Effect of *Lactobacillus rhamnosus* GG in Persistent Diarrhea in Indian Children

A Randomized Controlled Trial

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Aim: To evaluate the role of *Lactobacillus rhamnosus* GG (LGG) as probiotic in persistent diarrhea (PD) in children of North Bengal, India.

Setting: Hospital-based study.

Design: Randomized, double-blind controlled trial.

Patients and Methods: All patients of PD admitted over a period of 2 years were included in the study as per predefined inclusion criteria. They were randomized to receive oral rehydration solution (ORS) alone, or ORS plus LGG powder containing 60 million cells, twice daily for a minimum period of 7 days or till diarrhea has stopped along with correction of dehydration with ORS and/or intravenous fluids as per WHO protocol and antibiotics in culture positive patients. The duration and frequency of purge and vomiting were studied. Data were analyzed by SPSS-10 software. Statistical significance was calculated by Student *t* test and χ^2 test.

Results: The study comprised of 235 patients randomized into 2 groups, cases (117) and controls (118). Both the groups were similar with respect to age, number of breastfed infants, presentation with dehydration, degree of protein energy malnutrition, and distribution of infections. Stool culture was positive in 90 (38.3%) patients, *Escherichia coli* being the commonest organism followed by *Shigella* spp. and *Clostridium difficile*. The mean duration of diarrhea was significantly lower in the cases than in controls (5.3 vs. 9.2 d). The average duration of hospital stay was also significantly lesser in cases. No complication was observed from the dose of LGG used.

Conclusions: LGG (dose of 60 million cells) could decrease the frequency and duration of diarrhea and vomiting and reduced hospital stay in patients of PD.

Key Words: children, *Lactobacillus*, persistent diarrhea, probiotic

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In developing countries persistent diarrhea (PD) is a common cause of malnutrition. WHO has defined PD as an episode of diarrhea, which starts with an infection and lasts for at least 14 days.[1] The common causes of PD in the developing countries are persistent infection with one or more enteric pathogens, sequential enteric infections, and disaccharide/protein maldigestion/absorption. Lactose maldigestion/absorption is one of the main key factors of PD. The use of biotherapeutic agents or probiotics to treat a variety of infectious, most notably infections of mucosal surfaces are traditionally well known. With the encouraging results of feeding yogurt in lactose intolerant diarrheas, it was believed that probiotics may enhance lactose digestion and may produce antidiarrheal effect in PD. Several studies have supported this view.[2,3] In the last decade, there is a new thrust in the concept of friendly bacteria and a resurgence of use of probiotics in various diseases. A number of agents have been isolated and studied with a view to clinical use. *Saccharomyces boulardii*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* GG (LGG) have been found to be effective in diarrheal diseases.[4] The present study intends to evaluate the role of probiotics in PD, in Indian children.

PATIENTS AND METHODS

Study Setting

A randomized, double-blind, hospital-based controlled trial was conducted at the Department of Pediatrics, North Bengal Medical College. It was approved by the Hospital Ethics Committee.

Study Participants

All children who were admitted in Pediatric indoor with a diagnosis of PD over a period of 2 years (January 2003 to December 2004) were included in the study. The inclusion criteria were (1) history of diarrhea persisting for 14 days or more without any remission in between and (2) stool pH < 5.5 and stool reducing substance $> 1\%$.

The exclusion criteria were (1) presence of any systemic illness other than diarrhea on admission, (2) development of any systemic complication of diarrhea

during hospital stay, and (3) failure to give informed consent.

Sample Size

Considering the prevalence of PD in patients attending the Pediatric indoor of North Bengal Medical College, 2-tailed α of 0.05 and power (1-β) of 80%, we calculated the minimum sample size of 73 patients in each wing of the study.

Randomization

Randomization was performed by computer generated random numbers by the biostatistics division and patients were assigned to receive either oral rehydration solution (ORS)+LGG powder (cases) or only ORS (controls) by opaque and sealed envelops.

Technique of Blinding

Readymade disposable poly packs of ORS (100 mL in each poly pack) with and without LGG powder containing 60 million cells were prepared and numbered by hospital pharmacy. Both types of packets were similar in appearance. A master register was maintained by the pharmacy. After randomization, patients received packets of the same number as designated to them. These packets were distributed by the nursing staff of the Pediatric ward who were properly trained beforehand. Neither the nursing staff and the mothers nor the doctors/residents in direct contact with the patients were aware about the content of the ORS provided.

Study Procedure

The patients were thoroughly examined and worked up on admission. Degree of dehydration was assessed by WHO criteria.[5] Stool was examined microscopically for the presence of pus cells, RBC, mucus flakes, bacteria and cysts or trophozoites of *Entamoeba histolytica* and *Giardia lamblia* and sent for culture/sensitivity tests, rotavirus assay, and for the assessment of pH and reducing substances. Body weight was recorded before and after correction of dehydration to detect and classify any protein energy malnutrition (PEM) as per Indian Academy of Pediatrics (IAP) classification.[6] Detailed hematologic and biochemical investigations including hematocrit, total and differential count, serum albumin, urea, creatinine, and serum electrolytes were performed. Dehydration status was classified as no dehydration, some dehydration, or severe dehydration as per WHO criteria.[5] Dehydration was corrected following WHO plan A in no dehydration, plan B in some dehydration, and plan C in severe dehydration[5] using ORS and/or intravenous fluid provided from the hospital. After the initial correction of dehydration when the patients became clinically stable *Lactobacillus* supplementation was started. All the cases received *L. rhamnosus* strain GG (LGG) powder containing 60 million cells was dissolved in 100 mL of ORS twice daily for a minimum period of 7 days or till diarrhea stopped. The controls received an equal amount of the same ORS without LGG as placebo, twice daily for the same duration. On admission and thereafter everyday, mothers were provided with a piece of white paper and pen and were asked to make a stroke and a circle on a white paper for each purge and each vomit, respectively. Papers used to be collected after 24 hours. This was done in consideration of the poor literacy status of the mothers. All children were given hospital diet for their age. Yogurt was excluded from the diet and no food or drinks were allowed from outside. Breastfed children continued to receive breastfeeds. Daily calorie intake was calculated from the Indian Food Table.[7] Patients with positive stool cultures received antibiotics according to the sensitivity pattern. At the earliest sign of any complication—viz. electrolyte imbalance, septicemia, renal failure etc—the children were withdrawn from the study and treated accordingly. Patients were discharged when the diarrhea had stopped, and oral intake was adequate (as in prediarrhea state). Follow-up was performed weekly for a minimum period of 4 weeks.

Outcome Measures

The primary outcome measures were decrease in frequency and duration of diarrhea and vomiting. Secondary outcome measure was reduction in hospital stay.

Statistical Analysis

Data were analyzed by commercial software (SPSS-10) and statistical significance was calculated by Student *t* test and χ^2 test. *P* value < 0.05 was taken as statistically significant.

RESULTS

A total of 235 patients of PD completed the study after initial randomization (117 cases and 118 controls) (Fig. 1). Both the groups were comparable with respect to age, male:female ratio, body weight, literacy status of the mothers, calorie consumption per day, number of breastfed infants, and degree of dehydration at initial presentation (Table 1). A substantial number of patients had PEM; grade I PEM was present in 97 (45.8%), grade II in 84 (39.6%), and grade III in 31 (14.6%) children. None had grade IV PEM. Mean hemoglobin level was 8.7 ± 2.6 g/dL (range 7.0 to 11.2) and mean serum albumin level was 2.8 g/dL (range 2.2 to 4.1). Rest of the biochemical and hematologic investigations were unremarkable. Mean stool pH was comparable in both the groups (5.2 in cases vs. 5.3 in controls) and all were positive for reducing substances. The mean duration of diarrhea was significantly lower in the cases than in controls. The average frequency of purge decreased significantly from the fourth day onwards in the cases. Frequency of vomiting was similar in both the groups. The average duration of hospital stay was 11.4 ± 1.5 days with a significant difference between the cases and controls (Table 2). The distribution of infection was similar in both the groups. Stool culture was positive in 90 (38.3%) patients, *Escherichia coli* being the commonest organism (22/90) followed by *Shigella* spp. (16/90) and *Clostridium difficile* (14/90). Cysts and/or trophozoites of

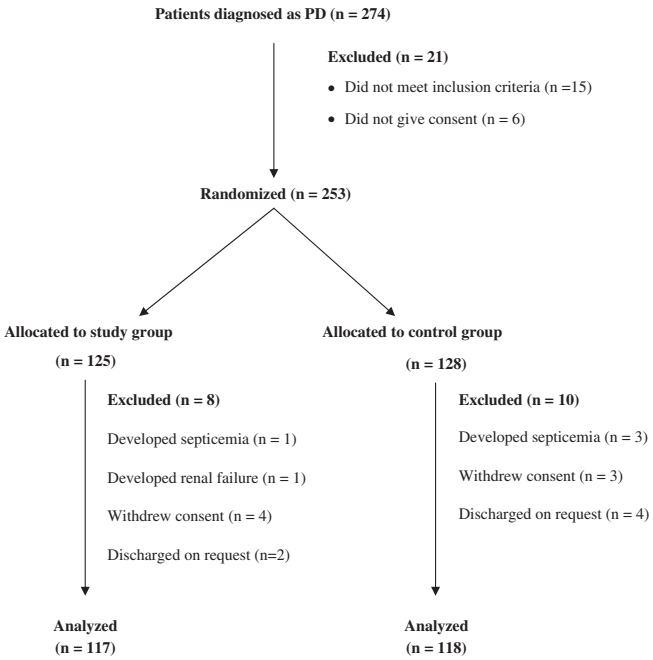

FIGURE 1. Flow of participants through each stage.

E. histolytica and *G. lamblia* were found in 16/90 and 13/90, respectively. Mixed infection was found in 9/90. The effect of *Lactobacillus* supplementation on different organisms was also assessed (Table 3). No significant difference was found in the mean duration of diarrhea and vomiting between the 2 groups except in patients with *C. difficile* diarrhea where the duration was significantly lesser in the cases ($P < 0.05$). No complication from the use of LGG could be documented. At discharge, proper dietary advice and vitamin supplementation were given specially to those with PEM. A total of 181 patients completed weekly follow-ups for 4 weeks. No complication could be identified in long-term follow-up.

DISCUSSION

Patients were given lactose-free diet to prevent lactose intolerance. Same diet was provided to all children from the same source (hospital kitchen) and nothing was allowed from outside, as many food items may have probiotic effect for example, yogurt, fermented foods, yeast containing foods, etc. The mean calorie consumptions of both the groups in all categories of diarrheas were comparable and adequate. This was ensured to avoid a bias on the probiotic effect between the cases and controls, as adequate oral intake maintains normal function of the gut.

In the present study, significant reduction in the duration and frequency of diarrhea was observed in the patients who received LGG. However, the effect was evident only after the fourth day. Probably, this was the time taken by the *Lactobacillus* for multiplication and colonization in the gut. In most of the previous studies, LGG was found to be efficacious in the treatment of sporadic, infectious diarrhea by reducing the duration of diarrhea by approximately 1 day, shortening the initial phase of watery stools, and reducing hospital stay in developed countries.[8] In the present study, more profound and significant benefit was seen as duration of diarrhea was shortened by approximately 4 days and total hospital stay was reduced by 8 days. This difference seen in our study was probably because our set of patients was different from the sporadic and acute infectious diarrhea group. Majority of our patients were malnourished and were suffering from PD as opposed to the type of cases in previous studies. PD has 2 components causing diarrhea; firstly extensive colonization by pathogenic organisms resulting from recurrent and chronic gut infections and secondly lactose intolerance, maldigestion, and malabsorption due to loss of villi of enterocytes. This was coupled with malnutrition, which results in poor local and systemic immunity. Thus the improvement of diarrhea in our group of patients was owing to correction of all these factors which was not so in the controls. We know from previous studies that probiotics can prevent or ameliorate

TABLE 1. General Characteristics of Patients

Characteristics	Cases (n = 117)	Controls (n = 118)
Age (y) (mean ± SD)	4.1 ± 1.8	4.2 ± 2.0
Male:female	1:0.90	1:0.87
Literacy status of mother/attendant		
Illiterate	106 (90.6)	109 (92.4)
Literate	11 (9.4)	9 (7.6)
No. breastfed infants	21 (17.9)	19 (16.1)
Degree of dehydration on admission		
No	68 (58.1)	65 (55.1)
Some	44 (37.6)	46 (38.9)
Severe	5 (4.3)	7 (5.9)
Patients with PEM (body weight < 80%expected)	107 (91.5)	105 (89.0)
Calorie intake in hospital (kcal/d) (mean ± SD)	920 ± 60.4	940 ± 68.0
Stool pH (Mean ± SD)	5.2 ± 0.1	5.3 ± 0.1

Figures in parentheses indicate percentage.
SD indicates standard deviation.

TABLE 2. Frequency and Duration of Diarrhea and Vomiting (Mean ± SD)

	Case (n = 117)	Control (n = 118)
Day 1		
D	10.4 ± 4.1	10.8 ± 4.3
V	1.2 ± 0.2	1.0 ± 0.3
Day 2		
D	11.3 ± 5.0	11.1 ± 4.9
V	1.0 ± 0.3	1.0 ± 0.4
Day 3		
D	10.4 ± 4.3	10.5 ± 4.5
V	0.0 ± 0.0	0.0 ± 0.0
Day 4		
D	5.8 ± 4.3	10.0 ± 4.2*
V	0.0 ± 0.0	0.0 ± 0.0
Day 5		
D	5.2 ± 2.1	10.2 ± 3.2*
V	0.0 ± 0.0	0.0 ± 0.0
Day 6		
D	1.0 ± 0.8	8.4 ± 4.0*
V	0.0 ± 0.0	0.0 ± 0.0
Day 7		
D	0.9 ± 0.2	7.5 ± 3.2*
V	0.0 ± 0.0	0.0 ± 0.0
Day 8		
D	0.9 ± 0.3	6.9 ± 3.0*
V	0.0 ± 0.0	0.0 ± 0.0
Day 9		
D	1.0 ± 0.2	4.3 ± 0.9*
V	0.0 ± 0.0	0.0 ± 0.0
Day 10		
D	0.8 ± 0.2	1.3 ± 0.9
V	0.0 ± 0.0	0.0 ± 0.0
Average duration of diarrhea (d)	5.3 ± 2.1	9.2 ± 2.8*
Average duration of vomiting (d)	2.0 ± 1.1	1.9 ± 1.2
Average duration of hospital stay (d)	7.3 ± 1.6	15.5 ± 1.5*

*Statistically significant ($P < 0.05$).
D indicates diarrhea; V, vomiting.

TABLE 3. Duration of Diarrhea and Vomiting in Organism Positive Cases (Mean ± SD)

Organism	Duration of Diarrhea (d)	Duration of Vomiting (d)
E. coli (n = 22)		
Case (n = 12)	5.8 ± 2.0	2.6 ± 1.1
Control (n = 10)	5.3 ± 1.8	2.2 ± 0.9
Shigella spp. (n = 16)		
Case (n = 9)	5.6 ± 2.2	2.4 ± 1.4
Control (n = 7)	5.2 ± 2.0	2.2 ± 1.9
C. difficile (n = 14)		
Case (n = 6)	3.2 ± 2.4	2.0 ± 1.0
Control (n = 8)	8.0 ± 2.8*	1.8 ± 0.8
E. histolytica (n = 16)		
Case (n = 7)	5.0 ± 2.2	2.3 ± 2.0
Control (n = 9)	4.8 ± 2.0	2.1 ± 1.9
G. lamblia (n = 13)		
Case (n = 5)	5.6 ± 2.1	2.4 ± 2.1
Control (n = 8)	5.4 ± 1.8	2.2 ± 1.8
Mixed infections (n = 9)		
Case (n = 6)	5.2 ± 1.9	3.0 ± 1.9
Control (n = 3)	5.4 ± 1.8	3.2 ± 2.0

*Statistically significant ($P < 0.05$).
SD indicates standard deviation.

diarrhea and inflammation through their local effects and/or their effect on the immune system. Modes of action of probiotic bacteria in the gut include, occupation of the binding sites on the gut mucosa, preventing pathogenic bacteria from adhering to the mucosa,[9,10] production of proteinaceous compounds, namely bacteriocins, that act as local antibiotics against more pathogenic organisms,[11] decrease in production of proinflammatory cytokines such as interferon-γ, tumor necrosis factor-α, and interleukin-12,[12,13] stimulation of IgA production[14] and production of acetic and lactic acid which inhibit the growth of bacterial pathogens.[9] It has also been postulated that probiotics compete with pathogens for nutrients and modify toxins produced by pathogens or toxin receptors found in the gut wall. Hence we postulate that the components of PD which were effectively tackled by LGG along with improved nutrition were likely the key factors in the significant reduction of duration of diarrhea was seen in our patients.

When organism wise evaluation was carried out, LGG was found to be significantly effective only in patients with *C. difficile* diarrhea, although *C. difficile* could be isolated only in a minority of patients of PD. Majority of patients of PD in the present study were culture negative (61.7%). This makes one to believe that the beneficial effect of LGG was not only by inhibition of *C. difficile*, but also owing to improvement in the digestive and absorptive functions of the gut (predominantly in the culture negative cases). Gaon et al[15] in their randomized double-blind trial found that *Lactobacillus* spp. and *Saccharomyces boulardii* could decrease the frequency and duration of diarrhea and vomiting in patients with PD. They mixed *Lactobacillus* with milk but we avoided milk in our study as many of our patients had lactose intolerance. Some other studies have also documented beneficial effect of *Lactobacillus* in *C. difficile* diarrhea.[16,17]

For a probiotic to be effective, it should be viable and metabolically active within the gastrointestinal tract, should itself be nonpathogenic, and act against pathogens by mechanisms different from that of antibiotics as many times it is coadministered with antibiotics. As there is no definite recommendation for the dose of probiotics, different studies have used different doses. We used a comparatively smaller dose (60 million cells of LGG) twice daily considering the fact that over 80% of our patients were malnourished. Some studies had used much higher doses.[18,19] Concerns are there over the deleterious effects of probiotics in severe malnutrition. Systemic infection, immunomodulation, gene transfer, and deleterious metabolic effects may occur. Immunomodulating properties may result in systemic infections in the immunocompromised patients.[20] In conclusion, *L. rhamnosus* GG when used with ORS in PD of Indian children effectively decreased the duration and frequency of purge and thus reduced the hospital stay. It is safe in long-term results. It may act as a valuable adjunct to ORS in the treatment of PD of children.

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