Original Article

Efficacy of Tyndalized *Lactobacillus acidophilus* in Acute Diarrhea

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Abstract. *Objective :* A double-blind randomized controlled-trial was done to evaluate the efficacy of tyndalized *Lactobacillus acidophilus* in acute diarrhea. ***Methods :*** All children from 6 months to 12 years with acute diarrhea were included. *Lactobacillus acidophilus*/placebo was given to the children for 3 days with ORS and feeds. Intake-output was recorded 4 hourly. Of the 98 children, 48 received *lactobacillus* and 50 the placebo. ***Results :*** ORS consumed, frequency of stools, duration of diarrhea, time for rehydration, hospital stay, weight gain and IVF needed were comparable in the two study groups. There were 4 treatment failures in the *lactobacillus* group and none in the placebo group (OR 0.92, 95%CI 0.84-0.99). In the rotaviral diarrhea and in those who had diarrhea of less then 60 hours the difference did not reach statistical significance. ***Conclusion :*** There is no significant benefit of tyndalized *Lactobacillus acidophilus* in acute diarrhea. **[Indian J Pediatr 2005; 72 (11) : 935-938]**
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In a meta-analysis of 18 studies[1] done recently it was concluded that probiotics could shorten the duration of acute diarrhea, especially due to rotavirus in children. Most of this work had been done in the developed world. The developing world has a distinct population, with perhaps greater heterogeneity with regard to nutritional status, and an etiological profile of diarrhea quite different from the advanced nations. This issue is further compounded by the fact that the majority of the studies,[2,3] have been done on live preparations of *Lactobacillus* sp which are not available in the Indian market. The tyndalized preparation, quite unlike the live one, available in the Indian market is being used as a surrogate instead. This increases the cost of management in acute diarrhea, without the evidence from well-controlled randomized trials. Recently, commercial mixtures of probiotics with ORS are flooding the market. Hence this study was undertaken to see the efficacy of tyndalized *Lactobacillus acidophilus* in acute diarrhea.

MATERIALS AND METHODS

This double-blind randomized controlled clinical trial was conducted on children aged 6 months to 12 years admitted to diarrhea training and treatment unit (DTTU) with acute watery diarrhea between April 2001 to September 2002. Children were excluded if they had evidence of systemic infection, encephalopathy and convulsions. Those patients with history of use of any pharmaceutical probiotics were also excluded. Prior antibiotics or probiotics were deemed to have been used if the attendant could name the drug(s), show supporting prescription or the drug(s) themselves. The placebo (puffed rice powder) and the lactobacilli (mixed with puffed rice powder) were packed in identical packets. Following simple randomization (done by a non-departmental colleague), a serial number was assigned to the packets. After informed consent the children received the numbered packets sequentially on enrolment into the trial. The investigators and the patients were thus both blinded to the contents of the packets. Children in lactobacilli group were receiving commercially available tyndalized *Lactobacillus acidophilus* (LA) *(Lactrol, Raptakos)* cells from the same pharmaceutical company throughout the period of study. Each child was advised to consume a packet daily for 3 days. Thus the children in the *LA* group were receiving 15 billion tyndalized *LA* cells daily for 3 days. All children were managed with WHO-ORS and feeding as per the guidelines of the IAP taskforce on diarrhea. Admission characteristics, anthropometry, frequency of stools, hydration status, amount of ORS consumed, duration of diarrhea and weight were recorded in a separate performa. Dehydration persisting for more than 72 hours, ORS consumed more than 8 litres in < 5 years or 10 litres in ≥ 5 years, and more than 200ml/kg of intravenous fluid needed throughout hospital stay were criteria for treatment failure. The data was recorded and analyzed for the purpose of treatment. The primary

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outcome measure was the duration of diarrhea (time from enrolment to first of the 3 consecutive semi-formed stools or till the last loose stool, subsequent to which there were no stools for 12 hours). Mean ORS intake, time taken to rehydrate, duration of hospital stay, weight gain and rate of treatment failure were the secondary outcome measures. Recovery and discharge criterion was: 3 consecutive semi-formed stools or no stools for 12 hours. Stools of all patients were collected on Cary Blair media for *vibrio cholerae* isolation. Additionally, 2-3ml of stool was collected in a clean dry pre-sterilized vial, free of detergent residue, for rota viral detection.

The required sample size was calculated to be 50 for experimental subjects. This was calculated to detect a difference of 25% during diarrhea, using a value obtained from an earlier work[4] from this centre of 31.1±14.24 hours, at significance level of 0.05 and power of 80% for a study design using t-test. Using SPSS (version 10) t-test was performed after log transformation[5] on all continuous variables showing non normal distribution, and chi-square analyses (and Fisher's exact) were done to compare proportions between the 2 independent groups.

RESULTS

Pre-admission Characteristics: Ninety eight children were included in the study, 48 in LA group and 50 in placebo group. The mean age, gender, hydration status, weight for age, height for age, weight for height, mean frequency of stools, mean frequency of vomiting and mean duration of diarrhea at admission of the 2 groups were comparable (Table 1). Of the total, 24 and 20 were infants in LA and placebo group respectively. Due to logistical constraints, stool specimens of only 22 children could be tested for rotavirus. Of these, 14 were positive for rotavirus. Of the total ninety-eight, eight were culture proven cholera.

Post-admission Characteristics: Mean duration of diarrhea, which was the primary outcome measure for this study (for which the sample size was calculated), was comparable in the LA and placebo groups (Table 2). The other secondary outcome measures like mean ORS consumed per kg body weight, mean time taken for rehydration and duration of hospital stay were less in the LA group but did not reach statistical significance. Mean intravenous fluid needed was more in the LA group but again the difference was insignificant (95% CI for ratio of geometric means 0.89 to 1.51). A statistically insignificant (95% CI 0.58 to 1.48) but higher weight gain was seen in the placebo group (Table 2). Four (3 in *LA* and 1 in placebo group) patients left before completion of the study period. Three of the cases left between 4 to 8 hours of therapy while one left after 24 hours of therapy. In addition, there were 4 treatment failures in the LA group *vs* none in the placebo group (OR 2.13, 95%CI 1.72-2.65). All the treatment failures were due to requirement of more than 200 ml/kg of IVF, and one of them also remained dehydrated beyond 72 hours. The data collected till the time these patients left the study has been analyzed for treatment. For these patients, outcome measures like duration of diarrhea, time taken to rehydrate and the hospital stay could not be analyzed since the end points were not available. The stool specimen of the 4 failed patients revealed: non-pathogenic organism, *vibrio cholerae, E.coli* and rotavirus. After excluding the failed subjects and those who left the study, there were 1 of 42 patients in *LA* and 2 of 48 in the placebo groups, with diarrhea of more then 5 days (OR 0.57 95% CI 0.05-6.5). Three cases each from the *LA* (42) and placebo (49)

Table 1. Baseline Characteristics of the Study Groups at Admission.

No	Characteristics N=48	*LA** N=50	Placebo
1	Age (mo) μ(SD)†	17.78 (2.34)	21.37 (2.39)
2	Z score for WFA μ(SD)	-3.29 (1.92)	-3.45 (2.15)
3	Z score for HFA μ(SD)	-4.98 (5.36)	-4 46 (5.52)
4	Z score for WFH μ(SD)	-1.81 (1.13)	-2.12 (1.33)
5	No of stools/24 hours μ(SD)†	20.89 (1.95)	17 78 (1.82)
6	Vomitings/24 hours μ(SD)†	10.09 (2.24)	8.57 (2.21)
7	Diarrhea duration (hours) μ(SD)†	20.56 (1.78)	18.62 (1.82)
8	Age groups (n)		
	6-36 mo	36	37
	37-72 mo	9	8
	73-144 mo	3	5
9	Gender (male) (n)	34	31
10	Severe Dehydration (n)	19	15
11	Use of antibiotics (n)	8	7
12	Vomitings (n)	43	45

* *lactobacillus acidophilus*

†data is the anti-log values after log transformation of the original data

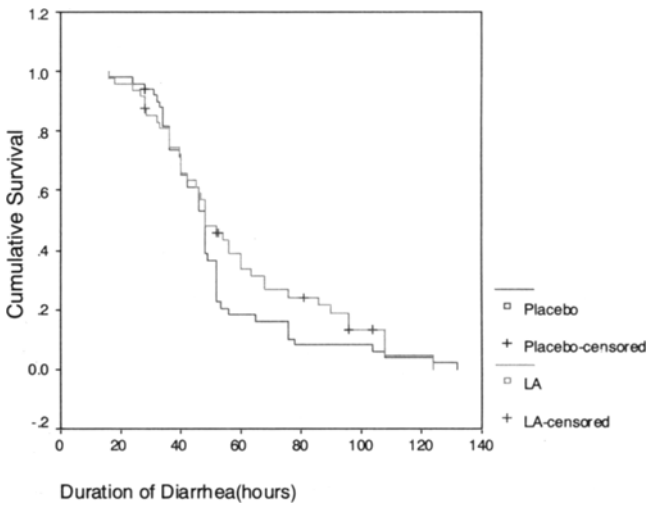

Fig 1. Kaplan Meier Survival Curve depicting comparable duration of diarrhea in the two study groups. (LA: lactobacillus acidophilus)

TABLE 2. **Comparison of the Post-admission Characteristics Between *LA* and Placebo Groups.**

Variables	Total cases (n=98)			Intervention within 60 hours (n=89)		
	*LA** (n=48) Mean (SD)	Placebo (n=50) Mean (SD)	Effect size (95% CI)	*LA** (n=42) Mean (SD)	Placebo (n=47) Mean (SD)	Effect size (95% CI)
No. of stools at 24 hours	15.84 (1.99)	14.79 (2.08)	1.06 (0.78-1.42)	16.33 (2.02)	14.91 (2.09)	1.09 (0.80-1.48)
No. of stools 24-48 hours	6.60 (2.63)	4.96 (3.52)	1.34 (0.72-2.48)	6.47 (2.66)	4.68 (3.46)	1.38 (0.73-2..60)
Duration diarrhea(hours)†	54.45 (1.48)	55.08 (1.51)	0.98 (0.83-1.16)	51.97 (1.44)	53.97 (1.48)	0.96 (0.81-1.13)
Re-hydration time(hours)†	6.76 (1.77)	6.90 (1.84)	0.96 (0.76-1.25)	7.02 (1.82)	6.99 (1.84)	1.04 (0.77-1.29)
Total ORS (ml/kg)	167.34 (2.49)	214.48 (2.01)	0.78 (0.55-1.09)	176.56 (2.45)	209.41 (1.98)	0.84 (0.60-1.17)
Total IVF needed (ml/kg)	108.74 (1.73)	93.68 (1.49)	1.16 (0.89-1.51)	107.37 (1.71)	93.66 (1.48)	1.14 (0.87-1.50)
Total fluid (ml/kg)	240.71 (2.04)	273.96 (1.72)	0.87 (0.67-1.14)	251.94 (1.98)	268.9 (1.70)	0.93 (0.72-1.21)
Hospital stay (hours)†	27.84 (1.98)	32.93 (1.79)	0.84 (0.65-1.09)	27.92 (2.03)	32.38 (1.77)	0.86 (0.65-1.12)
Weight gain (gm)	276.05 (2.91)	295.46 (2.81)	0.93 (0.58-1.48)	278.03 (2.97)	305.77 (2.78)	0.90 (0.56-1.46)

**Lactobacillus acidophilus*, † n (LA) = 41 & n (Placebo) = 49. Ratio of unity and confidence limit including 1 imply insignificant difference between geometric means.

groups were having watery stools at 72 hours (OR 1.17 95% CI 0.22-6.18). Mean duration of diarrhea (hours) in the Kaplan Meier survival analysis was 58.78 (95% CI 50.11-67.44) and 51.77 (95% CI 45.15-58.40) in the *LA* and the placebo group respectively (p=0.2333) (Fig 1).

***Subgroup Analysis*:** Analyzing the data after the exclusion of the failed subjects, it was found that all the above-mentioned variables showed similar trends and were statistically comparable in the 2 groups. Six of the *LA* and three of the placebo group had diarrhea of more than 60 hours before admission. On analysis of data after excluding these 9, no statistically significant beneficial effect of *LA* was found. On comparison of the data of the 13 rotavirus positive cases, it was noted that the beneficial effects in the *LA* group did not reach the level of significance.

DISCUSSION

Well-controlled clinical studies have shown that probiotics such as *Lactobacillus rhamnosus* GG, *L reuteri, L casei Shirota,* and *Bifidobacterium lactis* Bb12 shorten the duration of acute rotavirus diarrhea.[3,6,7] A multicentre trial[8] by ESPGHAN showed that probiotic administered in an oral rehydration solution significantly curtailed episodes of rotaviral diarrhea, whereas in non-specific or bacterial diarrhea no clear effect was found. Thus 2 important determinants of a successful outcome are: the probiotics strain used and etiology of diarrhea. Three meta-analyses[1-3]substantiate the consistent role of only Lactobacillus GG in shortening the duration of diarrhea particularly in rotaviral gastroenteritis. Except for one small trial from Pakistan, all other studies have been done in the developed world, the IAP taskforce therefore recommends withholding the usage of probiotics in diarrhea[9]. Though studies[10,11] with killed Lactobacillus acidophilus have demonstrated shortened duration of diarrhea, these studies have been done in overwhelmingly rotaviral positive patients and in cases with mild diarrhea. The present study was done in cases with moderate to severe diarrhea, including cases that needed IVF. Moreover, from the Kaplan Meier survival analysis it is evident that the duration of diarrhea, in both study groups, was relatively short. The effect of probiotics is dependant on colonization which may need longer diarrheal duration to have an appreciable difference. This has now been mirrored in other clinical trials.[12,13] Moreover, since only 18% of acute diarrhea in India is due to rotavirus, it may explain the lack of significant beneficial effect seen in the present study with *lactobacillus acidophilus*.[14] The limitations of the present study are the small sample size of rotavirus positive cases, lack of stool output measurement and the wide age range in the included sample. The first 2 limitations are due to financial constraints whereas the wide age range was decided to include the cholera patients to have the true representative sample of the etiological profile of acute childhood diarrhea in our country. The authors have found no statistically significant beneficial trends in the lactobacillus group during diarrhea, time taken for rehydration and total ORS consumed. Recent Brazilian[12] and Peruvian[13] studies have corroborated the findings of the present study.

CONCLUSION

Hence we conclude that tyndalized *Lactobacillus acidophilus* does not have any significant beneficial effect on diarrhea and should not be used in the management of acute diarrhea. Probiotic usage may not be harmful but would entail higher cost of treatment and, more importantly, may undermine the importance of ORS and

feeding in the management of acute diarrhea. There is a for need larger rotavirus positive samples to evaluate the efficacy of tyndalized *Lactobacillus acidophilus* in rotavirus diarrhea.

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