Sournal of Research PARIPEX		ORIGINAL RESEARCH PAPER ORAL SUPPLEMENTATION WITH PROBIOTICS IN PREVENTION OF NECROTIZING ENTEROCOLITIS IN PRETERM INFANTS WITH LOW BIRTH WEIGHT-A DOUBLE BLIND RANDOMIZED CONTROLLED STUDY		Pediatrics	
				KEY WORDS: NEC,Preterm,LBW,Probiotics	
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ABSTRACT	<ul> <li>Background &amp; Objectives : Necrotizing enterocolitis (NEC) is a devastating gastrointestinal (GI) disease that predominantly affects, preterm low birth weight (LBW) infants and its associated with high morbidity and mortality. Current evidence suggest that probiotics are effective in decreasing NEC in preterm infants. In this study the primary objective was to study the role of oral probiotic supplementation to preterm infants in reducing incidence and severity of NEC.</li> <li>Methods: Preterm infants &lt;2 Kgs were studied and randomly assigned to case and control group. The case group received the supplements of probiotics mixed with their feeds and the control group received only plain feeds without probiotics. The probiotic used was Lactid sachets (1g containing 109 spores of Saccharomyces boulardi , Lactobacillus rhamanosus, Lactobacillus acidophilus, Lactobacillus sporogenes, Bifidobacterium bifidum, Bifidobacterium longum). The primary outcome was incidence and severity of NEC.</li> <li>Results: The case and control groups did not differ significantly in the confounding factors and demographic and clinical variables. The incidence of NEC was 1.89% in test group and 6.38% in control group (p&lt;0.05).2% babies developed stage 1 NEC,3% babies developed stage 2 and 1% babies developed stage 3 disease. The incidence of sepsis in case group was 6.60% and 15.96% in control group (p&lt;0.05).</li> </ul>				
INTRO	<b>INTRODUCTION</b> colonization. <sup>12</sup> One of the strategies to prevent Necrotizing				

Necrotizing Enterocolitis (NEC) is the most common life threatening emergency of the gastrointestinal tract in the newborn period, and is primarily a disease of premature infants, although up to 10% of cases present in term and near-term babies.<sup>11</sup>Signs include abdominal distension, blood or bile-stained emesis, bloody stools, and pneumatosis intestinalis is the pathognomonic radiographic sign of the disease.<sup>16</sup>

Its sudden onset, rapid progression and ultimate toll on human life are daunting; equally challenging is the lack of any major breakthrough in preventing this ailment. Clinicians treating tiny premature infants live in fear, jumping at every sign of feeding intolerance and rounding of the abdomen. Clinicians also deal everyday with the many stubborn complications of NEC; strictures, short gut syndrome, stunted growth and poor neuro developmental outcomes to name just a few. For parents it is perhaps the most disheartening event, after weeks of slow but steady recovery from early complications of prematurity, many or all of the painstaking games are washed away by a mysterious illness that has no easy cure.

Medical management is largely supportive; however, surgery is required for intestinal necrosis and mortality may approach 35%.<sup>1</sup> Probiotic bacteria, such as Bifidobacteria and Lactobacillus, are live microbial supplements that colonize the intestines and provide benefit to the infant. The hope 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.<sup>22</sup>

The disease is characterized by various degrees of mucosal and transmucosal necrosis of the intestine. The cause of NEC remains unclear but is most likely multifactorial.<sup>4</sup>The triad of intestinal ischemia, enteral nutrition (metabolic substrate), and pathogenic organisms has classically been linked to NEC. The only factors that have been consistently demonstrated to be significantly associated with this disease are prematurity and enteral feeding.<sup>5</sup>

The gastrointestinal tract of newborn infant is sterile but colonized within 12-24 hours, first with maternal vaginal flora, followed by that of the external environment. The normal commensal flora – Lactobacilli and Bifidobacterium are found weeks later. The therapeutic strategies used in NICU – broad spectrum antibiotics and delayed initiation of enteral feeds contribute to the fact that the VLBW infant has a delayed and aberrant pattern of

colonization.<sup>12</sup>One of the strategies to prevent Necrotizing Enterocolitis is probiotics.Potential use of probiotics could lead to improvements in nutrition, reduced dependence on intravenous nutrition, a reduction in the incidence of sepsis and use of antibiotics and prevention of Necrotizing Enterocolitis.

### AIMS AND OBJECTIVES

- 1. The primary objective was to evaluate effect of probiotics in decreasing incidence of necrotizing enterocolitis.
- 2. The secondary outcomes were to study effect of probiotics in decreasing:
  - a.Neonatal sepsis
  - b.Feed intolerance
  - c.Duration of hospital stay
  - d.Mortality

### MATERIALS AND METHODS

Type of study- Randomized, double blind, placebo controlled trial

**Setting-** Neonatal Intensive Care Unit of MGM Hospital, Kalamboli attached to Mahatma Gandhi Mission University of Health Sciences, Navi Mumbai.

Study period- December 2013-December 2015(two years) Sample size -200

Ethics- The study was initiated after getting approval from ethical committee

**Consent**- Neonates were enrolled after obtaining written informed consent from the parent

### Inclusion criteria

- 1. Preterm neonates (gestational age <37 weeks)
- 2. Birth weight<2kg
- 3. Hemodynamically stable
- 4. Parent(s)' informed consent to participate in the study

#### **Exclusion criteria**

1. Cardiorespiratory instability

2. Parental refusal any time after having given informed consent earlier

### Materials used

Probiotic used: 'LACFID' sachets. Each sachet of 1 g contains 1.25 x10 <sup>9</sup> spores of Saccharomyces boulardi , Lactobacillus rhamanosus, Lactobacillus acidophilus,Lactobacillus sporogenes,Bifidobacterium bifidum, Bifidobacterium longum. It is manufactured by Serum Institute Of India Ltd,212/2,Hadapsar,

## VOLUME-6 | ISSUE-7 | JULY-2017 | ISSN - 2250-1991 | IF : 5.761 | IC Value : 79.96

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### METHOD OF COLLECTION OF DATA

Preterm neonates (gestational age <37 weeks) with birth weight <2kg who survive to feed enterally were included in the study. Of the 200 babies analyzed, 106 babies were randomized to test group and 94 to control group, after informed parental consents were obtained by picking up chits. Babies in the test group received a daily feeding supplementation with a probiotic mixure control group received no probiotics in their feeds.

A staff nurse who was allocated to pick up the chits either mixed a probiotic supplement with mother's milk or formula milk if mothers milk is not available or plain milk in the control group according to the chit picked up. The sterile containers with the feed were labelled with the infants name and identification number who is to be fed that particular feed with no indication of the study group. The other staff attending/feeding the infants and the doctors were blinded to the group assignments. At the feeding time the staff nurse would take out the container with the infants name and administer it with the regular feed.

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 was started at 10-20 ml/kg/day. The frequency of feeding was once in two hours. 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. The case group received 1gram (1.25x10°spores) of probiotics mixture mixed with their feeds as a single morning dose. 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). On admission to NICU a septic work up which included complete blood count, C-reactive protein and blood cultures was sent.

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 Staging Criteria.

#### RESULTS

Total of 200 preterm neonates were included in the study. They were randomly assigned to case or control groups. The case and control groups were compared regarding all the confounding factors like birth weight, gestational age, mode of delivery, antenatal risk factors, age of initiation of feeds, birth asphyxia etc. The case and control groups did not differ significantly in the confounding factors and demographic and clinical variables.

NEC	Gro	Total		
	case	Control		
No	104	88	192	
	98.11%	93.62%	96.00%	
Yes	2	6	8	
	1.89%	6.38%	4.00%	
Total	106	94	200	
	100.00%	100.00%	100.00%	
p- value: 0.02				

## Table 1: Incidence of NEC

The overall incidence of NEC and comparision between the two group were the primary objectives of the study. Out of the 200 babies included the overall incidence of NEC was 4%. In the control group 6(6.38%) babies had signs and symptoms of NEC, whereas in the study group only 2(1.89\%) babies had NEC. This was statistically significant (p=0.02). (Table 1)

#### Table 2: Bells staging of NEC

Bell Staging	Gro	Total	p value	
	case[106]	control [94]		
1A	1	0	1	0.34

	0.94%	0.00%	0.50%	
1B	1	2	З	0.49
	0.94%	2.13%	1.50%	
2A	0	1	1	0.28
	0.00%	1.06%	0.50%	
2B	0	2	2	0.13
	0.00%	2.13%	1.00%	
3A	0	1	1	0.28
	0.00%	1.06%	0.50%	
Total	106	94	200	
	100.00%	100.00%	100.00%	
p- value: < 0.05				

The babies who showed features of NEC were classified according to the Bells staging. Out of the total 8 babies who had NEC, 1(0.94%)baby from case group had stage 1A and none from the control group .Out of the 3(1.5%) babies in stage 1B 1(0.94%) was from case group and 2(2.13%)from control group.2(2.13%)babies and 1(1.06%) baby had stage 2B and stage 2A respectively from the control group whereas none from the case group had stage 2 disease. 1 baby (1.06%) from control group had stage 3A disease and none from the case group. There was no significant difference between the two groups when individual NEC stages were considered, but overall incidence in the two groups were statistically significant.(p<0.05).**(Table 2)** 

Out of the 8 babies with NEC 4 (2%) babies did not have any x-ray findings whereas 4(2%) babies had positive xray findings all of whom belonged to the control group and none to the case group. This was statistically significant.(p=0.03)

Out of the 200 babies, a total of 22 (11%) babies had culture positive sepsis whereas 178 babies did not have sepsis. Out of the 22 babies who had sepsis which was proven with positive blood cultures, 15(15.96%) babies belonged to the control group, (were not fed probiotics) compared to only 7 (6.6%) babies in the case group (supplemented with probiotics). Thus the administration of probiotics could significantly reduce the incidence of sepsis in the babies of the case group compared to the control group (p=0.04).

The mean age of onset of NEC were  $6.5 \pm 2.08$  days and  $10.32 \pm 3.54$  days in the case and control group respectively. This was statistically significant (p<0.05).But there was no significant difference between the two groups with regard to the mean duration of hospital stay (p=0.62)and were  $18.7 \pm 12.5$  days in the case group and  $19.8 \pm 13.4$  days in the control group.Out of the 8 babies who had NEC,1(12.5%) baby expired and 7(87.5%) babies survived.The baby who expired belonged to the control group.This was not statistically significant(p=0.2).

The final outcome of the babies in the two groups were also compared. Out of 200 babies 14(7%) babies expired. Out of this 6 babies were from the case group and 8 babies belonged to the control group. This was not a significant difference (p=0.57). The overall survival rate was 93%.

#### DISCUSSION

The primary objective of the study was to evaluate the effect of probiotics in decreasing the incidence of necrotising enterocolitis. In our study the incidence of NEC was 4% (8 neonates out of 200). Out of the 8 neonates 2 (1.89%) belonged to the case group and 6 (6.38%) were from the control group. There was statistically significant (p < 0.02). The result of previous studies available were compared with the result of present study. Studies by Lin et al , Bin-Nun et al, Manzoni et al, found a lower incidence of NEC in the probiotics group.

### (Table 3) Table 3:Incidence of NEC In various studies

1.Lin et al <sup>13</sup>	1.1% Vs 5.3% p=0.04
2.Dani et al <sup>®</sup>	1.4% Vs 2.7% p>0.05
3.Bin Nun et al <sup>6</sup>	4% Vs 16.6% p= 0.03
4. Costalos et al <sup>7</sup>	9.8% Vs 16% p=0.5
5. Manzoni et al <sup>14</sup>	2.6% Vs 4.9% p=0.51

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2

Further the babies who developed NEC were classified according to the Bells classification. Out of the 8 babies , 4 babies had stage 1 disease, 3 babies had stage 2 disease and 1 baby had stage 3 disease. The presence of x ray findings signifying more severe disease was significantly lower in the case group that the control group (0% Vs 4.26% p=0.03).

In our study out of 8 babies who developed NEC 7(87.5%) babies survived and 1 (12.5%) baby expired. This baby belonged to the control group. This was a non significant trend (0 out of 2 Vs 1 out of 6: p=0.2.). This implies that 2 deaths in the control group were due to NEC whereas there were no NEC related death in the case groups. Studies done by the Lin et al reported a reduction in overall mortality rate in the probiotics group but did not differentiate between the cause of the death .The study showed by the Angela hoyos MD<sup>3</sup> et al showed similar statistically significant result (35/1282 Vs 14/1237 p<0.005).

The secondary outcomes studied were , incidence of neonatal sepsis, feed intolerance or mean age of attaining full feeds, duration of hospital stay and mortality. In our study the incidence of sepsis was 15 out of 96 (15.96%) in control group compared to 7 out of 104 (6.6%) in case group. This was statistically significant (p=0.004). Probiotic administered orally primarily act in the gastrointestinal tract possibly by increased colonization of desirable gut flora. The study by Hung chin Lin et al reported lower incidence of sepsis in the probiotics group (22/180 vs 36/183 p=0.03). Studies by Dani et al and Bun Nun et al did not show any reduced incidence of sepsis but reported that sepsis was most often due to catheter related infections. The benefit of probiotics in the prevention of sepsis is not as clear. Two randomized trials, including a large trial by Manzoni and colleagues, have shown benefit of probiotics in reducing late onset sepsis . However, Manzoni and colleagues used the probiotic in combination with Lactoferrin and did not evaluate probiotics treatment alone .Other studies have shown no benefit, and one study showed an increased risk of sepsis with an RR of 1.67(95% CI 1.04-2.67) raising concerns regarding the risk of sepsis with probiotics therapy. The mean age of reaching full feeds were 12.98 ± 8.6 days in case group compared to  $13.624 \pm 8.2$  days in control group. This was a non significant trend (p=0.61) similar observations were reported by Lin et al, Bin Nun et al, Dani et al and Costalos et al.In our study the mean duration of hospital stay did not vary significantly between the two groups . It was 18.7  $\pm$  12.5 days case group 19.8 ± 13.4 days in control group studies by Lin et al and Angela B Hoyos et al also showed similar result.

Because the preterm gut demonstrates delayed commensal colonization and low bacterial diversity it may be particularly amenable to therapeutic manipulation by probiotics administration in keeping with with this idea several clinical studies have demonstrated the benefits of probiotics administration in reducing the incidence and severity of NEC .Most of these trials have used strains of probiotics from the genus lactobacillus or bifidobacteria, although the treatment regimen, including dose and duration of therapy, vary widely.

Numerous probiotics organisms have being studied in preterm infants, at varied dosages and duration of therapy. Several studies have used single agents, whereas several larger trials have used a combination of probiotics. Dosing the varies among the probiotics species and ranges from 10<sup>5</sup> to 10<sup>10</sup> colony forming units (CFU) per day.Most studies randomised infants and /or initiated therapy within a week after birth, and duration of therapy typically lasted beyond one month of age with several studies continuing therapy until hospital discharge. The two most commonly used probiotc strains were from the genera Bifidobacteria and Lactobacillus . A meta- analysis by Wang and colleagues, including several recent trials conducted in China compared the relative efficacy of these strains by pooling studies that used these probiotics agent, and found that bifidobacteria, lactobacillus, and the combination of 2 strains had similar efficacy in the prevention of NEC.

### VOLUME-6 | ISSUE-7 | JULY-2017 | ISSN - 2250-1991 | IF : 5.761 | IC Value : 79.96

Desphande and colleagues<sup>9</sup> evaluated 11 trials and found and overall pooled relative risk (RR) of 0.35(95% confidence interval [CI] 0.23-0.55) for NEC in infants randomized therapy compared with controls. Wang and colleagues<sup>23</sup>, as mentioned previously, included more recent trials and found a similar pooled RR of 0.33 (95% CI 0.24-0.46) form a total of 20 studies . The previously mentioned meta-analyses also demonstrated a decreased risk of death in infants randomized to probiotics treatment compared with control. It is likely that the mortality benefit is through a reduction in NEC- related and sepsis- related deaths, although the cause of death have not been routinely evaluated in clinical trials. The evidence based recommendations for use of probiotics by Desphande and colleague<sup>9</sup> are listed in (Table 4).

### (Table 4) :Evidence based recommendations for probiotic use

Selection of strains	Combination containing Lactobacillus and atleast one bifidobacterium species is preferable .Lactobacillus alone may not be effective
Dose	109 organisms per day,preferably in a single dose
When to start?	When the neonate is ready for enteral feeds preferably within 7 days
How long to continue?	At least until 35 weeks corrected age,or discharge
Supplementation	Stopping supplementation during

NEC remains a devastating complication of prematurity and new preventive therapies are urgently needed. Probiotic therapy has demonstrated promising efficacy for the prevention of NEC, as evidenced by a very strong treatment effect in favour of probiotic therapy in 2 recent meta-analysis by Deshpande et al9 and Wang et al23. However safety and dosing concerns continue to tamper widespread use, these concerns are likely to remain until large, multicenter trials adequately designed to address safety are completed.

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