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Original Article

Neonatology Section

Efficacy of Probiotics in Preterm Neonates in the Prevention of Necrotising Enterocolitis: A Randomised Controlled Trial

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ABSTRACT

Introduction: The most frequent and dangerous gastrointestinal emergency in newborns is Necrotising Enterocolitis (NEC). A novel and promising strategy for preventing NEC is enterally given probiotics.

Aim: To evaluate the efficacy of probiotics in preterm neonates in the prevention of NEC and to assess the time of achievement of full feeds and duration of Intensive Care Unit (ICU) stay.

Materials and Methods: The present study was a parallel design single-centre randomised controlled trial, conducted in Neonatal Intensive Care Unit (NICU), Department of Paediatrics, Kempegowda Institute of Medical Sciences, Bangalore, Karnataka, India, from November 2018 to April 2020. Total of 130 newborns were included after inclusion and exclusion criteria and were randomised into two groups: that is probiotic (group I, n=61) and non probiotic (group II, n=69) groups. The probiotic group was given probiotics with breastmilk and non probiotic group were given only breastmilk. Probiotic (Bifidobacterium breve M16V) 0.5 g was mixed with breastmilk and given twice daily till full feeds were reached. All neonates were followed-up on daily basis for the appearance of features of NEC. Other parameters like time of achievement of full feeds and duration of ICU stay were compared between the two groups. Descriptive and inferential statistical analysis was carried out in the present study.

Results: Out of total sample, majority of babies belonged to the gestational age group of 30-33 weeks i.e., 30 (49.2%) in group I and 30 (43.5%) in group II. There were 29 (47.5%) females and 32 (52.5%) males in group I and, 32 (46.4%) females and 37 (53.6%) males in group II. There was a significant reduction of incidence of NEC (p-value=0.024) and earlier achievement of full feeds in the probiotic group (p-value=0.003) when compared to non probiotic group. The mean duration of ICU stay compared between the two groups was not statistically significant (p-value=0.366).

Conclusion: Supplementation of probiotics to the preterm Low Birth Weight (LBW) babies helps in the reduction of incidence of NEC and also helps in earlier achievement of full feeds.

Keywords: Bifidobacterium breve, Gastrointestinal disorder, Newborn, Randomisation

INTRODUCTION

The NEC is a fatal gastrointestinal disorder that mostly affects preterm newborns. It is the major cause of gastrointestinal illness and death in premature newborns [1]. Transmural and mucosal necrosis of the gut in varying degrees are the disease's hallmarks. Although the exact aetiology of NEC is yet unknown, other factors are probably involved [2]. NEC is acute inflammatory damage to the small and frequently the large intestine's proximal portions. Segmental coagulative necrosis of the mucosa with localised bleeding is shown in the surgical pathology as proof of ischaemia. Three risk factors that are universally acknowledged are formula feeding, bacterial dysbiosis, and prematurity [2].

Incidence of NEC inversely correlates with gestational age at birth, with a higher incidence in babies born at lower gestational ages. There is also a correlation between gestational age at birth and length of interval between birth and onset of disease: the earlier an infant is born, the more time will pass between birth and onset of NEC [1]. This results in the highest NEC incidence between 28 and 33 weeks of corrected gestational age [1]. Babies with NEC display a variety of symptoms, some of which may appear gradually or suddenly and catastrophically. A wide range of illnesses exist, from moderate conditions with merely guaiac-positive stools to severe conditions with intestinal perforation, peritonitis, systemic inflammatory response syndrome, shock, and even death [3].

Modified Bell's criteria is used for the diagnosis of the NEC and treatment includes medical and surgical management depending on the stage and severity of NEC [4]. The two most promising strategies for NEC prevention are exclusive use of human milk and probiotic supplementation [5]. A novel and promising strategy for preventing NEC is enterally given probiotics. Probiotics may aid in restoring normal gut microbiota colonisation when given to premature newborns [2]. According to a meta-analysis, probiotic-fed newborns (such as *Lactobacillus GG, Bifidobacterium breve, Saccharomyces boulardii*, and *Lactobacillus acidophilus* had a more than 50% lower incidence of NEC than controls [6].

There are many uses of probiotics in the prevention of NEC, that is they compete against pathogenic bacteria hence, reduces the chances of infection, decrease the inflammation, upregulate the cytoprotective genes and tighten the gut barrier. But there are not many studies which showed the proven benefit of probiotics to reduce the incidence of NEC in preterm babies and hence it is not routinely recommended [7,8]. With this background, the present study was conducted with the primary objective of analysing efficacy of probiotics in prevention of NEC. The secondary objectives were assessing the incidence of NEC at the hospital and assessing the time of achievement of full feeds and duration of ICU stay. To evaluate the efficacy of probiotics in the prevention of NEC and to assess the time of achievement of full feeds and duration of ICU stay.

MATERIALS AND METHODS

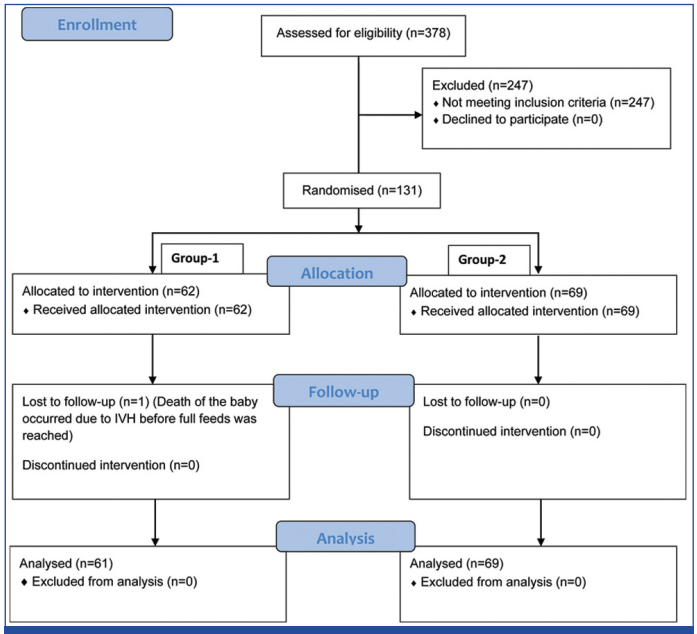
The present study was a parallel design single-centre randomised controlled trial with an allocation ratio of 1:1 conducted in NICU, Department of Paediatrics, Kempegowda Institute of Medical Sciences Hospital, Bangalore, from November 2018 to April 2020. After obtaining approval and clearance from the Institutional Ethics Committee (KIMS/IEC/D59/2018), the patients fulfilling the inclusion criteria were enrolled for the study after obtaining informed consent from the parents.

Sample size calculation: Sample size was calculated using Epi Info7 software based on previous study [9]. Considering 95% confidence interval, and 80% power with precision of 20%, it was calculated sample size of 50 in each group.

Inclusion criteria: Preterm neonates less than 37 completed weeks of gestation weighing less than or equal to 2 kg, both inborn and outborn neonates admitted in NICU and whose parents gave informed consent were included in the study.

Exclusion criteria: Neonates with chromosomal and congenital anomalies, neonates with gut malformations and outborn neonates who were already breastfed were excluded from the study.

A total of 131 neonates were randomised after excluding the neonates who did not meet the inclusion criteria. After randomisation, number of neonates allocated to group I and group II were 62 and 69, respectively. In that one baby in group I was lost to follow-up because the death of the baby occurred due to Intraventricular Haemorrhage (IVH) before full feeds was reached. So, finally 61 babies in group I and 69 babies in group II were analysed for the results [Table/Fig-1].



[Table/Fig-1]: CONSORT flow chart of study participants.
CONSORT: Consolidated standards of reporting trials

Study Procedure

The gestational age was determined by Last Menstrual Period (LMP) and by ultrasound scan of first trimester, and new Ballard scoring [10]. Preterm neonates and weight of the babies were classified as Low Birth Weight (LBW) and very LBW based on the World Health Organisation (WHO) classification [11]. LBW is defined as a birth weight of less than 2500 g. LBW is further categorised into Very Low Birth Weight (VLBW, <1500 g) and Extremely Low Birth Weight (ELBW, <1000 g) [11].

Newborns fulfilling the inclusion criteria were randomised using block randomisation technique with varying block sizes. Random allocation sequence was generated by the statistician using random allocation software version 1.0 to generate blocks. Sealed opaque consecutively numbered envelopes (prepared by the investigator) were used for allocation concealment. Participants were enrolled by the investigator and were allocated to intervention by the staff nurse based on the envelopes. Participants and the investigator assessing the outcomes were blinded after assignment to intervention.

Neonates allocated under group I was given probiotics along with breastmilk and those under group II were given only breastmilk without probiotics. Probiotic (Bifidobacterium breve M16V) was used for the current study. Each sachet of 0.5 g containing Bifidobacterium breve M16V (1 billion CFU/0.5 g) was diluted in 3 mL of expressed breastmilk and was given twice daily till the full feeds were reached [9]. The preparation was prepared freshly each time just before the feeds. And all the neonates were assessed by the investigator for the features of NEC like feeding intolerance, abdominal distension, blood in stools, erythema over the abdomen, and X-ray features of NEC like abnormal gas pattern consistent with ileus, bowel wall edema, pneumatosis intestinalis, gasless abdomen indicating ascites, portal or hepatic venous air, pneumobilia or pneumoperitoneum with the appearance of gas under the diaphragm [2]. Babies were followed-up on daily basis for appearance of features of NEC till the full feeds (100 mL/kg/day) was reached [9]. Effectiveness of probiotics was considered, if neonate did not show above signs and symptoms of NEC. Staging of NEC was done according to Modified Bell's criteria [4].

STATISTICAL ANALYSIS

The statistical software namely Statistical Package for the Social Sciences (SPSS) version 22.0, and R environment version 3.2.2 were used for the analysis of the data. Results on continuous measurements were presented on mean±Standard Deviation (SD) (Minimum-Maximum) and results on categorical measurements are presented as number and percentage (%). Significance was assessed at 5% level of significance. Student t-test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Intergroup analysis) on metric parameters. Leven's test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/Fisher's-exact test has been used to find the significance of study parameters on categorical scale between two or more groups, non parametric setting for qualitative data analysis. Fisher's-exact test was used when cell samples were very small.

RESULTS

The majority of babies belonged to the gestational age group of 30 weeks to 33 weeks, and the obtained p-value was 0.726, which is not statistically significant [Table/Fig-2].

Variables	Group I (n=61) n (%)	Group II (n=69) n (%)	p-value
Gender			
Female	29 (47.5%)	32 (46.4%)	0.96*
Male	32 (52.5%)	37 (53.6%)	
Gestational age (weeks)			
<30	5 (8.2%)	8 (11.6%)	0.726#
30-33	30 (49.2%)	30 (43.5%)	
34 and above	26 (42.6%)	31 (44.9%)	
Birth weight (kg)			
<1.0	3 (4.9%)	4 (5.8%)	1.000#
1.1-1.5	34 (55.7%)	39 (56.5%)	
1.6-2.0	24 (39.3%)	26 (37.7%)	

[Table/Fig-2]: Comparison of gender, gestational age and birth weight of neonates in the two groups.
*Chi-square test; #Fisher's-exact test

The variables like need for resuscitation at birth (bag and mask, intubation), ventilation {Synchronised Intermittent Mandatory Ventilation (SIMV), Continuous Positive Airway Pressure (CPAP)}, need for surfactant, sepsis variables (total count, platelet count, CRP) were non significant between the two groups [Table/Fig-3].

Among 130 babies included in the present study, a total of 18 (13.8%) babies had NEC that is the overall incidence of NEC in this study was 13.8%. The p-value of incidence of NEC in each group was 0.024 which was statistically significant. So in this study, NEC was more common in non probiotic group [Table/Fig-4].

Time of achievement of full feeds was 6-10 days in majority of babies in both the groups and the p-value was 0.012 which was

Variables	Group I (n=61)	Group II (n=69)	p-value
Need for resuscitation with bag and mask at birth, n (%)	3 (4.9%)	9 (13%)	0.110*
Need for intubation at birth, n (%)	4 (6.6%)	7 (10.1%)	0.463*
Ventilation: SIMV, n (%)	25 (41%)	34 (49.3%)	0.343#
Ventilation: CPAP, n (%)	14 (23%)	16 (23.2%)	0.974#
Need for surfactant, n (%)	24 (39.3%)	32 (46.4%)	0.419#
Sepsis variable: total count (cells/cumm), Mean±SD	12284.85±4987.21	13366.74±8609.23	0.390$
Sepsis variable: Platelets (lakh cells/cumm), Mean±SD	1.52±1.09	1.33±0.97	0.279$
Sepsis variable: CRP (mg/dL), Mean±SD	1.54±2.62	1.70±2.53	0.728$

[Table/Fig-3]: Comparison of other variables between the two groups.
SIMV: Synchronised intermittent mandatory ventilation; CPAP: Continuous positive airway pressure; CRP: C-reactive protein
*Chi-square test/#Fisher's-exact test/$Student's t-test

NEC	Group I (n=61) n (%)	Group II (n=69) n (%)	p-value
NEC	4 (6.6%)	14 (20.3%)	**0.024***
NEC stage			
• Stage 1A	4 (6.6%)	9 (13%)	0.423#
• Stage 1B	0 (0%)	5 (7.2%)	

[Table/Fig-4]: Comparison of incidence and severity of NEC in the two groups studied.
*Chi-square test/#Fisher's-exact test; The p-value in bold font indicates statisticlly significant values

statistically significant, which implied that probiotic group babies attained full feeds earlier when compared to non probiotic group [Table/Fig-5].

Variables	Group I n (%)	Group II n (%)	p-value
Time of achievement of full feeds (days)			
• 1-5	9 (14.8%)	5 (7.2%)	0.012#
• 6-10	48 (78.7%)	45 (65.2%)	
• 11-15	4 (6.6%)	16 (23.2%)	
• 16-20	0 (0%)	3 (4.3%)	
Duration of ICU stay (days)			
• 1-5	6 (9.8%)	3 (4.3%)	0.161#
• 6-10	9 (14.8%)	4 (5.8%)	
• 11-15	9 (14.8%)	18 (26.1%)	
• 16-20	12 (19.7%)	18 (26.1%)	
• >20	25 (41%)	26 (37.7%)	
Total	61 (100%)	69 (100%)	

[Table/Fig-5]: Comparision of time of achievement of full feeds (days), duration of ICU stay (days).
Fisher-Exact test (#)

The mean duration of time of achievement of full feeds in group I was 7.65±2.36 days when compared to group II where it was 9.13±2.99 days [Table/Fig-6].

Parameters	Group I (n=61) (Mean±SD)	Group II (n=69) (Mean±SD)	p-value
Time of achievement of full feeds (days)	7.65±2.36	9.13±2.99	**0.003**$
Duration of ICU stay (days)	18.68±9.98	20.24±9.56	0.366$

[Table/Fig-6]: Mean time of achievement of full feeds (days) and duration of ICU stay (days).
Values presented as mean±SD; $Student t-test

DISCUSSION

The NEC is a devastating disease of the gastrointestinal tract that contributes to morbidity and mortality in preterm infants [2]. Sterile Gastrointestinal (GI) tract gets rapidly colonised after birth [12]. Intestinal floras are acquired during the newborn period at the time of delivery [13]. Consequently, there may be some delay in acquisition among the infants due to treatment with antibiotics, total parental nutrition, etc., [14]. But the flora can be modified beneficially in the host through the introduction of desirable bacterial species through the use of probiotics [15]. Bifidobacterium is the most commonly found bacteria in the stool of term infants which is lacking in preterm babies. Studies have shown that earlier administration of bifidobacteria to the babies showed earlier appearance of bacteria in the faeces [16].

The overall incidence of NEC in the present study was 13.8% (18 babies out of 130 babies). The incidence of NEC in probiotic group was 6.6% (n=4) when compared to non probiotic group which was 20.3% (n=14) which was statistically significant (p-value=0.024). Similar observation was seen in the study by Chowdhury T et al., which showed the development of NEC was significantly lower in the study group than that of control group (1.9% vs. 11.5%; p-value=0.044) [17]. In the study by Braga TD et al., there were four confirmed cases of NEC stage ≥2 by Bell's criteria occurred only in the control group [18]. In study by Fernández-Carrocera LA et al., NEC incidence in the study group was 8% versus 16% in the control group, although the difference was not statistically significant (p-value=0.132) [9]. In the study by Oncel MY et al., neither the frequency of NEC stage 2 (4% vs 5%; p-value=0.63) nor the overall NEC or mortality rates (10% vs 13.5%; p-value=0.27) differed statistically significantly between the two groups [19]. A meta-analysis was done by Liu H et al., in which a total of 10 trials, which together comprised 3,227 patients, were selected for analysis [20]. Five of them made use of probiotics with multiple strains, and five others did so with just one. This meta-analysis revealed that probiotic treatment could lower mortality in underweight premature children {RR=0.81; 95% confidence interval (CI): (0.70, 0.94); Z-value=2.864; p-value=0.004} and feeding intolerance {RR=0.78; 95% CI: (0.67, 0.90); Z-value=3.280; p-value=0.001}. It could also lower the incidence of severe NEC {RR=0.66; 95% CI: (0.50, 0.87); Z-value=–2.978; p-value=0.003}.

The mean duration of time of achievement of full feeds in present study was 7.65±2.36 days in probiotic group when compared to non probiotic group which was 9.13±2.99 days (p-value=0.003). Similar observation was seen in the study by Chowdhury T et al., which showed, the age of achievement of full oral feeding was significantly earlier in the study group than that in the control group (14.88±3.15 and 18.80±4.32 days; p-value <0.001) [17]. Similar findings were seen in study done by Deshpande G et al., where babies in the probiotic group reached full feeds early (p-value=0.001) and in a study by Samanta M et al., where the number of days required to reach full enteral feeding (13.76±2.28 vs. 19.2±2.02; p-value <0.001) was significantly low in babies who received probiotics [21,22].

The mean duration of ICU stay in this study was 18.68±9.98 days in the probiotic group when compared to the non probiotic group which was 20.24±9.56 days (p-value=0.366). In the study done by Chowdhury T et al., the duration of hospital stay was significantly short in the study group compared to the control group (15.82±2.94 days vs 19.57±4.26 days; p-value <0.001) [17]. Similarly a study done by Samanta M et al., showed the duration of hospital stay was (17.17±3.23 days vs 24.07±4 days, p-value <0.001) also significantly low in the probiotic group compared with the control group [22]. Whereas in a study done by Fernández-Carrocera LA et al., there was no statistically significant difference between the study group and control group in terms of total days of hospitalisation 45 (13-134) days vs 40 (8-120) days, p-value=0.343 [9].

One of the reasons for the higher incidence of NEC in the present study Institution maybe, because it is a tertiary care centre and it receives many high-risk pregnancies, and babies stayed in ICU for more than 20 days mostly because of sepsis and prematurity rather than NEC.

Limitation(s)

The study conducted here is with a lesser number, hence further studies need to be done on a larger scale to conclude about the regular use of probiotics in the NICUs. And NEC being the multifactorial disease, other factors predisposing to the development of NEC have to be taken care of.

CONCLUSION(S)

The incidence of NEC was significantly lower in the probiotic group when compared to the non probiotic group and the babies belonging to the probiotic group reached full feeds early when compared to the non probiotic group. Hence, the present study implies that supplementation of probiotics in preterm LBW neonates helps in the reduction of incidence of NEC and helps in earlier achievement of full feeds. Although larger trials are needed to confirm the observations and regular supplementation of preterm babies with probiotics should be considered as a beneficial option in the neonatal ICUs.

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