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Efficacy of Lactobacillus Acidophilus DDS-1, Lactobacillus Bulgaricus, Bifidobacterium Infantis in the Prevention of Necrotizing Enterocolitis in Preterm Neonates

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ABSTRACT

Objectives: To compare the efficacy of combination of lactobacillus acidophilus DDS-1, lactobacillus bulgaricus, bifidobacterium infantis in the prevention of necrotizing enterocolitis in preterm neonates with placebo. **Study Design:** Randomized controlled trial. **Duration of Study:** May 2012 to October 2012. **Setting:** Department of Pediatric Medicine, Allied Hospital, Faisalabad. **Methodology:** Over 6 months, 220 preterm neonates meeting the inclusion criteria were selected and were assigned randomly to two groups after parental informed consent was obtained. The neonates in study group received probiotic with breast milk or preterm formula milk and neonates in control group received breast milk or preterm formula milk. Both groups were observed for the development of clinical evidence of NEC. The Data was statistically analyzed; Chi square test and Fischer's exact test was applied to compare both groups for presence of NEC. **Results:** The number of days required to reach full enteral feeding (study group 8.73±3.87 days vs control group 10.72±5.43 days; *p*-value 0.002) and duration of hospital stay was (study group 11.35±6.74 vs control group 15.35±10.29; *p*-value 0.001) significantly low in the probiotic-exposed group compared with the control. The incidence of NEC (study group 10.0% vs control group 22.72%; *p*-value 0.011) was significantly low in the probiotic-exposed group when compared with non-exposed group. **Conclusion:** Alteration of microbial florae following oral supplementation of probiotics along with human milk have beneficial effects in reducing the incidence of NEC especially in pre-term neonates.

Keywords: Preterm Neonates, Necrotizing Enterocolitis, Probiotics.

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INTRODUCTION

Necrotizing Enterocolitis (NEC) is common gastrointestinal emergency of newborns. It is the inflammation and necrosis of bowel which occur due to vascular, mucosal and metabolic insults to immature intestine.[1] NEC incidence is inversely related to gestational age and it affects 15% of very low birth weight (VLBW) preterm neonates in the neonatal intensive care unit (NICU). The overall mortality rate is 20-40%.[2]

Pathogenesis of NEC is not clear and it is considered a multifactorial disease; prematurity, bacterial colonization, enteral feeding and hypoxic ischemia of intestine are major risk factors.[3] It has been suggested that an inappropriate inflammatory response to colonizing pathogenic florae in the premature gastrointestinal tract plays a major role in the initiation of NEC. The inflammatory cascade promotes the spread of toxins or bacteria, resulting in ischemia, necrosis, and perforation.[4]

Some signs which are non-specific and may trigger the suspicion of NEC are feeding intolerance, apnea, abdominal distension/tenderness and blood in stools; definite signs picked by X-ray abdomen are pneumatosis intestinalis and portal venous gas.[1,5]

Modified Bell's Staging Criteria classifies NEC into 3 stages i.e. Stage 1 (suspected case), stage 2 (proven case), and stage 3 (severe case).[6]

Medical management of NEC consist of discontinuing oral feedings, nasogastric intubation

to decompress distended abdomen, intra-venous fluids, monitoring and correcting laboratory values.[7] Studies suggested that normal florae of GIT could reduce or inhibit inflammatory signaling in intestinal epithelia through inhibition of the NF-kB pathway.[8] These data suggest that probiotics, by modifying the occurrence of these cascades of events, may play a major role in reducing the incidence of NEC. Probiotics have been used in Necrotizing enterocolitis, inflammatory bowel disease, Celiac disease, Diarrhea and Lactose intolerance. "Probiotics are alive organism which when administered in proper amounts confer health benefit to the host". such as Sacchromyces boulardie, Bifidobacterium lactis, Bifidobacterium infantis, Lactobacillus rhamnosus, Lactobacillus acidophilus and Lactobacillus bulgaricus etc.[9] Recent studies have shown that probiotics treatment in preterm neonates decrease the incidence and severity of NEC at discharge. Incidence of NEC was present in 5.49% of the neonates treated with combination of probiotics (study group) as compared to placebo group (control group) in which 15.78% had NEC. [10]

The rationale of this study is to reduce the mortality, morbidity and financial burden of community in developing country like Pakistan from this severe illness by using very economic drug like Probiotics. Although a few studies have been performed on this issue but only a single Probiotic was used. First time three Probiotics were evaluated in combination in local study related to protective effect against NEC.

The aim of this study was to compare the efficacy of combination of lactobacillus acidophilus DDS-1, lactobacillus bulgaricus, bifidobacterium infantis in the prevention of necrotizing enterocolitis in preterm neonates with placebo.

METHODOLOGY

This Randomized controlled trial was conducted at Department of Pediatrics, Allied Hospital, Faisalabad, during a period from May 2012 to October 2012. The informed written consent were procured from parents/guardian of 220 preterm neonates having gestational age less than 37 weeks and having weight less than 2500 g, meeting the inclusion criteria (started feed enterally after birth and survived beyond 48 h of life). Preterm who had fetal chromosomal anomalies, cyanotic congenital heart disease, severe birth asphyxia (stage 3), congenital intestinal atersia, gastroschisis or omphalocele, whom enteral feeding could not be established in 1st week of life, neonates who were fed exclusively with formula feed and those who were fasted for > 3 weeks were excluded. Gestational age was assessed from history of last menstrual period and after birth by new Ballard scores. Patients were assigned randomly into 2 groups; study group (A) and control group (B) by using computer generated random number table.

The neonates in study group received 25 mg or 0.375 x 10^9 per dose of probiotic (HiFLORA powder for oral suspension 50 mg/5ml on reconstitution containing combination of Lactobacillus Acidophilus DDS-1(L. Acidophilus), Lactobacillus Bulgaricus (L. Bulgaricus), Bifidobacterium Infantis (B. Infantis) 50 mg or 0.75 x 10^9 viable cells manufactured by Bonney & Dennisgay Phyto Pharma) two times in a day with breast milk or preterm formula milk from first feed till discharge while neonates in control group received breast milk or preterm formula milk. The supplementation of probiotics with milk was given by trained staff nurse under the supervision of researcher. The rest of the management in both groups was as per standard protocol for the management of preterm neonates. The clinical variables that are potential risk factors for NEC were compared in both the study and the control group. Preterm neonates were weighed daily and monitored for daily increment in feed volume, abdominal girth, appearance of erythema of abdominal wall, loose stools with blood, vomiting and nasogastric aspiration. Stool for occult blood and Complete Blood Count by Medonic CA 620 for thrombocytopenia were performed in Pathology Department, Allied Hospital Faisalabad. Abdominal radiographs were done and reported by radiologist in neonates developing clinical signs of NEC. Primary outcome measures were noted down in terms of presence of NEC during hospital stay in both groups. NEC was diagnosed when 5 of the following 6 criteria were met: (1) Bradycardia (Heart rate less than 80 beats/min). (2) Nasogastric Aspiration. (3) Abdominal Distension. (4) Occult blood in stool. (5) Thrombocytopenia (Platelet count less than 150,000 per microliter of blood) (6) Dilated bowel loops or Pneumatosis Intestinalis on abdominal radiographs. All relevant data were noted using a structured performa.

The data were tabulated and analyzed using SPSS-19. Quantitative variables like age and weight of neonates at the time of enrollment, gestational age at the time of birth, full enteral feeding and length of hospital stay were presented by mean and standard deviation. Qualitative variable likes sex, mode of deliveries and presence of NEC were presented as frequency and percentage. Chi square test and Fischer's exact test was applied to compare both groups for presence of NEC. Independent sample t test was applied for variables like age and weight of neonates at the time of enrollment, gestational age at the time of birth, full enteral feeding and length of hospital stay. P-value of less than 0.05 was considered as significant; 95% confidence interval was computed for proportion of NEC.

RESULTS

Out of 220 preterm neonates, 110 were evaluated with probiotics and 110 were considered as control. Birth weight (study group 1931.36±365.60 g vs control group 1901.36±365.63 g; *p*-value 0.54) and gestational age (study group 31.75±1.96 weeks vs control group 31.39±1.85 weeks; *p*-value 0.17) were not significantly different. Other clinical variables of preterm neonates between the two groups were shown in Table-I.

The number of days required to reach full enteral feeding (study group 8.73±3.87 days vs control group 10.72±5.43 days; *p*- value 0.002) was significantly low in preterm neonates who received probiotics. Therefore, feeding tolerance was better in the probiotic exposed group. Duration of hospital stay was (study group 11.35±6.74 vs control group 15.35±10.29; *p*-value 0.001) also significantly low in the study group compared with the control. The incidence of NEC was significantly low in the study group when compared with control group (Table-II).

Primary outcome variable for different weight groups in the study and control groups was shown in Table-III.

Table 1: Clinical variables of preterm neonates (N=220)

Variable	Study group (n=110)	Control group (n=110)	*p*-value
Age at Enrollment (days)	4.28±0.62	4.35±0.88	0.48
Birth Weight (grams)	1931.36±365.60	1901.36±365.63	0.54
Gestational age (week)	31.75±1.96	31.39±1.85	0.17
Male n (%)	62 (56.36%)	58 (52.72%)	0.58
Cesarean section n (%)	64 (58.18%)	62 (56.36%)	0.78

None of the variables are statistically significant ($p > 0.05$)

p value by Independent sample t test or x^2 test

Table 2: Outcome variables after oral probiotics (n=220)

Variables	Study group (n=110)	Control group (n=110)	*p*-value
Full enteral feeding (days)	8.73±3.87	10.72±5.43	0.002
Hospital Stay (days)	11.35±6.74	15.35±10.29	0.001
Presence of NEC n (%)	11 (10.0%)	25 (22.72%)	0.011

All the variables are statistically significant ($P < 0.05$)

p value by independent sample t test or x^2 test

Table 3: Primary Outcome Variable for Different Weight Groups (N=220)

Outcome	1000 g to 1500 g (n=28)			1501 g to 2000 g (n=96)			2001 g to < 2500 g (n=96)		
	Study (n=12)	Control (n=16)	*P*	Study (n=48)	Control (n=48)	*P*	Study (n=50)	Control (n=46)	*P*
NEC n (%)	3(25.00)	10(62.5)	**0.067**	5(10.41)	13(27.08)	**0.065**	3(6.00)	2(4.34)	**1.0**

p value by *Fisher's exact test*

DISCUSSION

First time three Probiotics were evaluated in combination in local study related to protective effect against NEC among preterm neonates. Many variables have been suggested to be associated with the development of NEC; however, only low birth weight and prematurity have been consistently identified in different studies. [11]

Important first step in the development of NEC is the ability of bacteria to cross epithelial cell layers.[12] Bacterial interactions with the premature gut might play a major role in the pathogenesis of NEC; many studies suggest a strong relationship between proliferation of pathogenic florae and delay & low colonization of commensal florae in the immature gut, predisposing the preterm neonates to develop NEC.[13]

There is no standard dose schedule available for the use of probiotics in NEC. We have used a probiotic dose similar to that used in the study done by Bin Nun *et al*.[14] in Israel. In this study neonates received feeding supplementation with probiotic (Bifidobacterium infantis, Bifidobacterium bifidus, Streptococcus thermophillus) of 0.35×10^9 CFU once daily from first feed to 36 weeks. It was found that incidence of NEC was less in the study group than in the control group. Our results were also comparable.

In a study done by Dani C *et al.*[15], 585 neonates of <33 weeks gestational age or birth weight <1500 g were randomized to receive Lactobacillus Rhamnosus (6×10^9 CFU) once a day from first feed till discharge, or a placebo. There were no significant differences between the outcome of NEC in the probiotic and placebo groups. One major difference from ours is that we used L. Acidophilus DDS-1, L. Bulgaricus, B. Infantis, which are normal commensals of breast milk.[16] Bifidobacterium Infantis, Lactobacillus Acidophilus and Lactobacillus Bulgaricus have been shown to inhibit intestinal colonization of pathogenic microorganisms and promotes the integrity of enterocytes.[17] These characteristics support the use of L. Acidophilus DDS-1, L. Bulgaricus, B. Infantis as appropriate species of probiotics for the prevention of NEC.

In our study, probiotics significantly reduced the duration of hospital stay, duration for the establishment of full enteral feeding, and incidence of NEC in a study group. Similar results were shown by Samnta *et al.*[10] in India in which neonates received Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium infantis and Bifidobacterium longum.

In this study, no significant difference between the incidence of NEC in different weight groups but their number is less. Similar results were shown in a study conducted by Lin HC *et al.*[18] However further research with an adequate number of preterm neonates of different weight groups are required to define the beneficial effects of probiotics in each group.

Human milk feeding has been shown to reduce the incidence of NEC but cannot eradicate NEC because of deficiency of interleukin 10 (IL 10).[19] As in this study four out of 30 neonates in the study group and nine out of 30 neonates in the control group were receiving exclusive breast milk feeding but still developed NEC. Both lactobacillus and bifidobacterium has been shown to induce IL 10 production.[20] So human milk may have synergistic effects with L. Acidophilus DDS-1, L. Bulgaricus and B. Infantis to inhibit the inflammatory response in NEC and reduce the incidence of NEC.

CONCLUSION

The data from experimental studies appears to support that microbial invasion is an important factor in the pathogenesis of NEC. Therefore alteration of microbial florae following oral supplementation of probiotics specially containing Lactobacillus Acidophilus DDS-1, Lactobacillus Bulgaricus, Bifidobacterium Infantis along with human milk have beneficial effects in reducing the incidence of NEC especially in pre-term neonates.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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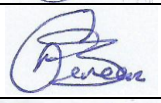
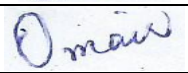
Dr. Saifullah Sheikh	Contributed in conception and interpretation of data and give his expert view of manuscript designing	
Dr. Syeda Umm-ul-Baneen Naqvi	Drafting the article and shares its expert research opinion and experience in finalizing the manuscript	
Dr. Ahmad Omair Virk	Data analysis and interpretation	
Prof. Dr. Muhammad Asghar Butt	Supervised the study and contributed in conception and shares its expert research opinion