

Placebo Controlled Introduction of Prophylactic Supplementation of Probiotics to Decrease the Incidence of Necrotizing Enterocolitis at Dhulikhel Hospital in Nepal

Dongol Singh S,¹ Klobassa DS,² Resch B,³ Urlesberger B,³ Shrestha RPB¹

¹Department of Paediatrics
Kathmandu University School of Medical Sciences
Dhulikhel, Kavre, Nepal.

²Department of Pediatric

³Division of Neonatology,
Medical University Graz,
8036 Graz, Austria.

Corresponding Author

Srijana Dongol Singh
Department of Paediatrics
Kathmandu University School of Medical Sciences
Dhulikhel, Kavre, Nepal.
E-mail: docsrijana@yahoo.com

Citation

Dongol Singh S, Klobassa DS, Resch B, Urlesberger B, Shrestha RPB. Placebo Controlled Introduction of Prophylactic Supplementation of Probiotics to Decrease the Incidence of Necrotizing Enterocolitis at Dhulikhel Hospital in Nepal. *Kathmandu Univ Med J.* 2017;60(4):319-23.

ABSTRACT

Background

Although recent reports suggest that the use of probiotics may enhance intestinal functions in premature infants, the mechanisms are unclear, and open questions remain regarding the safety and its efficacy.

Objective

The objective of this study is to evaluate the efficacy of probiotics on prevention of necrotizing enterocolitis in preterm infants in Nepal.

Method

We conducted a randomized, double blind, placebo controlled study of 72 hospitalized preterm infants. They were randomly allocated to receive probiotics (lactobacillus rhamnosus 35) at a dose of 0.8 mg in infants >1500 gms and 0.4 mg in infants <1500 gms in 2 ml of expressed breast milk two times daily or the same amount of expressed breast milk as placebo (without probiotics).

Result

Seventy-two patients were studied. The probiotics group (n=37) and placebo group (n=35) showed similar clinical characteristics. The incidence of necrotizing enterocolitis was found less frequently in the probiotic group (6/37, 16.2%) compared to the control group (10/35, 28.6%), this difference was not significant (p=0.16). This is 12.35% reduction in the incidence of necrotizing enterocolitis. Among the risk factors for necrotizing enterocolitis, pregnancy risk factors and perinatal risk factors were not significant. However neonatal risk factors were more frequent in the probiotic group (59.3%, n=32) than in the placebo group (40.7%, n=22), the difference was significant (p=0.02).

Conclusion

In the western world probiotics have been shown to be preventive in regard to necrotizing enterocolitis incidence. The present randomized trial showed a trend towards necrotizing enterocolitis minimal reduction in Nepal too. Further studies in a larger cohort are warranted to prove this effect for preterm infants.

KEY WORDS

Necrotizing enterocolitis, Placebo, Probiotics

INTRODUCTION

Necrotizing enterocolitis (NEC) is characterized by bowel wall necrosis of various length and depth.¹ It is primarily a disease of preterm infants, with the majority of cases occurring in very low birth weight (VLBW) infants.² No single unifying theory exists for the pathogenesis of NEC that explains all the clinical observations associated with the disorder. Proposed mechanisms for the development of NEC include immature intestinal mortality, digestion, circulatory regulation, barrier function, innate immaturity, and abnormal bacterial colonization.^{3,4} Diagnosis depends on Bell's staging criteria (table 1) that are based on radiographic evidence as bowel distension, ileus, pneumatosis intestinalis or bowel perforation.⁵

No consensus exists for the most effective approach for the management of NEC. On theoretical grounds, administration of probiotics to this vulnerable population could be an effective method to change gut colonization with the so-called healthy bacteria (Niekerk, 2011) while preventing the onset of NEC through balancing beneficial and harmful bacteria since birth.⁶ In 1965, Lilly and Stillwell first described probiotic in the literature as "live microorganism, which when administered in adequate amounts; confer a health benefit on the host".^{6,7} Among different strategies, none of the strategies have been really a breakthrough. The efficacy of probiotics in extremely low birth weight infants (<1000 gms) remains to be proven.^{8,9}

We therefore designed a randomized double-blind, placebo controlled clinical trial to evaluate the efficacy of lactobacillus rhamnosus on the incidence of NEC. Secondary endpoints were the identification of prenatal, perinatal and post natal risk factors in both groups.

METHODS

We undertook a randomized, double-blind, placebo controlled (1:1) trial from March 2013 to August 2015 at neonatal intensive care unit (NICU) of Dhulikhel Hospital, Kathmandu University Hospital. We screened 90 preterm newborns on the first or second days of life after birth in hospitals. Gestational age of infants was estimated using modified Ballard's methods and birth weight was recorded via a digital weighing scale.

We excluded sick infants (neonates with clinical or proven sepsis), those with congenital malformation especially (central nervous system) malformation and other such as gastrointestinal obstruction, gastrointestinal bleeding, gastroschisis, omphalocele, congenital heart defect and birth asphyxia (grade III). Outborn babies were also excluded in this study.

Premature neonates who met the inclusion criteria were divided into two groups of probiotic administration (intervention) and placebo using random selection by lottery. Prior to participation, effects of the administered

probiotic were explained to the parents of selected neonates, and written informed consent was obtained. In addition, information on sex, gestational age, birth weight, birth history any known infection during pregnancy or any maternal illness during pregnancy, cause of hospitalization, duration of NICU admission, and brief treatment modalities were recorded for all the neonates. In all included infants, expressed breast feeding (EBM) was initiated on second day of life. On the same day probiotics lactobacillus casei var. rhamnosus (LCR 35) 0.8 mg (half packet) dissolved in 2 ml of EBM in infant more than 1500 grams and 0.4 mg probiotics (1/4th packet) dissolved in 1 ml of EBM in infants less than 1500 grams was given twice a day until they reached full feeding. In all the infants both in the placebo and intervention group, feeding started from second day of life with 10% of total enteral feeding and then increased gradually by 10-20% until full feeding was reached.

Criteria for infants discharge from hospital in this study were:

- i) Complete treatment of the main morbidity
- ii) Ability to maintain own body temperature between 36.5°C-37.5°C
- iii) Well co-ordination of sucking and swallowing
- iv) Well feeding tolerance
- v) Well established breast feeding
- vi) Stable vitals sign for at least 2 days.

Intervention was instructed by the researcher and conducted by nursing staff of NICU. During the study, risk factors and co-morbidities of NEC were recorded in both groups. At the same time, incidence of NEC, treatment in brief, daily weight gain, mortality and causes of mortality were also recorded.

In this study, NEC was diagnosed according to Modified Bell's staging. According to this criteria staging depend on clinical, radiographic and gastrointestinal finding. Clinical finding were apnea, bradycardia, temperature instability, thrombocytopenia, metabolic acidosis, oligouria, hypotension, coagulopathy and shock. The radiographic finding were ileus, dilated loops, focal or wide spread pneumatosis, portal venous gas and pneumoperitoneum, Gastrointestinal finding like gastric residuals, abdominal distension, occult blood to gross blood in stool, absent bowel sound, abdominal wall erythema and bowel perforation.(Table 1)

Data analysis was performed in SPSS using the chi-square test, independent t-test and Fisher's exact test and a p value <0.05 was considered as significant.

Ethical clearance was obtained from the Institutional Review committee of Kathmandu University School of Medical Science and also from Nepal Health Research Council.

Table 1. Modified Bell's staging for NEC.

Review of Bell's stages	Clinical findings	Radiographic findings	Gastrointestinal findings
Stage I	Apnea and bradycardia, temperature instability	Normal gas pattern or mild ileus	Gastric residuals, occult blood in stool, mild abdominal distention
Stage II A	Apnea and bradycardia, temperature instability	Ileus gas pattern with one or more dilated loops and focal pneumatosis	Grossly bloody stools, prominent abdominal distention, absent bowel sounds
Stage II B	Thrombocytopenia and mild metabolic acidosis	Widespread pneumatosis, ascites, portal-venous gas	Abdominal wall edema with palpable loops and tenderness
Stage III A	Mixed acidosis, oliguria, hypotension, coagulopathy	Prominent bowel loops, worsening ascites, no free air	Worsening wall edema, erythema and induration
Stage III B	Shock, deterioration in laboratory values and vital signs	Pneumoperitoneum	Perforated bowel

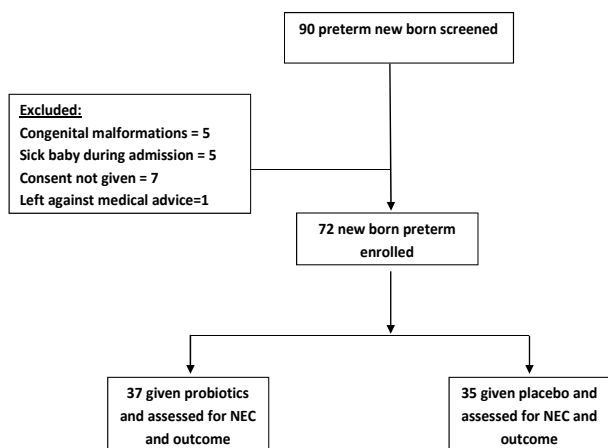


Figure 1. Participant flow through the trial

RESULTS

As shown in fig. 1, 90 newborns were screened in the study. Among 90 newborn 18 were excluded from the study (reason given in fig. 1), 37 of them received Lactobacillus casei. Rhamnosus(LCR 35)with expressed breast milk, 35 received placebo as expressed breast milk only. Both groups were comparable with regard to baseline characteristics such as sex of the child, gestational age, mode of delivery, prenatal steroids and birth weight. The probiotics group (n=37) and the placebo group (n=35) exhibited similar clinical characteristics.

Table 3 compares the pregnancy (prenatal), perinatal and neonatal risk factors between the intervention and placebo group. Pregnancy risk factors were considered as eclampsia, preeclampsia, premature rupture of membrane, gestational hypertension and clinical and histological chorioamnionitis. Pregnancy risk factors were

Table 2. Comparison of baseline characteristics

Variable	Probiotics (N=37)		Placebo (N=35)		
	No.	%	No.	%	
Sex	male	16	43.2	16	45.7
	female	21	56.8	19	54.3
Mean gestational age	32.6±2.2		32.6±2.2		
Birth weight groups					
i. Low birth weight (<2000 gms)	25	67.6	26	74.3	
ii. Very low birth weight (<1500 gms)	11	29.7	9	25.7	
iii. Extremely very low birth weight (<1000 gms)	1	2.7	0	0	
Perinatal steroid	25	67.6	24	68.6	
Mode of delivery					
i. Normal vaginal delivery	29	78.4	27	77.1	
ii. Instrumental delivery	0	0.0	1	2.8	
iii. Cesarean section	8	21.6	7	20.0	

Table 3. Risk factors for NEC

Variable	Probiotics (N=37)	Placebo (N=35)	p value
Pregnancy risk factor	8 (80%)	2(20%)	0.051
Perinatal risk factor (RDS/BA)	33(50.8%)	32(49.2%)	0.532
Neonatal risk factor (EOS,LOS)	32(59.3%)	22(40.7%)	0.020
Co-morbid condition (RDS,BA, NNS, hyper bilirubinemia)	34(50.74%)	33(45.25%)	0.53

RDS-respiratory distress syndrome, BA-birth asphyxia, EOS- early onset sepsis, LOS-late onset sepsis, NNS- neonatal sepsis.

four times more frequent in the intervention group than in the control group; however, the difference was not statistically significant. Respiratory distress syndrome and birth asphyxia were two main perinatal risk factors for NEC. There was no statistical significant difference in perinatal risk factors between intervention and control group. Neonatal sepsis was the main neonatal risk factor for NEC. Neonatal risk factor was 59.3% in the intervention group vs. 40.7% in the control group which was statistically significant (p value = 0.02).

The incidence of NEC was found less frequently in the probiotic group (6/37, 16.2%) compared to the control group (10/35, 28.6%), this difference was not significant (p=0.16). This was 12.35% reduction in the incidence of NEC. There was no significant difference between the two group regarding infants who expired during the study period, 8.1% in probiotic vs 8.7% in the placebo group (p=0.63).

The co-morbidities were also compared between the intervention and placebo group. The common co morbidities were respiratory distress syndrome, birth asphyxia, neonatal sepsis and hyperbilirubinemia. There was no significant difference between the two groups regarding co-morbidities (p=0.53). Among eight

mothers who had a pregnancy risk factor like eclampsia, preeclampsia, premature ruptures of membrane, gestational hypertension; two of them had histological chorioamnionitis(HC).

DISCUSSION

The present prospective, double-blind, randomized trial was designed to determine whether oral supplementation with probiotics(*Lactobacillus rhamnosus* 35) improved the gastrointestinal tolerance to enteral feeding in low birth weight infants. The overall incidence of NEC (22.22%) in current study was similar to other studies done in low birth weight infant, whose incidence of NEC was 23% and 25% respectively.^{10,11} Whereas it differs with the study performed in Canada, USA and most other western countries where the incidence of NEC was up to 5-7%.¹²⁻¹⁵

The analysis and classification of NEC done in this study was based on the Bell's staging criteria. The reduction in the NEC incidence in the treatment group was similar to another recent study done by Hojsak et al. where same probiotics had been used and NEC reduction was observed only by 7%.¹⁶

The study done by Ladd N and Ngo T in the year 2009 shown the incidence of NEC was 1.4% in the study group compared with 2.8% in the control group which was not statistically significant.¹⁷ In contrast with our study, supplementation with probiotics was associated with 75% relative risk reduction and 12% absolute risk reduction for the development of NEC. In addition, only 1% of the study group compared with 14% of the control group ($p=0.013$) has clinically significant NEC indicated by Bell's stage II or III.¹⁸ Differences in the probiotics strains used might have contributed to this discrepancy.

MN Shadkam et al. observed that 6.7% in the intervention group and 36.7% in the placebo group were diagnosed with NEC and there was a significant difference between the groups ($p=0.005$).¹⁹ In the same study, there was no significant difference between the intervention and placebo group in the incidence of co-morbidities like jaundice and sepsis, which is similar to our study.

Another meta-analysis done in Germany, examined the efficacy of probiotics in preterm infants in nine randomized controlled trials. They concluded enteral administration of probiotics significantly decreased the incidence of severe stage II-III NEC.²⁰

In the present study, we observed no significant differences between the two groups in term of co morbidities and mortality. Several recent studies done in 2015 and meta analysis done in 2010 also showed no significant differences between the infants receiving probiotics and placebo group regarding the incidence of neonatal co morbidities and mortality.^{16,19,20}

The present study failed to detect a significant reduction in the combined outcome of nosocomial sepsis and death. The most likely reason was insufficient statistical power from less number of involvements in the study as originally planned. This was comparable to the study done by Rouge et al done in two hospitals in France.²¹ The researcher concluded that use of probiotic did not show a benefit in prevention of NEC, sepsis or death.²¹

NEC is related to a multifactorial pathogenesis with multiple risk factors. There are certain prenatal, natal and post natal risk factors associated with development of NEC. In the present study there is no significant difference regarding pregnancy or prenatal risk factor ($p=0.051$) and perinatal risk factor ($p=0.0532$) between two groups however prenatal risk factor were four times more seen in the probiotics group. Among eight mothers who had different pregnancy (prenatal) risk factors like eclampsia, preeclampsia, premature ruptures of membrane and gestational hypertension, two of them had histological chorioamnionitis (HC). During our study periods both babies of those mothers developed NEC and expired as well. With respect to the association between histological chorioamnionitis and NEC, several studies in meta-analysis reported an association that reached a high level of statistical significance in chorioamnionitis with increased incidence of NEC.²²

Regarding neonatal risk factor, our study showed that the sepsis was more frequent in the probiotics group than in the placebo group. This was consistent with the finding of Dani D et al where bacterial sepsis was more frequent in the probiotic group (4.4%) than in the placebo group (3.8%) but the difference was not significant.²³ So neonatal sepsis may be an important risk factor for NEC. The role of postnatal systemic infection/inflammation including sepsis, in the pathogenesis of NEC is well recognized.^{24,25} Whenever neonates develop sepsis, they need to be treated with prolonged antibiotics. Prolonged administration of antibiotics further disrupts the colonization of the preterm gut and has been shown to increase NEC risk perhaps because of the destruction of good bacteria that compete with pathogenic bacteria.²⁶

Our study has some limitation. The sample size is small compared to most published studies abroad. We enrolled only inborn preterm neonates less than 32 weeks of gestation and also weight less than 1400 gram without any congenital anomalies. The other issue is that sometimes the probiotics could not be stored in appropriate temperature. Future studies in this area should address these limitations.

CONCLUSION

For the first time in Nepal prophylactic probiotics were introduced for study purpose. In the western world probiotics have been shown to be preventive in regard to NEC incidence. The present randomized trial could

not show a trend towards NEC reduction in Nepal. There was only 12.35 % reduction in the incidence of NEC. It remains unclear, whether this approach might be helpful in developing countries as well. Further studies in a larger

cohort are warranted to prove this effect for preterm infants. Our result suggests if the infant also had prenatal, natal and postnatal risk factors, then there is a high chance to have NEC.

REFERENCES

- Kefetzis DA, Skevaki C, Costalos C. Neonatal necrotizing enterocolitis: an overview *Curr Opin Infect. Dis* 2003; 16:349-55.
- Kosloske AM. Epidemiology of necrotizing enterocolitis. *Acta Paediatr Suppl.* 1994; 396:2-7.
- Gomella TI, Cunningham MD, Eyal FG, Lwnk KE. Necrotizing enterocolitis and spontaneous intestinal perforation In: Gomella TL editor. *Neonatology: Management, Procedures. On-call Problem, Diseases and Drugs* 5th ed. New York:Mc Grew-Hill Medical.2004;pp:482-7.
- Lin PW, Stoll BJ. Necrotizing enterocolitis. *Lancet.* 2006; 368(9543):1371-1283.
- Muralidhar H, Premkumar. Necrotizing enterocolitis. *Manual of Neonatal care.* 7th ed. pp.345 (340-349).
- Szajewska H. Probiotics and prebiotics in preterm infants: where are we ? Where are we going ? *Early Hum Dev.* 2010; 86 Suppl 1:81-6.
- Bernardo WM, Aires FT, Carneiro RM, Sa FP, RullVE, Burns DA. Effectiveness of probiotics in the prophylaxis of necrotizing enterocolitis in preterm neonates: a systematic review and meta-analysis. *J Pediatr (Rio J).* 2013; 89(1):18-24.
- Martin CR, Walker WA. Probiotics role in Pathophysiology and prevention in necrotizing enterocolitis. *Semin Perinatol.* 2008; 32:127-37.
- Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database syst Rec.* 2008; CD005496.
- Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics.* 2005; 115(1):14.
- Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien Ri, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics.* 2008; 122(4):693-700.
- Ballance WA, Dahms BB, Shenker N, Kliegman RM. Pathology of neonatal necrotizing enterocolitis: a ten- year experience. *J Pediatr.* 1990; 117(1):6-13.
- Guthrie So, Gardon PV, Thomas V ,Thorp JA, Peabody J, Clark RH. Necrotizing enterocolitis among neonates in the United States. *J Perinatol.* 2003; (4): 278-85.
- Horbar JD, Badger GJ, LewitEM ,Rogowski J Hospital and patient characteristics associated with variation in 28 day mortality for very low birth weight infant. *Pediatrics.* 1997; 99: 149-56.
- Hsueh W, Caplan MS, Qu XW, Tan XD, De Plaen IG, Gonzalez CF. Neonatal necrotizing enterocolitis: clinical consideration and pathogenetic concepts. *Pediatr Dec Pathol.* 2003; 6(1):6-23. Epub 2002 Nov 11.
- Hojdak I, Abdovic S, Szajewska H, Milosevic M, Krznicaric Z et al. Lactobacillus GG in the prevention of Nosocomial Gastrointestinal and Respiratory Tract Infections. *Pediatrics.* 2010; 125:1171-7.
- Ladd N, Ngo T. The use of probiotics in the prevention of necrotizing enterocolitis in preterm infants. *Pharmacology Notes.* 2008;14(14):2142-64.
- Sherry AL, Jacob TY, Healthier EW. Probiotics and Necrotizing enterocolitis: Finding the Missing Pieces of the probiotic Puzzle. *J Pediatr Pharmacol Ther.* 2012; 17(4):308-28.
- MN Shadkam, F Jalalizadeh, K Nasiriani. Effect of probiotic Lactobacillus Reuteri (DSM 17938) on the incidence of Necrotizing Enterocolitis in very low birth weight premature infants. *Iranian Jour of Neonatology.* 2015; 6(4).
- K Alfaleh, J Anabress, D Bassler. Probiotics reduce the risk of Necrotizing Enterocolitis in preterm Infants: A Meta-analysis. *Neonatology.* 2010; 97:93-99.
- Rouge C, Piloquet H, Butel JM, Berger B, Rochat F, Ferraris L. Oral supplementation with probiotics in very low birth weight preterm infants: a randomized, double-blind, placebo-controlled trial. *The American Journal of clinical Nutrition.* 2009; 89:1828-35.
- Gephart SM, McGrath JM, Effken JA, Halpern MD. Necrotizing enterocolitis risk: state of the science. *Adv Neonatal Care* 2012; 12:77-87.
- Been JV, Lievens S, Zimmermann IJ, Kramer WB, Wolfs GAM. Chorioamnionitis as a risk factor for Necrotizing Enterocolitis: A systematic review and Meta-analysis. *Journal of Pediatrics.* 2013;162(2):236-242.
- Martin CR, Bellomy M, Allred EN, Fichorova RN, Leviton A. Systemic inflammation associated with severe intestinal injury in extremely low gestational age newborns. *Fetal Pediatr Pathol.* 2013; 32:222-34.
- Markel TA, Engelstad H, Poindexter BB. Predicting disease severity of necrotizing enterocolitis: how to identify infants for future novel therapies. *J Clin Neonatol.* 2014; 3:1-9.
- Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sanchez PJ et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics.* 2009; 123(1):58-66.