [−16.41 to 2.41] compared to the control group, but the difference was not significant (Table 2).

Proportion of children requiring parenteral rehydration: There was no significant difference between the two groups 0.36 [0.06–2.01] (Table 2).

Table 1. ***Basal characteristics***

Descriptive data	SB group ($n = 30$)	Control group ($n = 30$)	*p*-value
Age, months (IQR)	25 (9.5)	23 (8.8)	0.45
Males (%)	17 (56.7)	18 (60)	0.79
Weight (kg) (IQR)	11.4 (2.7)	10.8 (3.1)	0.28
Median duration (IQR) of diarrhea before treatment (hours)	60 (36)	72 (24)	0.18
Stool consistency at the time of enrolment (%)			
Liquid	08 (26.7)	10 (33.3)	0.23
Semiliquid	05 (16.7)	05 (16.7)	
Loose	17 (56.7)	15 (50)	
Number (%) of children vomiting	20 (66.7)	21 (70)	0.78
Median (IQR) duration of vomiting (hours)	34 (22)	42 (25)	0.16
Number (%) of children with fever	14 (46.7)	16 (53.3)	0.6
Median (IQR) duration of fever (hours)	56 (38)	65 (40)	0.35
Dehydration status (%)			
No	14 (46.7)	15 (50)	0.77
Some	08 (26.7)	07 (23.3)	

Notes. IQR = Inter-quartile range; SB = *S. boulardii*.

Table 2. ***Outcome measures: primary and secondary***

Outcome	SB group ($n = 30$)	Control group ($n = 28$)	Difference (95% CI)
Median (IQR) hours of diarrhea[a]	60 (51–67)	89 (68–95)	–29 (–41.2 to –16.8)
Median (IQR) hours of hospitalization[a]	74 (64–90)	91 (76–105)	–17 (–33.46 to –0.54)
Median (IQR) duration of fever (hours)[a]	56 (48–67)	67 (55–81)	–11 (–23.04 to 1.04)
Median (IQR) duration of vomiting (hours)[a]	48 (39–56)	55 (43–61)	–7 (–16.41 to 2.41)
Proportion of children requiring parenteral rehydration[b]	2 (6.7)	5 (16.7)	0.36 (0.06 to 2.01)
Proportion of children having diarrhea lasting beyond day 7[b]	1 (3.3)	4 (14.3)	0.21 (0.02 to 1.98)

Notes.
[a]The difference between the two medians and calculation of 95% CI (confidence interval) has been done by the method proposed by Bonett and Price [17].
[b]Data expressed in odds ratio (OR) and 95% CI.

Proportion of children having diarrhea lasting beyond day 7: There was no significant difference between the two groups 0.21 [0.02–1.98] (Table 2)

Adverse events: There was no report of any adverse events in either of the groups.

DISCUSSION

In this randomized clinical trial, we found that SB in a dose of 250 mg orally twice a day for 5 days given to children aged under five during an acute episode of rotavirus diarrhea resulted in significant shortening in the diarrheal duration and duration of hospitalization without any adverse events. There was no effect on duration of fever or vomiting. No significant difference between the two groups was observed for the proportion of children requiring parenteral rehydration or having diarrhea persistent beyond day 7.

A meta-analysis of 22 randomized control trials (rotavirus = 4) including 2440 children (rotavirus = 301) of 1 months to 15 years, reported that SB was associated with significant reduction in the duration of acute rotavirus diarrhea [mean difference (MD) of −18.07 h] [18]. In general, no difference in vomiting duration was found (five of six trials) except in one trial that reported a shorter average time of vomiting in the SB group. Similarly, no difference in fever duration was found (three trials). Regarding the duration of hospitalization (two trials), one found a decrease in the duration whereas the other did not find any difference between the two groups. None of the included trial reported any serious adverse effects related to using of SB. None of the included trial was from India.

The published reports about use of probiotics in children with rotavirus diarrhea refer mainly to cases managed as outpatients. Like previously published trials, the present trial also provides evidence that probiotics is helpful in hospitalized children with <10% dehydration [13, 19]. It is worth noting that the total length of hospitalization was decreased in the probiotics supplemented group in contrast to previous trial [13]. We could not find any significant effect on the duration of vomiting. Similarly, some trials have reported no effects [20], whereas others have reported either a significant decrease [21, 22] or a transient effect on vomiting [23]. Like previous trial, we also could not find any effect on the duration of fever [13], which was in contrast to other trials [24]. We could not find any effect on the persistence of diarrhea beyond day 7 or requirement of rehydration, though these outcomes have not been reported in previous trials.

There are limited data on the mechanism of action of SB against viral diarrhea including the rotavirus [25]. The possible beneficial effects include stimulant action on the gut immunity, trophic action on the damaged gut mucosa as it produces substances like spermin, competition for pathogen adhesion sites and viral interaction, activation of C3 fragment of the compliment and starting the cascade, increase in the secretory component of IgA in the gut, increase in the Na+ dependent uptake of D-glucose and improvement of brush border, and

influence on the maturation, enzyme expression, membrane transport and the epithelial surface renewal in the intestine.

The strength of our study is that it evaluated the effect of SB in the Indian perspective, as the findings from western countries cannot be extrapolated on Indian children due to different hygienic conditions, higher breast feeding rate and different gut colonization status. We evaluated the effect on the persistence of diarrhea beyond day 7 or requirement of rehydration; both these outcomes are important from public health perspective. A prolonged diarrhea increases the chance of malnutrition, other morbidity and mortality. Requirement of rehydration is a surrogate marker of prolonged hospitalization, thereby increasing the cost of health care and risk associated with prolonged hospitalization. Potential limitations include: other etiologies for acute diarrhea were not explored. We also could not measure the volume of stool output (g/kg). Although we monitored common clinical symptoms and potential adverse effects of intervention, we did not monitor for any asymptomatic bacteremia due to SB.

CONCLUSIONS

To conclude, results of this trial support the efficacy and safety of *S. boulardii* in treating acute rotavirus diarrhea in a developing country setting like India.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Tropical Pediatrics* online.

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