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Original Research Article

To study the role of probiotics in the prevention of necrotizing enterocolitis in preterm neonates

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ABSTRACT

Background: Necrotizing enterocolitis is defined as an inflammatory bowel necrosis in premature infants and is major cause of morbidity and mortality in neonatal intensive care units throughout the world. We aim to study the role of probiotics in reducing incidence and severity of necrotizing enterocolitis in preterm neonates ≤34 weeks and its role on secondary outcomes like mortality, time to reach full feeds, daily weight gain, days of hospitalization and effect on nosocomial infections.
Methods: This study was a prospective randomized controlled interventional trial conducted in SGRDIMSAR, Amritsar. A sample size of 150 was selected. 75 were randomized to test group and 75 to control group by simple random sampling.
Results: The incidence of NEC was significantly lower in the test group compared with the control group (1 of 75 neonates vs 12 of 75 neonates; p=0.001). The severity of NEC, nosocomial sepsis and mean duration of hospital stay was significantly lower in the test group. Daily weight gain was significantly higher in the test group. There was no significant difference in mean age of onset of NEC, mortality and mean age to reach full feeds in two groups.
Conclusions: Incidence and severity of NEC was less in the probiotic group. Daily weight gain was better, nosocomial sepsis and mean duration of hospital stay were less in the probiotic group.

Keywords: Mortality, NEC, Probiotics, Sepsis

INTRODUCTION

Necrotizing enterocolitis is defined as an inflammatory bowel necrosis in premature infants and is major cause of morbidity and mortality in neonatal intensive care units throughout the world.[1,2] The incidence of NEC varies from 1-3 cases per 1000 live births, 2-5% of very low birth (VLBW) infants and 1-8% of all Neonatal Intensive care unit admissions.[3,4] Population studies from India show incidence to be 5.2% in babies less than 32 weeks gestation.[5] It usually affects preterm neonates (90%) and only 10% are term neonates. Necrotizing enterocolitis affects males and females equally.[6] The cause of this intestinal catastrophe is complex, but common factors associated with this disease are prematurity, immaturity of intestinal tract, intestinal ischemia, microbial colonization with pathogenic organisms and enteral feeding.[7,8] Inflammatory mediators like platelet aggregation factor (PAF) and interleukin 8 (IL-8) have been implicated in the development of NEC.[9-11] A proposed strategy for the prevention of NEC is the administration of oral probiotics. Emerging evidence suggests that probiotics may have a role in the control or prevention of NEC by reducing intestinal colonization with pathogenic organisms, reinforcing intestinal barrier and alleviating intestinal inflammation. Functions such as promotion of fermentation to produce organic acids and production of antimicrobial bacteriocins and fatty acids

add further theoretical support to their role in the protection of NEC. Lastly, their colonization might reduce the pro-inflammatory mediators responsible for the intestinal tissue damage.[7,8]

Present study was undertaken to study the role of probiotics in reducing incidence and severity of necrotizing enterocolitis in preterm neonates ≤34 weeks and its role on secondary outcomes like mortality, time to reach full feeds, daily weight gain, days of hospitalization and effect on nosocomial infections.

METHODS

This study was a prospective randomized controlled interventional trial conducted from January 2015 to June 2016 in Neonatal Intensive Care Unit of Department of Pediatrics at Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Sri Amritsar after ethical committee clearance. A sample size of 150 was selected. 75 were randomized to test group and 75 to control group by simple random sampling after informed parental consent.

Inclusion criteria

- Preterm neonates (gestational age ≤34 weeks)
- Hemodynamically stable.

Exclusion criteria

- Gestational age >34 weeks
- Significant Cardiorespiratory illness
- Parental refusal.

Gestational age was recorded according to LMP. Birth weight/weight on admission was recorded. Feeding was started when the infant had stable vital signs, normal bowel sounds without abdominal distension and no abnormal gastric aspirates. Depending on the birth weight and gestational age of the neonate, expressed breast milk was 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 upto 150-180 ml/kg/day. Feeding was stopped if there was any sign of feeding intolerance (defined as the presence of gastric aspirate in the amount that was more than half of previous feeding, or with abdominal distension, or occult blood in stools).

Prefeed aspirates, abdominal girth, bowel sounds, character of stools, daily weight, amount of feed/kg/day was noted daily. Investigations like hemoglobin, hematocrit, CBC, peripheral blood film, electrolytes and stool for occult blood was done. CRP (quantitative) was done using NycoCard CRP test. Blood cultures were done by using BACTEC and Vitek-2 system. Two blood cultures were sent, one on admission and the other after 5 days of admission. X-ray abdomen erect was done if indicated. Whenever a study neonate was suspected to have NEC, clinical status and abdominal films were reviewed and if the diagnosis of NEC was established, probiotic was stopped and the newborn was assigned a stage according to Modified Bell`s Staging Criteria.

The test group received their regular feeds plus daily probiotic supplement mixed with expressed breast milk, as soon as the enteral feeds were tolerated, 12 hourly for 2 weeks. The control group was fed with breast milk without the addition of probiotics. Follow up was done for 2 weeks and patient was called again if discharged earlier. Probiotic sachets were used in this study. Each sachet of 1g containing 1.25 billion cells of Saccharomyces boulardii, Lactobacillus rhamnosus, Lactobacillus acidophilus and Bifidobacterium longum was used.

RESULTS

In our study, 150 preterm neonates ≤34 weeks of gestational age were selected based on inclusion and exclusion criteria. They were assigned randomly to test group (75) and control group (75). Test group was fed with probiotics with breast milk and control group only with breast milk. Test and control groups were compared for their age at admission, gender distribution, gestational age, AGA/SGA/LGA, birth weight, mode of delivery.

Table 1: Demographic characteristics of two groups.

	Test (n=75)	Control (n=75)	p-value
Age at admission			
Day 1	75(100%)	72 (96%)	0.382
Day 2-6	0	3 (4%)	
Gender			
Male	54 (72%)	49(65%)	0.379
Female	21 (28%)	26(35%)	
Gestational age			
28-31 wks 6/7 days	6 (8%)	8 (10.66%)	0.807
32-34wks	69 (92%)	67(89.34%)	
Birth weight			
<1500gm	16(21.33%)	20(26.66%)	0.754
≥1500gm	59(78.67%)	55(73.34%)	
AGA	62(82.67%)	54 (72%)	0.119
SGA	13(17.33%)	21 (28%)	
Mode of delivery			
Vaginal delivery	14(18.67%)	13(17.33%)	0.832
Caesarean section	61(81.33%)	62(82.67%)	

75 babies (100%) in the test group and 72 (96%) in the control group were admitted on day one of their life. 3 babies (4%) in the control group were admitted between 2nd and 6th day of their life. There was no statistically significant difference in the age of admission between test and control groups (p=0.382).

In current study, the number of male babies was 54 (72%) in the test group and 49 (65%) in the control group whereas the number of female babies was 21 (28%) in the test group and 26 (35%) in the control group. There was

no statistically significant difference between the two groups in gender distribution (p=0.379).

In the present study, 69 babies (92%) in the test group and 67 babies (89.34%) in the control group were of 32-34 weeks gestational age. 6 babies (8%) in the test group and 8 babies (10.66%) in the control group were of 28-31 weeks 6/7 days gestational age. There was no statistically significant difference between the two groups in gestational age distribution (p=0.807).

In the present study, 59 babies (78.67%) in the test group and 55 babies (73.34%) in the control group were of birth weight ≥1500 gm. 16 babies (21.33%) in the test group and 20 babies (26.66%) in the control group were of birth weight <1500gm. There was no significant difference in the birth weight of babies between the test and control group (p=0.754).

Out of 150 preterm neonates, 62 (82.67%) were AGA in the test group and 54 (72%) were AGA in the control group. There were 13 (17.33%) SGA neonates in the test group and 21 (28%) SGA neonates in the control group. Difference was not statistically significant (p=0.119).

Caesarean section was done in 61 cases (81.33%) in the test group and 62 cases (82.67%) in the control group. Vaginal delivery was done in 14 cases (18.67%) in the test group and 13 cases (17.33%) in the control group. There was no statistically significant difference regarding mode of delivery in the test and control group (p=0.832).

Table 2: Antenatal risk factors.

Antenatal risk factors	Groups		p-value
	Test	Control	
Nil	53 (70.67%)	52 (69.33%)	
PROM (premature rupture of membranes)	8 (10.67%)	5 (6.67%)	
PIH (pregnancy induced hypertension)	3 (4%)	4 (5.33%)	
Magnesium Sulphate/ Methyl dopa	-	-	X^2 = 0.866 Df=2 Pvalue =0.649
IUGR (intrauterine growth retardation)	6 (8%)	8 (10.67%)	
Reversal of diastolic blood flow on USG foetal well-being and colour Doppler	1 (1.33%)	1 (1.33%)	
PNA (Perinatal asphyxia)	4 (5.33%)	5 (6.67%)	
Total	75 (100%)	75 (100%)	

p-value= 0.649 (Not significant)

Antenatal risk factors were absent in majority of babies in both groups, 53 (70.67%) in test and 52 (69.33%) in control group respectively. History of PROM was present in 8 cases (10.67%) in the test group and 5 cases (6.67%) in the control group. History of PIH was present in 3 neonates (4%) in test group and 4 neonates (5.33%) in control group. History of magnesium sulphate or methyldopa administration to mother was not elicited in both groups. There were 6 cases (8%) of IUGR in the test group and 8 cases (10.67%) in the control group. Reversal of diastolic blood flow was present only in 1 case (1.33%) each in test and control group on assessment of fetal well-being on ultrasonography and colour Doppler study. History of PNA was present in 4 neonates (5.33%) in the test group and 5 (6.67%) in the control group. There was no statistically significant difference in antenatal risk factors between test and control group (P=0.649).

Table 3: Incidence of NEC and Bell staging.

Staging	Groups		Total	p-value
	Test	Control		
No NEC	74 (98.67%)	63 (84%)	137 (91.33%)	
Stage IA	1 (1.33%)	8 (10.67%)	9 (6%)	
Stage IB	0	0	0	X^2 = 10.3 Df = 3 P value = 0.016
Stage IIA	0	3 (4%)	3 (2%)	
Stage IIB	0	0	0	
Stage IIIA	0	0	0	
Stage IIIB	0	1 (1.33%)	1 (0.67%)	
Total	75 (100%)	75 (100%)	150 (100%)	

p-value= 0.016 (Significant)

NEC developed in only one neonate (1.33%) in test group as compared to 12 neonates (16%) in control group. In test group, staging of NEC was IA whereas in control group, 8 cases (10.67%) were classified as stage IA, 3 cases (4%) as stage IIA and 1 case (1.33%) as stage IIIB. Overall incidence and severity was more in control group (p=0.016).

Table 4: Clinical manifestations of NEC.

Clinical manifestations of NEC	Test (n=75)	Control (n=75)	p-value
Stool for occult blood	1 (1.33%)	12 (16%)	0.001
Prefeed aspirates	1 (1.33%)	12 (16%)	0.001
Abdominal distension/signs	1 (1.33%)	12 (16%)	0.001
Abnormal x-ray abdomen	0	3 (4%)	0.080
Absent bowel sounds	0	3 (4%)	0.080

Stool for occult blood, prefeed aspirates and abdominal distension/signs were positive in only 1 case (1.33%) in

the test group and 12 cases (16%) in the control group which was statistically significant (p=0.001). X-ray abdomen was abnormal in 3 neonates (4%) and bowel sounds were absent 3 babies (4%) in control group while these findings were absent in test group cases. This difference was not statistically significant (p=0.08).

Table 5: Showing age of onset and secondary outcomes.

	Test (n=75) Days (Mean ±SD)	Control (n=75) Days (Mean± SD)	Unpaired 't-test' p-value
Age of onset of NEC	12±00	8.50±2.92	0.295
Time to reach full feeds	8.53±2.14	10.70±3.25	0.245
Duration of hospital stay	16.06±0.49	20.04±7.85	0.001

Using unpaired 't-test', there was no statistically significant difference in age of onset of NEC and time to reach full feeds between test and control group. However, there was statistically significant difference in duration of hospital stay between test and control group (p=0.001).

Table 6: Daily weight gain.

Daily Weight gain (gm)	Test	Control	Total	p-value
5-10	14 (18.66%)	37 (49.33%)	51 (34%)	X^2 =19.3 Df = 3 p-value =0.001
10-15	52 (69.34%)	37 (49.33%)	89 (59.33%)	
15-20	9 (12%)	1 (1.34%)	10 (6.67%)	
Total	75 (100%)	75 (100%)	150 (100%)	

p-value= 0.001 (significant)

Daily weight gain of 10-15 gm was observed in 52 cases (69.34%) in the test group and 37 (49.33%) in the control group. Daily weight gain of 5-10 gm was seen in 14 cases (18.66%) in the test group and 37 cases (49.33%) in the control group. Daily weight gain of 15-20 gm was observed in 9 cases (12%) and 1 case (1.34%) in test and control group respectively. Weight gain was statistically significant in the test group (p=0.001).

Definitive sepsis was seen in only 1 case (1.33%) in control group and none in test group which was statistically not significant. Probable sepsis was seen in 11 cases (14.67%) in the test group and 13 cases (17.33%) in control group which was statistically not significant. Nosocomial sepsis was seen in 2 cases (2.67%) in the test group and 21 cases (28%) in the control group which was statistically significant (p=0.001). Out of the two cases in test group, blood culture at fifth day of admission was positive for *Klebsiella pneumoniae* in both cases (100%). Out of 21 cases in control group, blood culture was positive for *E. coli* in 9 cases (42.86%), *Klebsiella pneumoniae* in 6 cases (28.57%), *Acinetobacter baumannii* in 6 cases (28.57%).

Table 7: Pattern of sepsis and mortality.

Sepsis	Test (n=75)	Control (n=75)	p-value
Definitive	0	1 (1.33%)	0.316
Probable	11 (14.67%)	13 (17.33%)	0.656
Nosocomial	2 (2.67%)	21 (28%)	0.001
Mortality	0/1	2/12	0.685

There was only 1 case of NEC in test group which survived. There were 12 cases of NEC in control group; out of which 10 survived and 2 died. This difference was statistically not significant (p=0.685).

DISCUSSION

In the present study, the incidence of NEC was significantly lower in the test group compared with the control group (1 of 75 neonates versus 12 of 75 neonates; p=0.001). Similar observation was seen in study by Bin-Nun A et al. who found a significantly lower incidence of NEC in the probiotic group (4% versus 16.6%; p=0.031).[12] Dani et al. found a lower incidence of NEC (1.4 vs 2.7%) in the probiotic group, but this did not reach statistical significance.[13]

In our study, mean age of onset of NEC in the test group was day 12 of life and in the control group was 8.5 (±2.92) days which was not statistically significant (p= 0.295). In study of Bin-Nun A et al. age at diagnosis of NEC was similar between groups (21±9 days in the study group and 21±14 days in the control group, p=1.00).[12]

In our study, the only case of NEC in test group (1.33%) and 8 cases (10.67%) in control group were in Stage IA. There were 3 cases (4%) in stage IIA and only one case (1.33%) in Stage IIIB in control group. Difference in severity of NEC in two groups was statistically significant (p-value = 0.016). The study by Lin HC et al. showed the incidence of NEC (≥stage 2) to be significantly lower in the study group when compared with the control group (4 of 217 vs 14 of 217, p=<0.02).[14] Similar observations were found in the study conducted by Bin-Nun A et al (1 of 72 (1%) vs 10 of 73 (14%), p =0.013).[12] In present study, there was only 1 case of NEC in test group and no mortality. There were 12 cases of NEC in control group; out of which 10 survived and 2

died. This is statistically not significant (p-value= 0.685). The study by Bin-Nun A et al. reported similar observations. They reported that 3 deaths in the control group were due to NEC, whereas there were no NEC related deaths among the test neonates (p = 0.87).[12]

In our study, nosocomial sepsis was seen in 2 cases (2.67%) in the test group and 21 cases (28%) in the control group which is statistically significant (p-value= 0.001). Out of the two cases in test group, blood culture at fifth day of admission was positive for *Klebsiella pneumoniae* in both cases (100%). Out of 21 cases in control group, blood culture was positive for *E. coli* in 9 cases (42.86%), *Klebsiella pneumoniae* in 6 cases (28.57%), *Acinetobacter baumannii* in 6 cases (28.57%). Sreenivasa B et al. performed a study in which incidence of sepsis was also significantly lower in the test group (28 of 100 versus. 42 of 100, p=0.038).[15] The study by Mario Rojas A et al. showed a trend toward a lower rate of nosocomial sepsis in the probiotic group (2.4% vs 5.0%; P = 0.06).[16]

In present study, the mean age to reach full feeds in test and control group were 8.53 ± 2.14 days and 10.70±3.25 days respectively. We found no significant difference in the mean age to reach full feeds in both test and control group (p-value=0.245). Similar observations were found in the study done by Bin-Nun A et al. They reported that full feeds were reached at similar ages in both test and control group (14.6 ±8.7 days versus 17.5±13.6 days, p=0.13).[12] Sreenivasa B et al. found that age to reach full feeds was similar in both test and control group (9.78±2.68 days vs 9.53±3.24 days, p=0.554).[15]

In this study, daily weight gain of 10-15 gm was observed in 52 cases (69.34%) in the test group and 37 (49.33%) in the control group. Daily weight gain of 5-10 gm was seen in 14 cases (18.66%) in the test group and 37 cases (49.33%) in the control group. 9 cases (12%) in the test group and 1 case (1.34%) in the control group had weight gain of 15-20 gm per day. Weight gain was statistically significant in the test group (p=0.001). The study by Hartel et al. showed that infants treated with probiotics had improved daily weight gain (20.8±6.6 vs 22.2±5.2, p<0.001).[17] Ravindra Sonawane et al. observed better weight gain among newborns receiving synbiotics (14.61±9.6 grams per day vs 2.97±3.81 grams per day, p=0.0000001).[18]

In current study, the mean duration of hospital stay in test and control groups were 16.06±0.49 days and 20.04±7.85 days respectively. There was significant difference in the mean duration of hospital stay (p-value=0.001). Chowdhury T et al. did a study which showed duration of hospital stay was significantly short in the study group compared to the control group (15.82±2.94 days versus. 19.57 ±4.26 days; p < 0.001).[19] Samanta et al did a study and concluded that prophylactic probiotic shortened hospital stay in VLBW babies (17.17±3.23 vs. 24.07±4, P<0.001).[20]

CONCLUSION

The incidence of NEC was significantly lower in the test group compared with the control group (1 of 75 neonates versus 12 of 75 neonates; p=0.001). The severity of NEC, nosocomial sepsis and mean duration of hospital stay was significantly lower in the test group. Daily weight gain was significantly higher in the test group. There was no significant difference in mean age of onset of NEC, mortality and mean age to reach full feeds.

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