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Effects of Probiotics on Late Onset Neonatal Sepsis in Preterm Infants: A Randomized Controlled Trial

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Abstract

Background And Objective: Late-onset neonatal sepsis frequently complicates prematurity, contributing to morbidity and mortality. Probiotics may reduce necrotizing enterocolitis (NEC) in preterm infants, with unclear effect on late-onset sepsis. This study aimed to determine the effect of administering a specific combination of probiotics in reducing the incidence of late onset neonatal sepsis in preterm infants. **Methods:** This is a prospective single center, double-blinded, placebo controlled, randomized trial compared daily administration of a probiotic with a placebo.

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Each probiotic capsule contains (500 mg blend)- Lactobacillus acidophilus- 2 billion cfu, Lactobacillus bulgaricus- 1 billion cfu, Bifidobacterium bifidum-1 billion cfu, Fructo-Oligosaccharide- 100 mg and each placebo capsule contains (500 mg blend) Pregelatinised Starch (Starch-1500). Infants born <35 completed weeks, weighing <2000 g admitted in NICU are included in this study. The primary outcome was at least 1 episode of late-onset sepsis. Secondary outcomes include: feeding intolerance, mortality, time to establish full enteral feeds, days required to physiological weight gain, patent ductus arteriosus, intraventricular hemorrhage, retinopathy of prematurity, bronchopulmonary dysplasia, duration of hospital stay. **Results:** Between 2019 and 2021, 119 preterm infants from NICU, BSMMU were randomized. Rates of definite late-onset sepsis (35.6 %), rates of LONS (definite and clinical) (44.1 %), mortality (13.6 %), feeding intolerance (11.9 %) were statistically significant low in probiotics group than the placebo group. There is more hospital stay, more days to reach full enteral feeds and less weight gain in placebo group and it is statistically significant. **Conclusions**: This randomized, double blinded, placebo controlled trial has power to demonstrate clinically significant effects of the chosen probiotic mixture on the rate of late-onset sepsis in LBW infants. A large clinical trial is required to address outstanding issues regarding safety and efficacy in this vulnerable population.

Keywords: Probiotics; Late Onset; Neonatal Sepsis; Preterm Infants.

1. Introduction

World health organization estimates the number of babies born with low birth weight, currently 25 million babies annually (WHO, 2020) and more than 80% of neonatal deaths were in low birth weight babies [1]. Late onset sepsis is a frequent complication of prematurity associated with increased mortality and morbidity. The commensal bacteria of the gastrointestinal tract play a key role in the development of healthy immune responses. Healthy term infants acquire these commensal organisms rapidly after birth. However, colonization in preterm infants is adversely affected by delivery mode, antibiotic treatment and the intensive care environment. Altered microbiota composition may lead to increased colonization with pathogenic bacteria, poor immune development and susceptibility to sepsis in the preterm infant [2]. Preterm infants are more susceptible to infection as they have an immature immune system, which may in part be due to the abnormal development of their gastrointestinal microflora (microbiome). Preterm infants largely acquire their colonizing gastrointestinal bacteria from the NICU environment, rather than their mother's genital tract flora, skin, or breast milk. In addition, gastrointestinal colonization with normal bacterial flora (eg, Bifidobacterium spp and Lactobacillus spp) is delayed and lacks biodiversity, whereas colonization with potentially pathogenic bacteria is increased (eg, Escherichia coli, Enterococcus spp, and Klebsiella pneumoniae). This is exacerbated by treatment with broad-spectrum antibiotics that alter the composition of the intestinal flora and may predispose very preterm infants to both late-onset sepsis and necrotizing enterocolitis [3]. Sepsis caused by intestinal bacteria is likely to result from the direct translocation of the bacteria over the intestinal barrier into the bloodstream [4]. It has been suggested that the overgrowth of pathogens might be prevented by inducing the colonization of the bowel with nonpathogenic bacteria (probiotics) of species normally resident in the gut of preterm and full-term infants. In particular, probiotics compete with other microbes for binding sites and substrates in the bowel and produce a wide range of antimicrobial substances such as bacteriocins, microcins, reuterin, hydrogen peroxide and hydrogen ions [5]. Probiotics are live exogenous micro-organism delivered

enterally that improve mucosal defences of the gastrointestinal tract and potentially provide benefit to the host. The common microorganisms used as Probiotics are (i) Lactobacillus (ii) Bifidobacterium [6]. There is increasing interest in the potential health benefits of protective colonization of the gastrointestinal tract of preterm infants [7]. Probiotics safety record renders it an attractive alternative to many of the more aggressive therapeutic options; it represents a simple, non-invasive attempt to recreate a natural or normal flora rather than a disruption of nature; and it appears to be effective in preventing a major source of morbidity in low birth weight infants. Postulated mechanisms by which they may protect the host from gastrointestinal and urinary infections include: increasing resistance of the mucosal barrier to migration of bacteria and their toxins by strengthening intestinal cell junctions, modification of host response to microbial products, augmentation of immunoglobulin A mucosal responses, enhancement of enteral nutrition to inhibit the growth of pathogens; production of bacteriocins (small proteins which kill bacteria); and competitive exclusion of potential pathogens [8]. Although evidence is accumulating that administration of probiotics to very preterm infants reduces necrotizing enterocolitis (NEC) and all-cause mortality, its effect on late-onset sepsis is less clear. We, thus, hypothesized that probiotics supplementation could reduce the incidence of sepsis in preterm infants.

2. Methodology

Patients and Study Design: This RCT was conducted in Department of Neonatology of BSMMU after approval by institutional review board over a period of eighteen months. Between 2019 and 2021, 119 infants born ≤35 weeks gestation, weighing ≤ 2000 g were randomized to the probiotics group (n = 59) or the placebo group (n = 60). On admission enrolled newborns was randomly assigned into two groups. Criteria for exclusion were the presence of major congenital malformations, chromosomal anomalies, and lack of parental consent. Randomization and Study Intervention: Study newborns was assigned either Group A or Group B. Group A was the intervention group which received probiotics along with regular breast feeding and standard care; and Group B was the control group which received placebo with regular breast feeding and standard care. Computerized randomization was done. Participants and investigators were unaware about group allocation. After completion of study, pharmaceutical company reveals that, which group get probiotics. The probiotic volume was 0.5ml containing 3×10^9 CFU and introduced once daily from first feeding by dropper or tube till discharged. Each probiotic capsule contains Lactobacillus acidophilus- 2 billion cfu, Lactobacillus bulgaricus- 1 billion cfu, Bifidobacterium bifidum-1 billion cfu, Fructo-Oligosaccharide- 100 mg and each placebo capsule contains Pregelatinised Starch (Starch-1500). The principal investigator and the research assistant was in charge in caring of the infants during their hospital stay. All the clinical care was given as per protocol. Sepsis was diagnosed on the basis of clinical features along with septic workup report. Of each infant, gestational age, birth weight, sex, type of delivery were recorded. Diagnosis of sepsis was based on clinical and laboratory data (total leukocyte count, total neutrophil count, immature-to-total neutrophil ratio, C-reactive protein concentration, peripheral blood film) confirmed by positive blood cultures. Primary and Secondary Outcomes: The primary outcome was the incidence of at least 1 episode of definite late onset sepsis during hospital stay. An episode of definite late-onset sepsis was defined as the sepsis >72 hours of postnatal age and when a pathogen was isolated from blood culture. Clinical sepsis was diagnosed either when a blood culture was negative, but the total count of WBC < 5000/cu mm or > 30,000/cu mm, C-reactive protein was > 6 mg/L, the immature-to-total neutrophil ratio was > 0.2, Absolute neutrophil count < 1500/ cu mm, Peripheral blood film shows features of septicemia.

Infants with late onset neonatal sepsis (definite and clinical) defined as both clinical and culture proven sepsis. Secondary outcomes were the mortality, time to reach enteral feeds of 120 mL/kg per day for ≥3 days, days required to physiological weight gain, feeding intolerance, duration of hospital stay, patent ductus arteriosus, intraventricular hemorrhage, retinopathy of prematurity and bronchopulmonary dysplasia. Sample Size and Statistical Analysis: After collection, data were entered into a personal computer then edited, analyzed, plotted and were presented in tables; categorical scale was analyzed by using Chi square test and continuous scale was analyzed by t test. Data was analyzed using the statistical package for social sciences (SPSS) version 25. P value <0.05 was considered as level of significance.

3. Results

Between 2019 and 2021, 119 infants born ≤35 weeks gestation, weighing ≤ 2000g were randomized to the probiotics group (n = 59) or the placebo group (n = 60). The baseline characteristics of the 2 groups are shown in Table 1.

Table 1: Baseline Characteristics of enrolled neonates (N=119)

	Probiotics group, n= 59	Placebo group, n= 60	P value
Gestational age, wk, mean (SD)	31.49 ±2.18	31.82 ± 1.90	0.39
≤32 wk, n (%) >32- 34wk, n (%) >34 wk, n (%)	36(61) 22(37.3) 1(1.7)	37(61.7) 22(36.7) 1(1.7)	0.99
Birth weight, g, mean (SD)	1417.80 ± 306.91	1502.03 ± 265.19	0.11
<1500 g, n (%) 1500- 1800 g, n (%) ≥ 1800 g - ≤ 2000g, n (%)	35(59.3) 19(32.2) 5(8.5)	30(50) 24(40) 6(10)	0.59
Male, n (%)	24(40.7)	32(53.3)	0.17
Multiple births, n (%)	13(22)	15(25)	0.70
Cesarean delivery, n (%)	47(79.7)	48(80)	0.96

Table 2: Late-onset sepsis outcomes (N=119)

	Probiotics group, n= 59	Placebo group, n=60	95% CI	P value
Infants with at least 1 episode of definite late-onset sepsis with pathogens, n (%)	21(35.6)	34(57.6)	0.41(0.19-0.85)	**0.01**
Infants with clinical late-onset sepsis, n (%)	5(8.5)	6(10.0)	0.83(0.24-2.89)	0.77
Infants with late-onset sepsis (definite and clinical), n (%)	26(44.1)	38(63.3)	2.19(1.05-4.57)	**0.03**

There is significant difference in the number of infants with at least 1 episode of definite late-onset sepsis (35.6% vs 57.6; (95% CI- 0.19 to 0.85), P = 0.01) in probiotics and placebo group. There is also significant difference in the number of total definite LONS and clinical sepsis (44.1% vs 63.3%; CI- 1.05-4.57), P= 0.03) (Table 2).

Table 3: Other secondary outcomes and morbidities (N=119)

	Probiotics group, n= 59	Placebo group, n=60	95% CI	P value
Mortality, n (%)	8(13.6)	17(28.3)	2.52(0.99-6.4)	**0.048**
Hospital stay, days, mean±SD	12.66 ± 6.69	17.85 ± 10.28		**0.016**
Days to full enteral feeds, mean (SD)	7.96 ± 4.63	13.60 ± 7.55		**0.003**
Weigh gain, n (%)	29 (49.2)	12(20.0)	0.26(0.11- 0.58)	**0.001**
Feeding intolerance,n(%)	7 (11.9)	17(28.3)	2.93(1.11-7.73)	**0.025**
PDA, n (%)	5(8.5)	5(8.3)	0.98(0.27-3.59)	0.978
IVH, n (%)	3(5.1)	3(5.0)	0.92(0.19- 5.08)	0.983
ROP, n (%)	8(13.6)	9(15)	1.12(0.40- 3.15)	0.822
BPD, n (%)	1(1.7)	2(3.3)	2(0.18-22.67)	0.569

Mortality, time to reach full enteral feeds, feeding intolerance, duration of hospital stay is less in probiotics group and it is statistically significant. Weight gain is statistically significant more in probiotics group. There is no significant differences in patent ductus arteriosus, intraventricular hemorrhage, retinopathy of prematurity, bronchopulmonary dysplasia, in between two groups (Table 3).

4. Discussion

Late onset sepsis is associated with high mortality and morbidity in preterm infants. Abnormalities in their intestinal microbiota development, which has important immune functions, may explain their increased susceptibility to infection. It has been hypothesized that very preterm infants, who have less microbial diversity in their GIT, may benefit from colonisation with exogenously administered probiotics [9]. Probiotics are defined as live microorganisms, which when administered in adequate amounts may confer health benefits on the host (FAO, 2001). The most recently published systematic review of probiotic supplementation in preterm infants concluded that enteral probiotics significantly reduces the incidence of severe Necrotising Enterocolitis and mortality [10]. In this study, we found that 13.6% mortality in probiotics group and 28.3% in placebo group which is statistically significant. Other favourable effects reported include reduced time to full enteral feeds, improved weight gain, colonisation rates and immune responses in the probiotic versus placebo group [11,12]. In this study we found feeding intolerance and hospital stay was less in probiotics group which is statistically significant. More days required to reach full enteral feed in case of placebo group. Weight gain is significantly more in probiotics group, in this study group. Administration of probiotics as 'live microbial feed supplements that beneficially affect the host by improving its microbial balance could be useful in the prevention of neonatal infections due to intestinal bacteria by several mechanisms [13,14,15]. It is known that lactobacilli can reduce the number of intestinal pathogens competing for their adhesion sites. The effect of probiotics on the incidence or severity of sepsis has been equivocal. This may be due to the heterogeneity of the RCTs included in the most recent Cochrane review with regard to the variation in probiotic strains studied, the method of administration (dosage, frequency, duration, etc.) or differences in local nursery guidelines for the diagnosis and treatment of sepsis. There is significant difference in the number of infants with at least 1 episode of definite late-onset sepsis (35.6% vs 57.6%; RR 0.41; (95% CI 0.19 to 0.85), P = 0.01) in probiotics and placebo group. There is also significant difference in the number of total definite LONS and clinical sepsis (44.1% vs 63.3%; RR 2.19 (1.05-4.57), P= 0.03). We found that probiotics decrease the incidence of bacterial sepsis. Neonatal infection is a

high priority area of research. Research on immunotherapy has provided very few leads. To the best of our knowledge, at present there are no proven interventions beneficial in preventing sepsis in LBW infants, apart from exclusive breastfeeding and practice of hygiene. Ohlsson and Lacy (2013) [16], provides an indication that microbial interference by beneficial bacteria is helpful in decreasing neonatal morbidity.

5. Conclusions

In conclusion, our study showed that oral supplementation of probiotics is effective in reducing the incidence of sepsis in preterm infants. We also found feeding intolerance, mortality is less in probiotics group. Time to reach full enteral feed, hospital stay more in placebo group. However, these results could represent an important preliminary work and justify further evaluation of the clinical effects of probiotics in a population with lower birth weight.

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