

Role of probiotics in prevention of necrotizing enterocolitis in preterm neonates

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ABSTRACT

Background: Necrotizing enterocolitis (NEC) is one of the common emergencies in preterm neonates which are associated with high morbidity and mortality despite recent advances in neonatal care. Probiotics may be one of the most effective therapies for the prevention of NEC. **Objective:** The objective of this study is to evaluate the role of probiotics in reducing the incidence of NEC in preterm newborns. **Methods:** This was a prospective randomized control study conducted at tertiary care teaching hospital. A total of 140 preterm newborns of gestation ≤ 34 weeks of age were enrolled in the final analysis. The recruited newborns were randomized into intervention group and control group by simple random sampling method. The intervention group was fed probiotics mixed with expressed breast milk, and the control group was fed with milk alone. **Result:** The incidence of NEC in probiotics group was significantly lower than in the control group (2.86% vs. 11.43%), ($p=0.04$). Although there were no significant differences in the initial presentation of NEC between the two groups, those in the study group who developed NEC had less severe disease, based on Bell's staging criteria. There was no significant difference in terms of age in diagnosis of NEC and in age at which birth weight is gained between the two groups. However, there was a statistically significant difference in duration of hospital stay (15.62 ± 2.84 vs. 23.54 ± 3.43 days; $p < 0.001$) and time to reach full feed (15.82 ± 3.15 vs. 20.22 ± 2.14 ; $p < 0.001$). There was no significant difference in incidence of overall mortality ($p=0.209$; relative risk [RR] 0.25, 95% confidence interval [CI]: 0.029–2.18) and sepsis rate ($p=0.673$; RR 1.15, 95% CI: 0.593–2.243) between the two groups. **Conclusion:** Probiotics supplementation reduced the incidence and severity of NEC in the preterm neonates. This resulted in shorter duration of hospital stay and faster achievement of full oral feeds.

Key words: *Necrotizing enterocolitis, Preterm, Probiotics, Sepsis*

Necrotizing enterocolitis (NEC) is one of the most common gastrointestinal emergencies in preterm newborns [1]. Despite recent advances in neonatal care, the incidence of NEC and the associated mortality and morbidity remain high [2]. NEC occurs more commonly in the premature infants. The incidence of NEC increases with decreasing gestational age and birth weight. The incidence of NEC varies among NICUs worldwide but ranges from 3% to 28% with an average of 7% in infants with birth weight < 1500 g [3]. The overall mortality related to NEC ranges between 20 and 30%, with the highest rate among infants requiring surgery [4]. The mortality rate in infants weighing < 1500 g can be as high as 45% [5].

Both medical and surgical management play important role in the management of NEC, but prevention is definitely a better choice to reduce overall morbidity and mortality. Probiotics may be one of the most effective therapies for the prevention of NEC. It may prevent NEC by promoting colonization of gut with beneficial organism, preventing colonization by harmful bacteria, improving the gut maturity and mucosal barrier function, and modulating immune system.

Many western studies have indicated the beneficial effects of probiotics in preterm neonates without any significant adverse

effects [6-8]. Studies based on the use of probiotics in preterm neonates in the prevention of NEC from India are limited [9,10]. Therefore, this study has been undertaken to assess the efficacy of probiotics in prevention of NEC in preterm neonates and its role on secondary outcomes such as length of hospital stay, age of onset of NEC, regaining birth weight, and time to reach full feed.

MATERIALS AND METHODS

This prospective randomized controlled interventional study was conducted in a tertiary care teaching hospital from March 2014 to August 2015, for 1½ year. The study was carried out after obtaining prior approval from the Institutional Ethical Committee. An informed consent was taken from parents. Clinically stable preterm neonates ≤ 34 weeks of gestation admitted to neonatal unit were included in the study. Sick preterm neonates not on oral feeds or newborns with major congenital anomaly were excluded from the study. Based on an estimated reduction in the incidence of NEC from 11% in non-intervention group to 5% with treatment, to achieve the power of 80% and α error of 5%, we calculated that a minimum of 140 sample size was required. Fig. 1 shows the study inclusion flowchart.

Detailed history and examination, with special emphasis to known risk factors associated with NEC, was carried out. The recruited newborns that were fulfilling the above criteria were randomized into intervention group and control group by simple random sampling method. The intervention group had received probiotics within 1st 10 days of life when the infant is ready for enteral feeds and continued for a minimum period of 7 days or till the corrected gestational age of 35 weeks. Probiotics were mixed with expressed breast milk and fed twice daily in infants with study group. The control group was fed with milk alone.

The progress of the neonate giving special importance to feed intolerance (defined as the presence of gastric aspirate in the amount that was more than half of the previous feeding, or with abdominal distension by monitoring abdominal girth or occult blood in stools) was carried out every day. If the diagnosis of NEC was established, feeding as well as probiotics was stopped and the newborn was assigned a stage according to Modified Bell's Staging Criteria [11]. Probiotic sachets were used in this study amounted each sachet of 1gm, which was a combination of probiotics not <1.25 billion cells of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces boulardii*.

The data analysis was performed using the SPSS version 17 and MicroSoft Excel sheet. Parametric data were presented as mean±standard deviations. Relationships between variables were analyzed using Chi-square test and independent sample t-test. The $p < 0.05$ was considered statistically significant.

RESULT

During 1½ year of the study period, a total number of 145 preterm newborns were included in the analysis. They were assigned randomly to interventional group (72) and control group (73). Of these, one newborn from intervention group could not receive a minimum of 7 days of probiotics and hence excluded (Fig. 1). Two newborns from the control group were discharged against medical advice, and one patient from each group had withdrawn consent before completion of the study. Table 1 presents the baseline characteristics of population studied. There was no significant difference between two groups in demographic profile ($p > 0.05$). There were no large for gestational age babies in both groups.

The risk factors associated with NEC had shown in Table 2. There was a slightly higher number of newborns treated for patent ductus arteriosus (PDA), who received packed RBC transfusion and were given mixed feeding in probiotics group. However, none of the risk factors is statistically significant in terms of a number of preterm newborns assigned in each group ($p > 0.05$). This means that risk factors for NEC had not influenced outcomes of the study in either group. All babies with PDA were treated with syrup Ibuprofen.

The efficacy of probiotics in reducing the incidence of NEC has shown in Table 3. Of 140 newborns studied, 10 (7.14%) had developed NEC. The incidence of NEC in probiotics group and control group was 2.86% and 11.43%, respectively, which was statistically significant ($p = 0.04$). Seven of 10 (70%) newborns

had stage I NEC. Three babies in control group had stage 2 NEC but none in probiotics group. There were no cases of NEC of stage \geq III in either group.

Table 4 summarizes the effect of probiotics on the secondary outcomes. There was no significant difference in age in the diagnosis of NEC and the age of gaining of birth weight between two groups. However, there was a statistically significant difference in duration of hospital stay and time to reach full feed between interventional and control group ($p < 0.001$).

Overall culture-proven sepsis rate was 20%. As shown in Table 5, there was no difference in the incidence of sepsis between

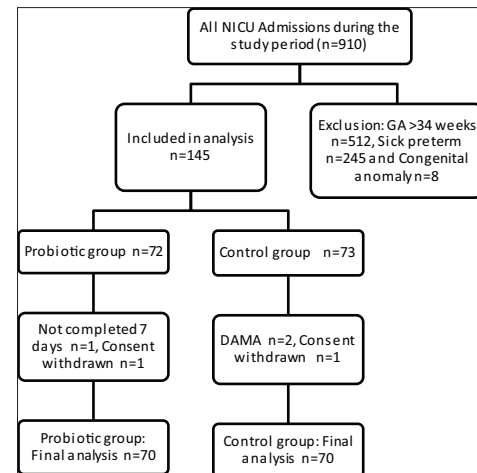


Figure 1: Study inclusion flowchart. GA: Gestational age, DAMA: Discharged against medical advice

Table 1: Population characteristics of two groups

Characteristics	Interventional group (n=70) (%)	Control group (n=70) (%)
Gender		
Male	38 (54.29)	41 (58.57)
Female	32 (45.71)	29 (41.43)
Gestational age (weeks)		
<28	2 (2.86)	3 (4.29)
28–32	35 (50.00)	36 (52.43)
>32	33 (47.14)	31 (44.29)
Birth weight (g)		
<1000	2 (2.86)	3 (4.29)
1000–1500	41 (58.57)	38 (54.29)
>1500	27 (38.57)	29 (41.43)
Weight for gestation		
SGA*	10 (14.29)	14 (20.00)
AGA*	60 (85.71)	56 (80.00)
Mode of delivery		
Vaginal	42 (60.00)	38 (54.29)
Cesarean	28 (40.00)	32 (45.71)
Mural status		
Inborn	63 (90.00)	59 (84.29)
Out born	7 (10.00)	11 (15.71)

*SGA - Small for gestational age, AGA - Appropriate for gestational age

probiotics and control group ($p=0.673$; relative risk [RR] 1.15, 95% confidence interval [CI]: 0.593–2.243). *Staphylococcus aureus* 13 (46%) were the most common bloodstream isolates, followed by *Klebsiella pneumoniae* 9 (32%), *Acinetobacter baumannii* 3 (11%), and *Candida species* 3 (11%). There was no significant difference between two groups in organism isolated. There was almost equal risk of death among preterm neonates with NEC (one from probiotic group and 4 from control group with NEC). This means that the use of probiotics did not reduce the overall mortality rate ($p=0.209$; RR 0.25, 95% CI: 0.029–2.18).

DISCUSSION

NEC is one of the life-threatening emergencies in premature neonates. Data obtained from large multicenter study showed a mean prevalence of 7% in infants weighing <1500 g with an estimated mortality of 15–30% [12]. Most of the infants in whom NEC develops were previously fed, and the disease usually occurs after a week after initiation of enteral feeding. However, orally fed probiotics may be a promising new approach for the prevention of NEC in preterm neonates.

Wang *et al.* conducted a meta-analysis of 20 updated randomized controlled trials of probiotics use in preterm infants with very low birth weight in reducing NEC and mortality, and they had found that the overall incidence of NEC of stage 2 or higher was 3.0% and 7.4%, respectively, for probiotics group and control group [7]. Another meta-analysis conducted by Yang *et al.* concluded that, regardless of gestational age and NEC stage, probiotic supplementation could significantly reduce the risk of NEC in preterm infants. Analysis also indicated that such supplementation did not increase the incidence risk of sepsis or mortality. Further, the study showed that probiotics use may have no adverse effect on normal feeding and growth [13].

However, the results of the study performed by Sari *et al.* [14] and Dani *et al.* [15] suggested no significant difference in reduction of NEC or death in preterm neonates <33 weeks or birth weight <1500 g. The use of single probiotics agent rather than multiple agents may explain the smaller treatment effect in their study.

In the present study, the incidence of NEC in probiotics group was much lower compared to control group (2.86% vs. 11.43%) which was statistically significant ($p=0.04$). Although there were no significant differences in the initial presentation of NEC between the two groups, those in the study group who did develop NEC had less severe disease, based on Bell's staging criteria. Similar observation was made by Arora *et al.* (1.33% vs. 16%; $p=0.001$) who used a similar strain of probiotics as we did in our study [9]. Another study by Bin-Nun *et al.* who used different strain of probiotics had found reduced incidence (4% vs. 16.4%; $p=0.031$) and severity of NEC (Bell's criteria 2.3 \pm 0.5 vs. 1.3 \pm 0.5; $p=0.005$) in probiotics supplemented group [16].

In our study, there was no significant difference in age in the diagnosis of NEC and age of gaining birth weight between two groups which was similar to other study [16]. However, there was a statistically significant difference in duration of hospital stay and time to reach full feed between interventional and

Table 2: Risk factors for NEC

Characteristics	Probiotics group (n=70) (%)	Control group (n=70) (%)
PROM	16 (22.86)	18 (25.71)
Incomplete or no antenatal steroids*	6 (8.56)	8 (11.43)
Reversal of diastolic blood flow on USG	1 (0.14)	0
Pregnancy-induced hypertension	15 (21.43)	18 (25.71)
Birth asphyxia	4 (5.71)	3 (4.28)
PDA treatment	11 (15.71)	9 (12.86)
UAC	6 (8.57)	4 (5.71)
Packed RBC transfusion	13 (18.57)	10 (14.29)
Type of feeding		
Only breast milk	52 (74.29)	57 (81.43)
Mixed (breast and formula)	11 (15.71)	7 (10.00)
Only formula	7 (10.00)	6 (8.57)

PROM: Premature rupture of membrane, PDA: Patent ductus arteriosus, UAC: Umbilical artery catheterization, RBC: Red blood cell, USG: Ultrasonography
*Incomplete course of antenatal steroid is defined as mother had received antenatal steroids <24 h or >7 days before delivery. NEC: Necrotizing enterocolitis

Table 3: Incidence NEC

Bell's staging	Probiotics (n=70)	Control (n=70)	Total (n=140)
No NEC	68 (97.14)	62 (88.57)	130 (92.86)
Stage IA	1 (1.42)	5 (7.14)	6 (4.29)
Stage IB	1 (1.42)	0	1 (0.71)
Stage IIA	0	2 (2.86)	2 (1.43)
Stage IIB	0	1 (1.42)	1 (0.71)
Stage IIIA	0	0	0
Stage IIIB	0	0	0

NEC: Necrotizing enterocolitis

control group ($p<0.001$). Similarly, Chowdhury *et al.* reported significantly shorter duration of hospital stay (15.82 \pm 2.94 days vs. 19.57 \pm 4.26 days; $p<0.001$) and shorter age of achievement of full feeding (14.88 \pm 3.15 vs. 18.80 \pm 4.32 days; $p<0.001$) in the study group [17].

In our study, there was no significant difference in the incidence of overall mortality ($p=0.209$; RR 0.25, 95% CI: 0.029–2.18) and sepsis rate ($p=0.673$; RR 1.15, 95% CI: 0.593–2.243) between the two groups. Sreenivasa *et al.* reported (28 of 100 vs. 42 of 100, $p=0.038$) significantly lower incidence of sepsis in probiotics group [10]. In contrast, Lin *et al.* found that probiotics use may increase the risk of sepsis in infants weighing <750 g [18].

In the present study, there was no significant difference in demographic and clinical variables of two groups studied. Antenatal risk factors also did not differ significantly between two groups. We included only clinically stable neonates in our study. Hence, our data cannot be applied to sick preterm neonates, though NEC is more common in these sick newborns.

Table 4: Effect of probiotics on secondary outcomes

Characteristics	(Mean±SD)		p
	Probiotics group (n=70) days	Control (n=70) days	
Length of hospital stay	15.62±2.84	23.54±3.43	<0.001
Age of onset of NEC	10.52±2.14	9.98±2.62	0.18
Time to reach full feed*	15.82±3.15	20.22±2.14	<0.001
Birth weight regained	12.53±1.24	12.84±2.22	0.31

*Full enteral feeding was defined as an enteral milk intake of 150 ml/kg/day. SD: Standard deviation, NEC: Necrotizing enterocolitis

Table 5: Effect of probiotics on sepsis and mortality

Characteristics	Probiotics group (n=70)	Control (n=70)	Total (n=140)
Sepsis	15 (21.43)	13 (18.57)	28 (20.0)
Mortality	1 (1.43)	4 (5.71)	5 (3.57)

CONCLUSION

Probiotics supplementation reduced the incidence and severity of NEC in the preterm neonates. It was also associated with shorter duration of hospital stay and faster achievement of full oral feeds. There was no significant difference in mean age of onset of NEC, mortality, and sepsis rate.

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