

## Original Research Article

# Role of probiotics in clinical course and outcome of preterm neonates

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### ABSTRACT

**Background:** In a quest to improve the outcome of preterm neonates and to reduce neonatal mortality a lot of research has been done in the field of neonatology; one such intervention is the use of probiotics. We evaluated the role of probiotics in reducing the incidence and severity of NEC, sepsis, hyperbilirubinemia and in improving enteral feeds in preterms.

**Methods:** A prospective randomized control trial was conducted in preterm neonates <37 weeks of gestation from December 2017 to November 2019. They were randomized into test and control groups. The neonates in the test group were fed with probiotics with breast milk twice daily till they reach full feeds. Babies in the control group were fed with breast milk alone. Various parameters were compared between two groups like incidence of NEC, sepsis etc.

**Results:** Sixty preterm neonates were enrolled, 30 in the test group and 30 in the control group. The demographic and clinical variables were similar in both groups. The incidence of NEC and sepsis in the test group is less compared to the control group. Duration of phototherapy was less in test group ( $1.1 \pm 0.3$  days) compared to control group ( $1.9 \pm 0.9$  days). Duration of hospital stay in test group was less ( $8.9 \pm 2.1$  days) compared to the control group ( $11.4 \pm 3.7$  days).

**Conclusions:** Prophylactic probiotics have a beneficial role in prevention of NEC and sepsis, reducing duration of phototherapy and duration of hospital stay in preterm neonates.

**Keywords:** Probiotics, Preterm neonates, NEC, Sepsis

### INTRODUCTION

11% of all infants are born premature, and this population thus represents 12.9 million infants per year worldwide.<sup>1</sup> India ranks number one among top 10 countries with greatest number of preterm births with about 3.5 million each year.<sup>2</sup> Neonatal mortality rate (NMR) is 21.7 deaths per 1000 live births in the year 2019.<sup>3</sup> 41% of under-five deaths is constituted by neonatal deaths. 75% of neonatal deaths occur within first week. Complications of preterm birth is the important cause for under-five deaths. Respiratory distress syndrome, sepsis, NEC, apnea of prematurity, retinopathy of prematurity, intraventricular hemorrhage and patent ductus arteriosus are some of the complications of preterm.

NEC occurs in 2-5% of all NICU admissions and 5-10% of VLBW babies.<sup>4</sup> It is estimated that up to 20% of the neonates develop sepsis and approximately 1% die due to sepsis related causes.<sup>5</sup> Of late growing interest has been shown in the field of probiotics in improving the survival and prevention of dreaded complications in preterm.

During the early neonatal period, the relation between intestinal micro flora and nutrition is the most important factor for normal intestinal and immune system development, particularly in the premature infant. Early colonization of the infant's gastrointestinal tract with non-pathogenic intestinal micro biota is crucial for the overall health of the infant and may prevent the development of intestinal tract inflammation. With the advancement of

modern methods of neonatal care in NICU settings it has been shown that there is a delayed or deficient colonization with non-pathogenic microflora and increased colonization with coli form organisms. The other advantage of probiotics is improvement in feed tolerance in preterm including those with IUGR who have a higher risk of developing infections. The exact mechanism of how probiotics help is not clear. The commonly used probiotics like lactobacillus and Bifidobacterium species act by producing lactic acid and propionic acid which lower the intestinal PH and help in establishing gut flora. The other postulated mechanisms include that they act at the level of immune system through apoptotic mechanisms, stimulating cell survival, cell adhesion and angiogenesis.

Our objective is to study the effectiveness of probiotics in prevention of NEC and sepsis, and in reducing the duration of hospital stay and mortality.

## METHODS

This study is a prospective randomized controlled trial conducted at NICU of tertiary care teaching hospital, Karimnagar, Telangana from December 2017 to November 2019. This trial was conducted to study the role of probiotics in clinical course and outcome of preterm neonates. Based on the previous study experiences and consultation with experts, a sample size of 60 was selected by simple random sampling.

### Inclusion criteria

Preterm neonates (gestational age <37 weeks). Birth weight <2500 grams. Hemodynamically stable. Intervention to be given for at least 7 days.

### Exclusion criteria

Severe cardiopulmonary illness. Gestational age >37 weeks. Birth weight >2,500 grams. Presence of perinatal asphyxia. Congenital anomalies. Parental refusal.

Sixty babies were selected strictly based on inclusion and exclusion criteria. Of the 60 babies analyzed, 30 babies were randomized to test group and 30 to control group, after obtaining informed parental consent. Babies in the test group received probiotics and were compared with the control group. The test group received their regular feeds plus daily probiotic supplements 1 gram/day (2.5 billion cells) (*lactobacillus acidophilus*, *lactobacillus rhamnosus*, *bifidobacterium longum*, *saccharomyces boulardii*) in two divided doses mixed with expressed breast milk from the onset of enteral feedings till the baby reached full feeds. The control group was fed with breast milk without the addition of probiotics. Feeding was started when the infant had stable vital signs, normal bowel sounds without abdominal distension and no bile or blood from nasogastric tube. A strict feeding protocol was followed for all study neonates. Depending on the birth weight and

gestational age of the neonate, expressed breast milk is started at 10-20 ml/kg/day. The amount of feeding was advanced slowly if tolerated with no more than a 20 ml/kg increment per day up to 150-180 ml/kg/day. Feeding was stopped if there were any signs of feeding intolerance (defined as the amount of gastric aspirate that was more than half of previous feeding or with abdominal distension). Standard practice guidelines as followed in our NICU for the care of these babies were carried out in both groups. On admission to NICU a septic work up which included complete blood count, C-reactive protein and blood cultures were done for all the babies. Whenever a study infant was suspected to have NEC, clinical status and abdominal films were reviewed and if the diagnosis of NEC was established, the newborn was assigned a score according to the Bell's staging criteria.

Results were analyzed by Fischer exact 't' test and one-way ANOVA for primary outcomes like incidence and severity of NEC in test vs. control groups and secondary outcomes like neonatal mortality, sepsis, time to establish full enteral feeds (days), duration of phototherapy(days) and duration of hospitalization (days).

All the statistical methods were carried out through the Statistical package for social sciences (SPSS) for Windows (Version25).

## RESULTS

A total 93.3% (28) of cases and 90% (27) of controls were admitted on day 1 of life. 6.7% (2) of cases and 10% (3) of controls were admitted on day two of life. Among 30 cases, 23 (76.7%) were male babies and 7 (23.3%) were female babies. Among 30 controls, 19 (63.3%) were male babies and 11 (36.7%) were female babies. 7 (23.3%) cases and 11 (36.7%) controls had birth weight below 1.5 kgs. 18 (60%) cases and 12 (40%) controls had birth weight between 1.5 and 2 kgs. 5 (16.7%) cases and 7 (23.3%) controls had birth weight above 2 kgs. 5 (16.7%) cases and 8 (26.7%) controls were born at <32 weeks. 20 (66.7%) cases and 17 (56.7%) controls were born between 33 and 35 weeks. 5 (16.7%) cases and 5 (16.7%) controls were born at >36 weeks.

A total 16 (53.3%) cases and 10 (33.3%) controls were SGA babies. 13 (43.3%) cases and 19 (63.3%) controls were AGA babies. 1 (3.3%) case and 1 (3.3%) control were LGA babies. 14 (46.7%) cases and 16 (53.3%) controls were born to primi mothers. 16 (53.3%) cases and 14 (46.7%) controls were born to multiparous women (Table 1).

A total 15 (50%) cases and 13 (43.3%) controls were born to mothers with complications like preeclampsia, PROM, eclampsia, GDM, hypothyroidism and dengue fever. 22 (73.3%) cases and 19 (63.3%) controls were delivered by LSCS. 8 (26.7%) cases and 11 (36.7%) controls were delivered by NVD. The mean age of initiation of feeds in test group is 2.0±0.9 days and in control group is 1.9±0.7

days. Mean age to reach full feeds in test and control group were  $7.2 \pm 1.8$  days and  $8.2 \pm 2.8$  days respectively (Table 3).

A total 1 (3.3%) case and 4 (13.3%) controls developed NEC which is statistically highly significant ( $p < 0.001$ ). Mean age of onset of NEC in the test group was 5 days and in the control group was 4.3 days. More severe NEC i.e. stage 2 and stage 3 were seen in control group. NEC was less severe in the probiotic group. There is no significant difference between the test and control group in different stages of NEC ( $p > 0.05$ ). Although the difference was not significant, it is observed that the baby who died with NEC was from the control group (Table 2).

A total 5 (16.67%) cases and 13 (43.3%) controls developed sepsis which was statistically significant ( $p < 0.05$ ). 10 (33.3%) cases and 12 (40%) controls developed jaundice. The mean age of onset of jaundice in probiotic group is  $4.0 \pm 1.6$  days and in control group is  $3.6 \pm 1.2$  days which is statistically not significant ( $p > 0.05$ ). The mean duration of phototherapy in case group is  $1.1 \pm 0.3$  days and in control group is  $1.9 \pm 0.9$  days which is statistically significant (P value of 0.01). The mean duration of hospital stay in test and control groups were  $8.9 \pm 2.1$  days and  $11.4 \pm 3.7$  days respectively, which is statistically significant ( $p < 0.01$ ) (Table 3).

**Table 1: Distribution of cases and controls for a given parameter.**

		Cases (%) Total=30	Controls (%) Total=30
Age of admission	Day 1	28 (93.3)	27 (90)
	Day 2	2 (6.7)	3 (10)
Sex	Male	23 (76.7)	19 (63.3)
	Female	7 (23.3)	11 (36.7)
Birthweight	<1.5 kgs	7 (23.3)	11 (36.7)
	1.5 - 2 kgs	18 (60.0)	12 (40.0)
	>2 kgs	5 (16.7)	7 (23.3)
Gestational age (GA)	<32 weeks	5 (16.7)	8 (26.7)
	33 - 35 weeks	20 (66.7)	17 (56.7)
	$\geq 36$ weeks	5 (16.7)	5 (16.7)
Weight for GA	SGA	16 (53.3)	10 (33.3)
	AGA	13 (43.3)	19 (63.3)
	LGA	1 (3.3)	1 (3.3)
Parity	Primi	14 (46.7)	16 (53.3)
	Multi	16 (53.3)	14 (46.7)
Antenatal risk factors	Preeclampsia	8 (26.7)	4 (13.3)
	PROM	3 (10.0)	3 (10.0)
	Eclampsia	1 (3.3)	4 (13.3)
	GDM	1 (3.3)	1 (3.3)
	Dengue	2 (6.6)	0 (0.0)
	Hypothyroid	0 (0.0)	1 (3.3)
	No risk factors	15 (50.0)	17 (56.7)
Mode of labour	LSCS	22 (73.3)	19 (63.3)
	NVD	8 (26.7)	11 (36.7)

**Table 2: Neonatal outcome in case group and control group.**

	No. of cases (%)	No. of controls (%)
NEC incidence	1 (3.3)	4 (13.3)
Bell's staging	Stage I	1 (3.3)
	Stage II	0 (0.0)
	Stage III	0 (0.0)
Mortality due to NEC	0 of 1 NEC affected (0.0)	1 of 4 NEC affected (25)
Sepsis incidence	5 (16.7)	13 (43.3)
Incidence of jaundice	10 (33.3)	12 (40.0)

**Table 3: Neonatal outcome in case group and control group**

	Cases	Controls
Age of initiation of feeds (days)	$2.0 \pm 0.9$	$1.9 \pm 0.7$
Age to reach full feeds (days)	$7.2 \pm 1.8$	$8.2 \pm 2.8$

Continued.

	Cases	Controls
<b>Age of onset of NEC (days)</b>	5.0	4.3
<b>Age of onset of jaundice(days)</b>	4.0±1.6	3.6±1.2
<b>Duration of phototherapy (days)</b>	1.1±0.3	1.9±0.9
<b>Duration of hospital stay (days)</b>	8.9±2.1	11.4±3.7

## DISCUSSION

In the present study, majority of neonates were between 1.499-1.999 kg (60% in the test versus 40% in the control) but did not show statistically significant difference between groups. Most of the neonates were admitted on their first day of life. Majority of neonates were between 33-35 weeks of gestation in both groups. Antenatal risk factors also did not differ between two groups. Age of initiation of feeds didn't differ much between test and control group with mean age of initiation of feed being 1.9 days in both the groups.

### *Incidence of NEC*

In our present study, the incidence of NEC was significantly lower in the test group compared with the control group (1 of 30 neonates versus 4 of 30 neonates;  $p < 0.001$ ). Similar observations were seen in studies by Lin et al, Angela, Bin-Nun et al, Lin et al, Janvier et al and Khurana et al.<sup>8,13,14,17,18,20</sup> However studies conducted by Dani et al, Costalos et al and Manzoni et al found a lower incidence of NEC in the probiotic group, but this did not reach statistical significance.<sup>7,15,16</sup>

### *Age of onset of NEC*

In our study, mean age of onset of NEC in the test group was 5 days and in the control group was 4.3 days which was not statistically significant. Two studies done by Lin et al showed similar observations.<sup>18</sup> According to literature, the postnatal age at onset of NEC is inversely related to birth weight and gestational age with a mean age at onset of 12 days.

### *NEC and Bell's staging*

In our study, out of the 5 babies that developed NEC, 3 babies developed stage I NEC, 1 baby developed stage II NEC and 1 baby developed stage III NEC, which was statistically not significant ( $p > 0.05$ ). The study by Lin et al showed more severe NEC (>stage 2) in the control group.<sup>17</sup>

### *NEC and mortality*

In our study, one baby that died with NEC was from the control group. There is a non-significant trend towards less NEC-related mortality in the probiotic group (0 of 1 versus 1 of 4;  $p = 0.065$ ). The study by Nun et al reported similar observations.<sup>13</sup> The study done by Hoyos et al showed NEC-associated mortality is more in the non-probiotic

group (35/1282 vs. 14/1237;  $p < 0.005$ ) which was statistically significant.<sup>14</sup> But here the test group was compared with historic controls. Studies done by Lin et al and Manzoni et al reported a significantly lower mortality rate in the probiotic group but did not differentiate between death attributed to NEC versus other causes.<sup>7,17</sup>

### *Incidence of sepsis*

In our present study, the incidence of sepsis in the test group is less (16%) than the control group (43%), which is statistically significant ( $p = 0.024$ ). The study published by Lin et al, Samanta et al and Uberos et al showed decreased rate of sepsis in probiotic group compared to the control group.<sup>6,18,19</sup> The mechanism for the efficacy of probiotics in reducing the incidence of sepsis in VLBW infants is probably similar to NEC and possibly a result of increased colonization of desirable microflora supplemented through probiotics. But studies by Dani et al and Nun et al did not show any reduced incidence of sepsis in the probiotic group.<sup>13,15</sup> Their studies reported that sepsis was due to pathogens most often related to catheter related infections in both groups and probiotics will act locally in the gut. The primary effect of orally administered probiotics is in the gastrointestinal tract. Hence it is not surprising to see the lack of beneficial effects on other organs such as lungs and central nervous system.

### *Age to reach full feeds*

In our study, the mean age to reach full feeds in test and control group were 7.2±1.8 days and 8.2±2.8 days respectively with no statistical significance ( $p = 0.104$ ). Similar observations were found in the study done by Nun et al, Lin et al, Dani et al and Costalos et al.<sup>13,15-17</sup> Similarly a study done by Shashidhar et al reported the mean (SD) time to reach full enteral feeding was 11.2 (8.3) days in probiotic versus 12.7 (8.9) in no probiotic group ( $p = 0.4$ ).<sup>22</sup>

### *Incidence of jaundice*

In our present study, the incidence of jaundice in the test group is 33% and in the control group is 40% without statistical significance ( $p = 0.592$ ).

### *Age of onset of jaundice*

In our study, the mean age of onset of jaundice in test and control group was 4.0±1.6 days and 3.6±1.2 days respectively without statistical significance ( $p = 0.504$ ).

### Duration of phototherapy

In our study, the mean duration of phototherapy in test and control group was  $1.1 \pm 0.3$  days and  $1.9 \pm 0.9$  days respectively which is significant ( $p=0.01$ ). This view was also echoed by study done by Torkaman et al.<sup>9</sup> Demirel et al reported that the administration of probiotics is effective in reducing the duration of phototherapy in very low birth weight (VLBW) newborns of gestational age  $\leq 32$  weeks with jaundice.<sup>10</sup> Liu et al reported that therapy with probiotics in term newborns with jaundice lowered the serum bilirubin levels significantly in the treatment group compared to the control group.<sup>11</sup>

In a RCT conducted by Chandrasekhar et al on a total of 1043 babies with a gestational age of more than 35 weeks treatment with probiotic reduced the risk of need for phototherapy by 44% (RR 0.56, 95% CI 0.32, 0.99).<sup>12</sup> The median duration of phototherapy in the intervention group was 18 hours (IQR 16.50-24.00) and that of control group was 24 hours (IQR 18.00-48.00) which is statistically significant.

### Duration of hospital stay

In the present study, the mean duration of hospital stay in test and control group was  $8.9 \pm 2.1$  days and  $11.4 \pm 3.7$  days respectively, which is statistically significant ( $p < 0.002$ ). Similar observations were made by Raguz et al, Torkaman et al and Khurana et al.<sup>8,9,21</sup> However Studies done by Lin et al and Lin et al showed no significant difference in the hospital stay between test and control group.<sup>17,18</sup>

### Limitations

The choice of probiotic mixture, the dose and the frequency of dosing need to be discussed because each probiotic organism has a variable rate of colonization. The adverse effects of probiotic supplementation like probiotic associated sepsis could not be analysed in our study. NEC is a multifactorial disease. The other factors contributing to the development of NEC is not analysed in our study. Further studies with larger sample size are needed.

### CONCLUSION

The present study found that probiotic supplementation has reduced incidence of NEC, incidence of sepsis, duration of phototherapy and mean duration of hospital stay in the preterm neonates  $< 37$  weeks of gestational age, but there were no significant differences between test and control groups in age to reach full feeds and age of onset of jaundice. However more research is required involving more sample size to support the use of probiotics in preterm neonates.

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