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ROLE OF ORAL PROBIOTICS IN THE PREVENTION OF NECROTIZING ENTEROCOLITIS IN PRETERM NEONATES

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ABSTRACT Necrotizing enterocolitis (NEC) is the most commonly acquired intra-abdominal emergency and causes significant mortality and morbidity. A prospective randomized control trial was conducted in preterm neonate (<34 weeks of gestation) at in NICU of Jawaharlal Nehru Medical College Hospital, Bhagalpur, Bihar, India. The neonates in the test group were fed with Probiotics Darolac' sachets with breast milk twice daily till they reach full feeds. The neonates in the control group were fed with breast milk alone.The incidence of NEC was significantly lower in the test group (3 of 100 vs. 10 of 100). The incidence of severe NEC was also significantly lower in the test group when compared with the control group (0 of 100 vs. 7 of 100). We conclude that Probiotics fed enterally with breast milk reduced both the incidence and severity of NEC. However, the data are insufficient to comment on their short and long term safety.

KEYWORDS : Preterm neonates; Probiotics; Necrotizing enterocolitis; Sepsis.

INTRODUCTION

Necrotizing enterocolitis (NEC) is primarily a disease of preterm neonates and prematurity is the single greatest risk factor. This causes significant mortality and morbidity in preterm neonates with mortality approaching 20%-40% 1. The causes of this intestinal catastrophe is complex, but common factors associated with this disease are prematurity, immaturity of intestinal tract, intestinal ischaemia, microbial colonization with pathogenic organisms and enteral feeding.[2]

NEC is likely to be initiated with intestinal mucosal injury from any number of factors. Following this injury, bacteria in the gut proliferate with formula or breast milk as a substrate. The bacteria invade the damaged mucosa causing inflammation and, ultimately, necrosis of the infected area.[3]

The signs of NEC include abdominal distension, blood-stained or bile-stained emesis, bloody stools, and pneumatosis intestinalis is the pathognomic radiographic sign of the disease3. Medical management is largely supportive; however, surgery is required for intestinal necrosis .

Because of the association of feeding and bacterial infection with NEC, prevention strategies have focused on manipulating the feeding of premature infants as well as trying to manipulate the bacterial environment of the intestine.

Recent interest has focused on giving probiotic bacteria to premature infants. Probiotic bacteria, such as Bifidobacteria and Lactobacillus, are live microbial supplements that colonize the intestines and provide benefit to the infant. The idea is to prevent the overgrowth of pathogenic organisms that have been associated with NEC. There have been a modest number of studies that primarily have looked at the safety of probiotics in newborns; and to date, it appears safe to administer these bacteria.[4]

Although the use of probiotics to prevent NEC appears attractive, at this time it is too early to recommend their general use. Hence this study was done to determine whether the probiotics decrease the incidence of NEC and to identify the possible side effects.

MATERIAL AND METHODS

A Prospective randomized control study of one year duration (March 2019 – March 2020) was done in NICU of Jawaharlal Nehru Medical College Hospital, Bhagalpur, Bihar, India.

Hemodynamically stable preterm neonates (gestation age <34 week) were included while seriously ill preterm neonates and those whose parents/guardians did not give consent were excluded in the study . Two hundred babies were selected strictly based on inclusion and exclusion criteria, through simple random sampling. Of the 200 babies analyzed, 100 babies were randomized to test group and 100 to control group, after informed Parental consents obtained. Babies of test group received their regular feeds plus daily probiotic supplement of 125 mg/kg/dose twice daily mixed with expressed breast milk from the onset of enteral feeding till the baby reached full feed.(each sachet was mixed with 20 ml of expressed breast milk so that each ml contain 100 mg of Probiotic). Dose was calculated according to the weight of baby (125 mg/kg/dose). The control group was fed with breast milk without the addition of probiotics.

Probiotic used was 'Darolac' sachets, each sachet of 2gm, manufactured by ARISTO Pharmaceuticals pvt.Ltd.(India). Bell's staging was used to assess the severity of NEC. The results were analyzed by using unpaired t-test used to test the statistical significance of difference in mean score between the two groups.

Chi-square test/Fischer exact test was used to test the significance of difference in proportions.

The p value of <0.05 was considered to be significant. The analysis was done using SPSS.

Ethical approval was obtained from Institutional Ethics Committee.

RESULTS

In the present study, out of 200 preterm neonates 99% of babies each in the test group and control group were admitted on day one of their life. Rest one percentage of babies were admitted on day two of life. In our study 52% were males and 48% were females in each group. Of the 200 preterm neonates, 10% were less than 1 kg, 60% between 1.01-1.499 kg, 25% between 1.5 -2 kg and 5% > 2 kg. Regarding gestational age, out of total babies included in the study, 10% babies were < 28 weeks of gestational age, 40.5% babies between 28-30 weeks, 35% between 31-32 weeks and 14.5% between 33-34 weeks. Further among the total babies, 90% were appropriate for gestational age, 8% were small for gestational age and 2% were large for gestational age. The mean age of initiation feeds in test group was 2.33 ±0.711 and in control group was 2.14 ± 0.40 which was statistically significant (p=0.021).

In current study, 3 babies (3%) in test group and 10 babies in the control group (10%) developed NEC. The incidence of Necrotizing Enterocolitis in both groups was statistically significant (p=0.044). Incidence of NEC was less in the test group compared to controls. Mean age of onset of NEC in the test group was 4±1.41 and in the control group was 3.80±1.98. The difference in the age of onset of NEC was not found to be statistically significant. Out of the 13 babies

developing NEC, 6 babies developed stage I NEC, 5 babies with stage II NEC and 2 babies with stage III NEC. More severe NEC i.e. stage 2 and stage 3 were seen in control group while it was less severe in the probiotic group. There was significant difference between the test and control group in different stages of NEC (p=0.014). In our study, three of the 13 babies with NEC died; all three of the NEC associated deaths were from the control group. There was no significant difference in mortality associated with NEC (p-value =0.528). Although the difference was not significant, it was observed that the three babies who died with NEC were from the control group vs no babies from the test group. The incidence of sepsis in the test group was 28% and in the control group was 42%. It was found that the incidence of sepsis was less in test group, which was statistically significant (p=0.038). Mean age to reach full feeds in test and control groups were 9.78±2.687 and 9.53±3.248 respectively. There was no significant difference in the mean age to reach full feeds in both test and control groups (p=0.554). The difference in mean duration of hospital stay in test and control groups was not found to be statistically significant.

DISCUSSION

In our study, 200 preterm neonates <34 weeks of gestational age were selected based on inclusion and exclusion criteria. They were assigned randomly to test group (100) and control group (100). Study group was fed with probiotics with breast milk and control group only with breast milk.

In the present study, majority of neonates were between 1.01-1.499 kg. Most of the neonates were between 28-32 weeks of gestations in both test and control groups. Out of 200 babies 90% were appropriate for gestational age.

Many different species of bacteria and fungi have been used as probiotics. In our study the probiotics used contains Bifidobacterium longum, Lactobacillus rhamanosus, Lactobacillus acidophilus, Saccharomyces boluardii while the studies done by different randomized controlled trials had used different strains of probiotic organisms.5,6 The incidence of NEC was significantly lower in the test group compared with the control group. Similar observations were seen in study by Lin et al. They reported a lower incidence of NEC in the probiotic group (1.1% Vs 5.3%; p=0.04).7 The study by Bin-Nun et al. also found a significantly lower incidence of all cases of NEC in the probiotic group (4% Vs 16.6%; p=0.031).8 Manzoni et al. also reported a non-significant trend towards less severe NEC in the probiotic group (2.6% Vs 4.9%; p=0.51).[5]

In our study, mean age of onset of NEC in the test group was 4±1.41 and in the control group was 3.80±1.98 which was not statistically significant. Two studies done by Hung–Chin Lin et al. showed similar observations in the age of onset of NEC which were statistically non-significant.6,7According to literature, the postnatal age at onset is inversely related to birth weight and gestational age with a mean at onset of 12 days.[9]

NEC was found to be significantly higher in control group. Similar finding was reported by Lin et al.[6,7]

In the present study, more severe NEC were found in the control group. Similar study done by Lin et al. reported 6 cases of severe NEC in the control group versus none in the probiotic group (p=0.003).7According to the study done by Dani et al. a lower incidence of NEC were seen in the probiotic group (1.4% Vs 2.8%) but this did not reach statistical significance. But in this study, patients were not stratified by severity.10Study done by Manzoni et al. reported a non-significant trend towards less severe NEC in the treatment group. (2.6% Vs 4.9%; p=0.51).[5]

In our study, there is a non-significant trend to towards less NEC-related mortality in the probiotic group . The study by Bin Nun et al. reported similar observations. They reported that three deaths in the control group were due to NEC, whereas there were no NEC- related deaths among the test neonates (p = 0.87).8The study done by Hoyos MD et al. showed NEC-associated mortality is more in the non probiotic group (35/1282 Vs 14/1237; p-value<0.005) which was statistically significant. But here the test group was compared with historic controls.11 Studies done by Lin et al. and Manzoni et al. reported a significantly lower mortally rate in the probiotic group but did not differentiate between death attributed to NEC version other cases.[5,6,7]

In the current study it is found that the incidence of sepsis is significantly less in the test group as compare to control group.The study published by Hung–Chin Lin et al. in 2005 reported a lower incidence of sepsis in the probiotic group (22/180 Vs 36/183; p=0.03).7The 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 done by Dani et al. and Bin Nun et al. did not show any reduced incidence of sepsis in the probiotic group. Their studies reported that the pathogens were most often related to catheter related infections in both groups.[8,10]

We found no significant difference in the mean age reached full feeds in both test and control group.Similar observations were found in the study done by Bin Nun et al. They reported that full feeds were reached at similar ages in both test and control group (p=0.13).[8]

The study published by Lin et al in 2008 also reported similar observations.12 Studies by Dani et al. and Costalos et al. reported observations which are similar to the present study.[10,13]

There was no significant difference in the mean durations of hospital stay.

Study done by Hung–Chin Lin et al. in 2008 showed similar observations that no significant difference in the hospital stay between test and control group 46.7 ± 27.1 in test and 46.5 ± 26.1 in control group). Study by Lin et al in 2005 also showed similar findings (46.4 ± 24.2 in test and 43.3 ± 21.0 in control; p=0.16).65,69Study done by Angela B Hoyos et al. also showed similar findings.[52]

We conclude that enterally fed probiotics are beneficial in the prevention of both incidence and severity of NEC in preterm neonates < 34 weeks of gestation. Probiotic supplementation has also reduced the incidence of culture proven sepsis in the preterm neonates but there were no significant differences between test and control groups in age reached full feeds and mean duration of hospital stay. However more research is required involving more sample size to support the use of probiotics in preterm neonates.

Our study has some limitations. First, the choice of probiotic mixture, the dose and the frequency of dosing need to be discussed because each probiotic organisms has variable rate of colonization. Second, NEC is a multifactorial disease but the other factors contributing to the development of NEC is not analysed in our study.

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TABLES

Table I: General characteristics of study subjects (N=200)

Characteristics		Group		Frequency(%)
Age of admission (days)		Test(100)	Control(100)	
	1	99	99	198(99)
	2	1	1	2(1)
Sex	Male	52	52	104(52)
	Female	48	48	96(48)
Birth weight(kg)	<1	12	8	20(10)
	1.01-1.499	62	58	120(60)
	1.5-2	20	30	50(25)
	>2	6	4	10(5)
Gestational age(weeks)	<28	16	8	24(12)
	28-30	45	36	81(40.5)
	31-32	33	37	70(35)

	33-34	6	19	25(12.5)
Gestational age	AGA	87	93	180(90)
	SGA	11	5	16(8)
	LGA	2	2	4(2)

Table II: Showing stages of NEC (Bell Staging) in test and control groups

Staging	Groups		Total	p-value
	Test	Control		
No NEC	97	90	187 (93.5%)	0.014
Stage I	3	3	6 (3%)	
Stage II	0	5	5 (2.5%)	
Stage III	0	2	2(1%)	
Total	100	100	200	

Table III: Showing Mortality and sepsis associated with NEC

Characteristics		Groups		Total	p-value
		Test	Control		
Mortality cases with NEC		0	3	3	0.528
Sepsis	Present	28	42	70	0.038
	Absent	72	58	130	

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