RESEARCH PAPER

Probiotics for Promoting Feed Tolerance in Very Low Birth Weight Neonates — *A Randomized Controlled Trial*

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Objective: To measure the efficacy of a probiotic formulation on time to reach full enteral feeds in VLBW (very low birth weight) newborns.

Design: Blinded randomized control trial.

Setting: A tertiary care neonatal intensive care unit (NICU) in Southern India between August 2012 to November 2013.

Participants: 104 newborns with a birth weight of 750-1499 g on enteral feeds.

Intervention: Probiotic group (*n*=52) received a multicomponent probiotic formulation of *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum and Saccharomyces boulardii* once a day at a dose of 1.25×10^9 CFU from the time of initiation of enteral feeds till discharge and the control group (*n*=52) received only breast milk.

Outcome measure: Time to reach full enteral feeds (150 mL/kg/day).

Results: The mean (SD) time to reach full enteral feeding was 11.2 (8.3) days in probiotic *vs*. 12.7 (8.9) in no probiotic group; (*P*=0.4), and was not significantly different between the two study groups. There was a trend towards lower necrotizing enterocolitis in the probiotic group (4% *vs*. 12%).

Conclusion: Probiotic supplementation does not seem to result in significant improvement of feed tolerance in VLBW newborns.

Keywords: *Bifidobacterium, Infant feeding, Lactobacillus, Necrotizing entrocolitis*.

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Enteral feeding intolerance is a major issue in premature infants, resulting in prolonged hospitalization and a predisposition to serious complications due to prolonged use of parenteral nutrition. A delay in reaching full enteral feedings is also associated with a poorer mental outcome in preterm neonates at 24 months corrected age [1].

As the maturation of motor activity in premature infants lags behind that of digestive and absorptive functions, it is most frequently a disorder of gastrointestinal motility that limits the use of enteral feeding in this population. An adequate establishment of the intestinal flora after birth is strictly related to motility maturation and plays a crucial role in the development of gut barrier function and the innate and adaptive immune system [2].

Enteral supplementation of probiotics prevents severe necrotizing enterocolitis (NEC) and all-cause mortality in preterm infants [3]. Moreover, among the many strategies tried for prevention of feeding intolerance, probiotics are the most promising. However, they are yet to be used as standard of care. The objective of this trial was to determine the efficacy of probiotics on feed tolerance in very low birthweight (VLBW) neonates.

Methods

All neonates with a birth weight between 750 g to 1499 g admitted to the NICU in whom enteral feeds were started were eligible for enrolment. Neonates with gastrointestinal anomalies, severe congenital malformation, and those not started on enteral feeds by day 14 of life were excluded. The study was a double blind randomized controlled trial (RCT) conducted in a tertiary care NICU between August 2012 to November 2013.

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We hypothesized that by establishing a normal intestinal flora probiotics could reduce the incidence of feed intolerance. The study protocol was approved by the Institutional Ethics Committee and a written informed consent was taken from the parent/guardian before enrollment.

Outcomes: The primary outcome of the study was the time taken to reach full enteral feeds. Secondary outcomes were episodes of feed intolerance, incidence of NEC stage 2 or more, duration of hospital stay, days on total parenteral nutrition (TPN), weight gain and

mortality during hospital stay.

Feed intolerance was defined as presence of any one of the following four features - abdominal distension ≥2 cm from the previous measurement; or vomiting ≥2 episodes in the past 6 hours or blood stained or bilious; or gastric aspirate >2 episodes of voluminous gastric aspirates in a 6 hr period. Voluminous gastric residuals were defined as >50% of previous feed volume if ≥6 mL/feed; or 2 episodes of >50% in a 6 hr period or single residue of 100% if feed volume <6 mL/feed.

Sample size: The sample size was determined based on data from a pilot study done in the same clinical unit. To achieve a significant mean difference of 3.37 days (with SD 5.05 and 6.6 in the 2 groups) in time to reach full feeds with a Type 1 error of 5% and a Power of 80% (2 sided), the sample size required was 47 (in each group) which was inflated by a further 10% based on the usual percentage of deaths and discharges against medical advice in the VLBW group in our Unit.

The subjects were randomly allocated into two groups using computer generated random numbers by an investigator not directly involved in the study. We followed a parallel group design and block randomization was done with block sizes varying from 8 to 12. Sequentially numbered opaque sealed envelopes were used for allocation concealment. The two groups were coded as A and B and the group code was kept off site in an opaque sealed envelope and opened only after the final analysis was done.

Feeding protocol: Feeding was initiated, advanced, stopped and restarted as per unit protocol derived by consensus for the purpose of the study. The protocol was attached to all the study case files to ensure compliance. Trophic feeds *i.e* 10 to 20 mL/kg/day at 2 hourly interval of either colostrum (if available) or donor breast milk feeds were initiated in hemodynamically stable infants. Feeds were advanced by 20 mL/kg/day (in babies 750-1249 g and those with abnormal antenatal Doppler) or by 35mL/kg/day (in babies 1250-1499g and well). Feeds were given every 2 hourly and pre-feed aspirates were measured in babies on gavage feeds. Feeds were withheld if there were signs of feed intolerance, hemodynamic instability, suspected NEC or voluminous gastric residuals. Feeds were restarted when all the above mentioned signs were resolved. Parenteral nutrition was continued till 100 mL/kg/day of feeds were reached. Full feeds were defined as 150 mL/kg/day. Oral feeds were initiated in babies more than 30-32 weeks with good suck reflex and otherwise well. 5% Dextrose was used if milk was not available. Human milk fortifiers were used as per NNF India recommendations.

The weights were checked daily on a calibrated digital weighing machine with a sensitivity of ±5 g. A best gestational age was given for each infant based on LMP and corroborated by early first trimester ultrasound when available. NEC was defined and staged as per modified Bell's staging. The infants were discharged as per unit protocol which is baby maintaining hemodynamic stability without support, on full oral feeds-either by breast milk or paladay, showing consistent weight gain of 15 to 20 g/kg/day for 3 days and 1.4 kg or more. The management protocols, clinical practices, equipment, infrastructure, and key personnel were unchanged during the study period. The baseline illness severity documented by Score for Neonatal Acute Physiology Perinatal extension (SNAPPE-II) was used in both groups [4]. Adequate antenatal steroids were defined as an interval of at least 24 hrs after the initial dose.

Probiotic group received a multicomponent probiotic formulation of *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum* and *Saccharomyces boulardii* in the form of powdered sachets of 1g each (Darolac; Aristo Pharmaceuticals Pvt. Ltd.). The intervention was administered once a day at a dose of 1.25×10^9 CFU starting within 24 hours of initiation of feeds. It was given as a powder form dissolved in breast milk and from day 2, given at a fixed time of the day. Fresh suspensions of supplements were individually prepared in the pantry under strict asepsis by study nurses who were not directly involved in routine patient care for each study infant. The probiotic supplementation was continued till discharge given once a day if the volume of feeds was 2 mL or more, and in two divided doses if the baby received <2 mL/feed. It was stopped when feeds were withheld for any reason. The no probiotic group received only breast milk and served as the control. No placebo was used.

The probiotic supplementation did not change the physical appearance of the milk, and the syringes were labeled only with the patient's name and identification number with no indication of study group assignment. Attending physicians and nurses caring for the infants were blinded to the group assignments. To ensure blinding, the mixing was done after the milk required for each study infant was gathered away from the patient care area in the NICU irrespective of the assigned group. The probiotic was stored as per manufacturer's guidelines and was prescribed to all infants enrolled in the study to ensure blinding.

Nurses followed strict asepsis during preparation and compliance was monitored regularly by one of the investigators. The infants were clinically monitored daily

by the consultants for feed intolerance and sepsis. A septic screen followed by blood culture was done as per clinical suspicion.

Statistical analysis: Continuous variables were compared by using Student's t test or the Mann-Whitney U test when appropriate; chi square analysis or Fisher's exact test when appropriate, was used to ascertain significant differences in categorical variables between groups. All tests were 2-tailed. Significance was defined as $P<0.05$. Intention to treat analysis of data was performed. The final statistical analysis was performed using SPSS 20.0 software (SPSS Inc, Chicago, IL, USA)

RESULTS

Of the 162 VLBW babies admitted to the NICU during the study period, 104 VLBW newborns were enrolled; 52 in each group. ***Fig.* 1** shows the flow of study subjects through the phases of the study.

The baseline characteristics are depicted in ***Table* I**. The groups were comparable except for slightly more number of Caesarean deliveries in the no probiotic group (***Table* I**). There were 5 extremely preterm babies (4 in control and 1 in probiotic group) and 15 extremely low birth weight babies (10 in control and 5 in probiotic group). Enteral feeding was initiated at a similar postnatal age in the probiotic and no probiotic groups. Oral supplementation with probiotics began in parallel with enteral feeding (<24 h after initiation of feeds). A mean duration of 26.3 (17.6) days of probiotic supplementation was received in the intervention group. 22 babies in the probiotic group and 25 in the control group were exclusively fed with breast milk.

The primary outcome of time to full enteral feeding was 11.2 (8.3) days in probiotic *vs* 12.7 (8.9) in no probiotic group and was not significantly different (***Table* II**). The secondary outcomes were also comparable between the groups. Treatments such as duration of ventilation and antibiotic usage, and other co morbidities like intraventricular hemorrhage, patent ductus arteriosus, respiratory distress syndrome and bronchopulmonary dysplasia were not significantly different between the two groups.

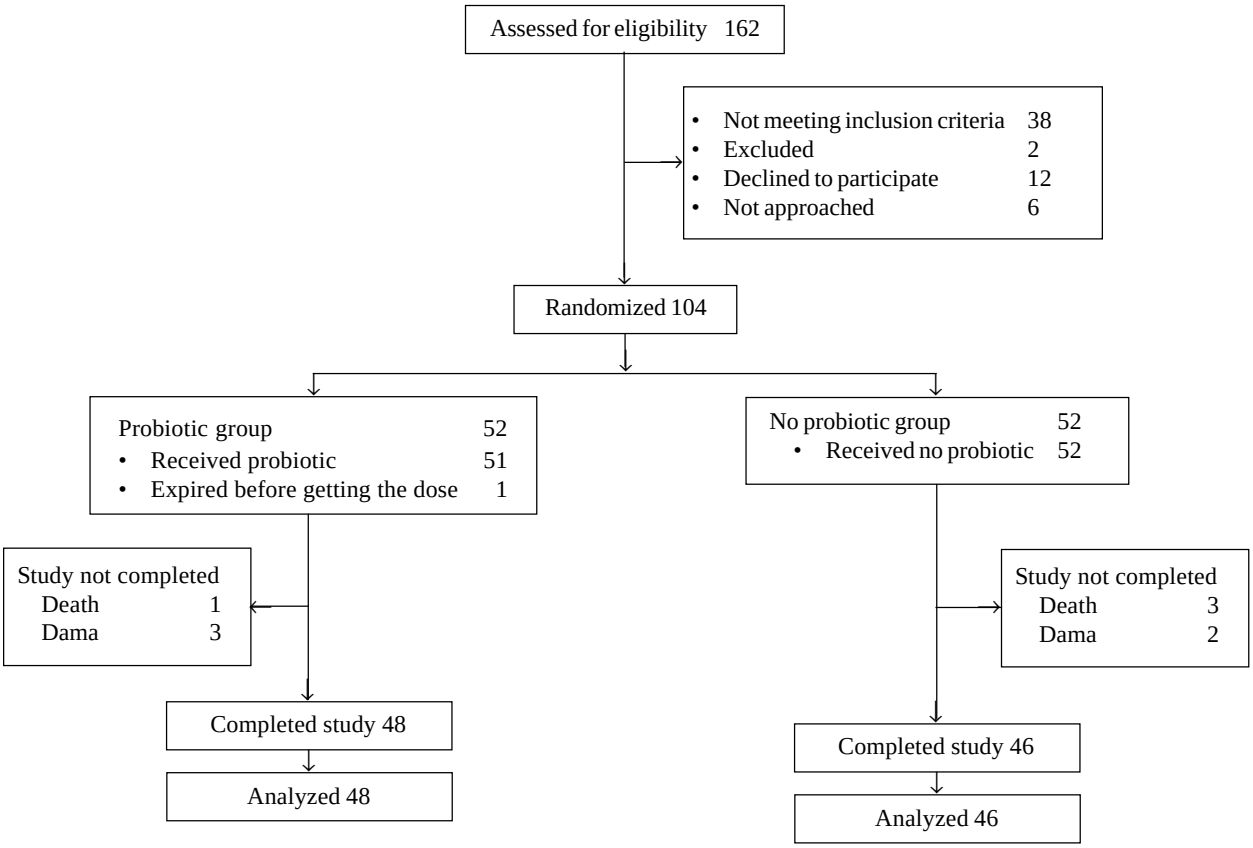

FIG. 1 *Trial flow.*

TABLE I BASELINE CHARACTERISTICS OF PARTICIPANTS

Characteristics	*Probiotic (n=52)*	*No probiotic (n=52)*
Gestational age *(wk)	31.2 (2.1)	31 (2.1)
Sex (M:F)	27:25	20:32
Birth weight *(g)	1256 (185)	1190 (208)
Out born, *n* (%)	10 (19.2)	8 (15.3)
Primigravida, *n* (%)	30 (57.6)	31 (59.6)
Small for gestation, *n* (%)	18 (34.6)	19 (36.5)
Gestational hypertension, *n* (%)	21 (40.3)	31 (59.6)
Abnormal doppler, *n* (%)	12 (23)	9 (17.3)
SNAPPE score*	6.7 (7.9)	8.3 (9.9)
Caesarean delivery, *n* (%)	27 (51.9)	38 (73)
Adequate antenatal steroids, *n* (%)	27 (51.9)	27 (51.9)
APGAR at 1 min*	6.6 (2.6) {*n*= 45}	6.7 (1.5) {*n*= 46}
APGAR at 5 min*	8 (0.8) {*n*= 45}	8 (1.0) {*n*=46}
Age at initiation of enteral feeds# (hrs.)	15 (6,51)	17 (9,47.5)

**Mean (SD); #Median (IQR).*

No unexpected adverse events were observed during the course of the study. There was no significant difference in the incidence of nosocomial infection, including fungal sepsis, in the probiotic group.

DISCUSSION

The present RCT comparing use of probiotic *versus* no probiotic did not observe any significant reduction in the time to reach full enteral feeding. However, VLBW infants receiving probiotics reached full feeds 1.5 days earlier, which was similar to the result of recently published systematic review [9].

The strength of the study was the blinding used. We did not evaluate the successful colonization of infants' gut by the organism. The sample size derived from our pilot study was probably over-optimistic on the expected effect size, as the difference observed was much smaller.

The results of our study were similar to other studies [6-12]. The RCTs which have shown a positive effect on time to full feeds are the ones from India [13] and France [6]. However, the Indian study enrolled infants at 5 days and allocation concealment, and blinding of intervention and outcome was not adequately described [14], and the French study [6] showed a benefit only in >1000 g babies. In our RCT, feeds were initiated at a median of 15 and 17 hours, respectively, in the probiotic and no probiotic group which was comparatively early compared to other RCTs which report a mean of about 3 to 5 days. Though many trials and the Cochrane review [14] have shown a favorable impact on time to reach full feeds with probiotics, *i.e.* three days earlier than the control group (95% CI: 2.78 to 3.69 days, $P<0.001$), many recent trials cited earlier have shown no improvement. The guidelines for use of probiotics have been published [15] a few months after initiation of our study. However, on review, our methodology was consistent with most of the recommendations. There could be several explanations for the observed results. It could be because of predominant use of human breast milk in our NICU which is a rich natural source of probiotic organisms and protects against NEC [16]. Most studies showing benefit in NEC have had a significant use of non-human milk. The second reason could be cross-contamination resulting in nosocomial acquisition of probiotic strains by the other group in the unit as evidenced by Kitajima [17] resulting in narrowing of differences between the two groups. Cross-contamination in the control arm is expected to underestimate the true effects of probiotics. A significantly higher number of caesarean deliveries in the

TABLE II OUTCOMES IN PRETERMS IN PROBIOTIC AND CONTROL GROUPS

Parameter	*Probiotic group (n=48)*	*No probiotic group (n=48)*	*Mean difference (95% CI)*	*P value*
Time to reach full feeds in days[1]	11.2 (8.3)	12.7 (8.9)	-1.5 (-4.9,1.9)	0.4
Duration of hospital stay (days)*	27.6 (18.5)	31.2 (22.9)	-3.6 (-11.7,4.5)	0.4
Duration of total parenteral nutrition (days)*	9.5 (8.3)	10.5 (9)	-1.0 (-4.4,2.4)	0.5
Number of episodes of feed intolerance#	1 (0,2)	1(0,2)	0 (0.7,0.7)	1.0
Number of withheld feeds#	21 (1,40.5)	12 (0,48)	2.7 (-14.7,20)	0.8
Weight gain per week (g)*	31.1(27)	39.5 (32.3)	-8.4 (-20,3.2)	0.2
Necrotizing enterocolitis ≥stage II (%)	2 (4.1)	6 (12.5)	3 (0.6,14.2)$	0.3
Mortality, *n* (%)	1(1.9)	3 (5.7)	3 (0.3,27.9)$	0.6

**Mean(SD); #Median (IQR); $Relative risk (95% CI).*

no probiotic group (52% *vs* 73%) could have narrowed the differences. The intestinal flora of caesarean delivered infants is altered and characterized by a substantial absence of *Bifidobacteria sp*., and vaginally delivered is characterized by predominant groups such as *B. longum* and *B. catenulatum*. Therefore, the infants who would have probably benefited more by probiotics were in the control arm. It may also be due to the different strains used in our study which were chosen on the basis of availability and existing literature of the time.

In conclusion, in the present double-blind randomized trial, oral supplementation with multicomponent probiotic formulation of *L. acidophilus, L. rhamnosus, B.longum* and *S. boulardii* did not improve the gastrointestinal tolerance to enteral feeding in very-low-birth weight infants, but was a safe intervention. We suggest larger multicentric trials of probiotic supplementation to achieve early feed tolerance before accepting/rejecting probiotics for wider clinical use.

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