



Efficacy of Probiotics in Preventing Necrotizing Enterocolitis in Preterm Neonates: An Interventional Study

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Abstract

Background

Necrotizing enterocolitis (NEC) is a common devastating neonatal condition that has now become a priority for research. Studies on the efficacy of probiotics in reducing NEC and its complications in developing countries like India are lacking. Providing probiotics to preterm new-borns has a potential to normalize abnormal colonization pattern, thus preventing the development of NEC.

Aim

The aim of this study is to know the efficacy of probiotics in reducing the incidence of NEC in preterm neonates.

Methods

This interventional randomized study was done at a tertiary care centre among 200 preterm neonates in the department of pediatrics' at NRI Institute of Medical Sciences, Visakhapatnam, Andhra Pradesh, from January 2022 to June 2022. Infants with cardio respiratory illness, congenital malformations, neurological problems, gestational age above 34 weeks were excluded. 100 neonates belonged to test group and 100 belonged to control group. Test group was given probiotics and breast milk. Age, gender, birth weight, development of NEC, and stage of NEC were assessed and compared between both groups.

Results

Most of the neonates belonged to 1 day of age. There is no significant difference in the mean age, mean birth weight, mean APGAR scores at 1, 5 min and gender between two groups. NEC is more commonly found in control group who were given only expressed breast milk. There is no significant difference in the NEC stage among the test and control groups.

Conclusion

Expressed breast milk with probiotics effectively prevented NEC onset compared to expressed breastmilk alone. So, we highly recommend providing breastmilk and probiotics to neonates who were born before 34 weeks of gestational age to prevent the onset of NEC.

Keywords

Probiotics, Necrotizing Enterocolitis, Preterm, Neonates, Breast milk.

Introduction

Necrotizing enterocolitis (NEC) is a common devastating neonatal condition that has now become a priority for research.^[1]In spite of various great advances in neonatal care, morbidity, mortality and healthcare costs related to NEC are significant. NEC is a multifactorial disease. The most important risk factor was prematurity. 90 % of the neonates who develop NEC are born preterm.^[2]NEC occurs due to comorbidities in prematurity like usage of broad-spectrum antimicrobials, immunosuppression, delayed enteral feeding and decreased availability of human milk. Probiotics are living microorganisms that, when taken in adequate amounts, provide health-benefit to the host by interaction with gut microbiota.^[3]Gut microbiota undergoes multiple dynamic changes during childhood. Gut colonization in preterm babies

happen differently compared to healthy term neonates^[4], and preterm infants commonly have delayed and divergent acquisition of “normal” flora. Studies done on preterm neonates showed that amniotic fluid and meconium are not sterile, indicating the intrauterine origin of gut microbiota^[5,6]. After birth, the preterm baby’s immature intestine gets exposed to a unique environment and to various manipulations. Subsequent intestinal dysbiosis is the main risk factor for the development of NEC: Preterm neonates with NEC have decreased bacterial gut diversity and abnormal bacterial strains compared to controls.^[7] In this view, providing probiotics to preterm neonates has the potential to “normalize” abnormal colonization patterns, thus preventing the development of NEC.^[8]Using probiotics for preventing NEC in preterm babies was previously investigated in certain randomized controlled trials. They found that probiotics decrease NEC development and decrease mortality rate.^[9,10]But, the American Academy of Paediatrics highlighted the need for more research to address unanswered questions on the dose of probiotics or type of probiotics to be used.^[11]The benefits of probiotics are strain-specific. Studies on the efficacy of probiotics in reducing NEC and its complications in developing countries like India are lacking. Hence this study was conducted. The aim of this study is to determine the effect of probiotics in reducing NEC and its complications in preterm neonates.

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Materials and Methods

Source of data and type of study: This interventional comparative study was done on preterm

neonates in the department of pediatrics, at a tertiary center named NRI Institute of medical sciences, Visakhapatnam, Andhra Pradesh, India.

Study Duration

The study was done for 6 months from March 2022 to September 2022.

Sampling

Simple random sampling method was used to select the study population.

Sample size: 200 preterm neonates

Sample Size Calculation

As per the systematic review done by Stollet al.^[12] the global prevalence of NEC among infants was 11%. The sample size is estimated as follows:

$$N = Z^2 PQ / E^2$$

N = Sample size

P = Prevalence

$$Q = 1 - P$$

$$N = 167$$

Confidence levels - 90% (power)

Error - 4%

167 is the minimum sample size. So, we included 200 neonates in our study.

Inclusion Criteria

- Preterm newborns with gestational age below 34 weeks.

- Neonates who are hemodynamically stable.
- Neonates of any gender, for whom consent was taken from legally accepted representatives.

Exclusion Criteria

- Neonates with cardiorespiratory illnesses
- Neonates with perinatal asphyxia
- Neonates with congenital malformations.
- Neonates with neurological problems.

Materials

Probiotic used is ‘Pedistine TM’ sachets. Each sachet contains 1 gram of *Saccharomyces* 282.50 mg with 250 mg of yeast, *Lactobacillus acidophilus* 0.24 billion, *Lactobacillus rhamnosus* 0.24 billion, *Streptococcus thermophilus* 0.24 billion, *Bifidobacterium longum* 0.24 billion.

Methodology

After getting informed consent from parents, one hundred neonates were selected as per the eligibility criteria. Test group neonates received regular expressed breast milk and daily Probiotics supplements daily two times until the neonate reach full feeds. The control group received only expressed breast milk without Probiotics.

Age in days	Test	Control
1-2 DAYS	90	84
3-4 DAYS	8	10
5-6 DAYS	2	4
7 DAYS	0	2
MEAN AGE	1.01±0.4days	1.04±0.3 days
P VALUE	0.54, T value=0.60	

Statistical Analysis

Data analysis was done using Epi Info software version 7.2.5. The results were expressed as mean \pm S.D, percentages, and all parameters were compared between two groups using students T test or chi-square test.

P value $<$ 0.05 was considered significant.

Ethical considerations

Ethical committee approval was taken before conducting the study. The informed consent form was taken from every parent of neonates who participated in the study.

Results

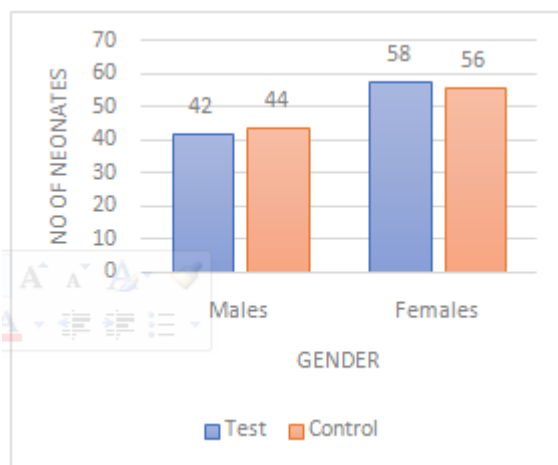
Age

None of the neonates is aged more than 7 days. There is no significant difference in the mean age of neonates between the two groups. Hence the comparison is justifiable.

Table 1 shows age of neonates in days

Gender

Most of the neonates were females. There is no significant difference in gender in both groups (p=0.88). Graph 1 shows gender distribution in both groups

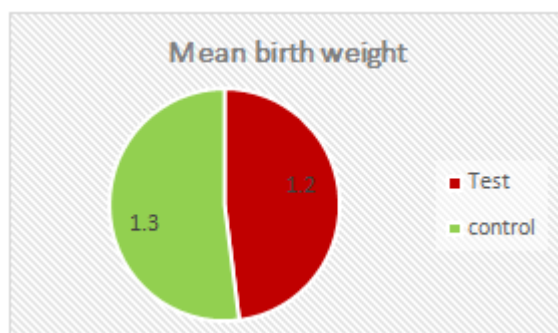


Birth Weight

There is no significant difference in the mean birth weight between two groups, as per T test

(p=0.27). The mean birth weight in test group was 1.2 ± 0.4 kgs and the mean birth weight in control group was 1.3 ± 0.5 kgs.

Graph 2: Mean birth weight in both groups



APGAR scores at 1 min and 5 min

There is no significant difference in the mean

APGAR scores at 1 min and 5 min between two groups.

Table 2: APGAR scores in both groups

Group	Test	Control	P value
1 min	6.7±1.1	6.9±1.9	0.52
5 min	8.1±2.1	8.4±2.2	0.48

Presence of NEC

NEC was developed in 2 neonates in the test group and 14 neonates in the control group. There is a significant difference in the incidence of NEC in both

groups as per the chi-square test. (P=0.001). The presence of NEC is more in the control group who were given only expressed breast milk without Probiotics.

Table 3: Presence of NEC in both groups

Presence of NEC	Test	Control
Yes	2	14
No	98	86
P value	0.001	

Stage of NEC in Both Groups

More severe NEC was seen in control group neonates but there is no significant difference in the

stage of NEC between test and control groups as per chi-square test. (p=0.158).

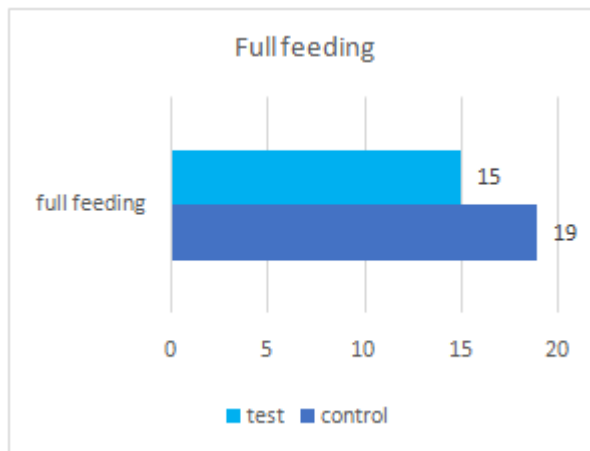
Table 4: Stage of NEC in both groups

NEC Stage	Test	Control
No	98	86
Stage I	2	8
Stage II	0	4
Stage III	0	2
P value	0.158	

Age of achievement of full feeding

Full feeding was achieved in 19 ± 3.4 days in the control group and in 15 ± 3.2 days in the test group.

There is a significant difference in the mean age of achieving full feeding, as per T-test ($p=0.0001$). Graph 3: Age of achievement for full feeding



Duration of Hospital Stay

The mean duration of hospital stay in control group was 20.2 ± 4.5 days and it was 16.1 ± 3.5 days in

the test group. There is a significant difference in mean duration of hospital stay, as per T-test ($p=0.0001$).

Table 5: Duration of hospital stay

Duration of hospital stay	Test	Control
Mean	16.1 ± 3.5	20.2 ± 4.5
P value	0.0001	

Side effects: No neonate was dead in the current study. No neonate developed sepsis.

Discussion

The study included 200 neonates who belonged to test and control groups, containing 100 in each group. There was no significant difference in the mean APGAR scores at 1 and 5 min, mean age, mean birth weight and gender between two groups. Development of NEC was more commonly seen in control group who were given only expressed breast

milk. The duration of hospital stay and no. of days for full feeding were more in control group. This implies that probiotics play vital role in preventing NEC and decreasing the duration of hospital stay and no. of days for full feeding.

In the study of Lin HC et al^[13] 360 very low birth weight infants were included. 180 belonged to the study group who were given probiotic along with breast milk and 187 belonged to the control group who were given only breastmilk. There was no

significant difference in age and gender between groups, similar to our study. The incidence of death or NEC above stage 2 was significantly less in study group. None of the neonates with positive blood culture grew *Lactobacillus* or *Bifidobacterium* species.

In the study of **Jacobs et al.**^[14] incidence of late-onset sepsis was 16.2%, NEC stage 2 was seen in 4.4%. Mortality rate was 5.1%. In contrast in our study, mortality rate and sepsis were nil. The difference could be due to sample size. Authors reported that probiotics *B infantis*, *Thermophilus*, and *B lactis* significantly decreased the incidence of NEC stage 2 or more in preterm babies.

One study done in India also reported a high efficacy of probiotics in preventing NEC in neonates with a birth weight of less than 1.5 kgs.^[15]

Many other studies done in western countries like Canada, North America, Germany reported good efficacy of probiotics in preventing NEC.^[16-20]

The strength of this study was we provided effective prophylactic option to prevent development of NEC among risky neonates.

Main limitation is small sample size. And we didn't do blood culture for neonates and it is a painful invasive procedure.

Conclusion

In the current study, we tested the efficacy of probiotics among neonates suffering from necrotizing enterocolitis. Results showed that there more incidence of NEC and severe NEC in test neonates who were given only expressed breast milk. This implies that probiotics play a vital role in preventing NEC. We highly recommend giving expressed breast milk along with probiotics for neonates who were

born preterm or before the gestational age of 34 weeks.

The study is self-sponsored.

There were no conflicts of interest.

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References

1. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med.* 2011 Jan 20;364(3):255-64. doi: 10.1056/NEJMra1005408. PMID: 21247316; PMCID: PMC3628622.
2. Gephart SM, McGrath JM, Effken JA, Halpern MD. Necrotizing enterocolitis risk: state of the science. *Adv Neonatal Care.* 2012 Apr;12(2):77-87; quiz 88-9. doi: 10.1097/ANC.0b013e31824cee94. PMID: 22469959; PMCID: PMC3357630.
3. Sanders ME, Guarner F, Guerrant R, Holt PR, Quigley EM, Sartor RB, Sherman PM, Mayer EA. An update on the use and investigation of probiotics in health and disease. *Gut.* 2013 May;62(5):787-96. doi: 10.1136/gutjnl-2012-302504. Epub 2013 Mar 8. PMID: 23474420; PMCID: PMC4351195.
4. Martin CR, Walker WA. Probiotics: role in pathophysiology and prevention in necrotizing enterocolitis. *Semin Perinatol.* 2008 Apr;32(2):127-37. doi: 10.1053/j.semperi.2008.01.006. PMID: 18346537.
5. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, Kim CJ, Erez O, Edwin S, Relman DA. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS*

- One. 2008 Aug 26;3(8):e3056. doi: 10.1371/journal.pone.0003056. PMID: 18725970; PMCID: PMC2516597.
6. Mshvildadze M, Neu J, Shuster J, Theriaque D, Li N, Mai V. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr.* 2010 Jan;156(1):20-5. doi: 10.1016/j.jpeds.2009.06.063. PMID: 19783002; PMCID: PMC3628625.
7. Wang Y, Hoenig JD, Malin KJ, Qamar S, Petrof EO, Sun J, Antonopoulos DA, Chang EB, Claud EC. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. *ISME J.* 2009 Aug;3(8):944-54. doi: 10.1038/ismej.2009.37. Epub 2009 Apr 16. PMID: 19369970; PMCID: PMC2713796.
8. Murguía-Peniche T, Mihatsch WA, Zegarra J, Supapannachart S, Ding ZY, Neu J. Intestinal mucosal defense system, Part 2. Probiotics and prebiotics. *J Pediatr.* 2013 Mar;162(3 Suppl):S64-71. doi: 10.1016/j.jpeds.2012.11.055. PMID: 23445850.
9. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics.* 2010 May;125(5):921-30. doi: 10.1542/peds.2009-1301. Epub 2010 Apr 19. PMID: 20403939.
10. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev.* 2014 Apr 10;(4):CD005496. doi: 10.1002/14651858.CD005496.pub4. Update in: *Cochrane Database Syst Rev.* 2020 Oct 15;10:CD005496. PMID: 24723255.
11. Thomas DW, Greer FR; American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Gastroenterology, Hepatology, and Nutrition. Probiotics and prebiotics in pediatrics. *Pediatrics.* 2010 Dec;126 (6):1217-31. doi: 10.1542/peds.2010-2548. Epub 2010 Nov 29. PMID: 21115585.
12. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD. *Pediatrics.* 2010;126(3):443-456. doi: 10.1542/peds.2009-2959. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
13. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics.* 2005 Jan;115(1):1-4. doi: 10.1542/peds.2004-1463. PMID: 15629973.
14. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, Morley CJ, Garland SM; ProPrams Study Group. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. *Pediatrics.* 2013 Dec;132(6):1055-62. doi: 10.1542/peds.2013-1339. Epub 2013 Nov 18. PMID: 24249817.
15. Samanta M, Sarkar M, Ghosh P, Ghosh Jk, Sinha Mk, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. *J Trop Pediatr.* 2009 Apr;55(2):128-31. doi: 10.1093/tropej/fmn091. Epub 2008 Oct 8. PMID: 18842610.
16. Hunter C, Dimaguila MA, Gal P, Wimmer JE Jr, Ransom JL, Carlos RQ, Smith M, Davanzo CC.

- Effect of routine probiotic, *Lactobacillus reuteri* DSM 17938, use on rates of necrotizing enterocolitis in neonates with birthweight < 1000 grams: a sequential analysis. *BMC Pediatr.* 2012 Sep 4;12:142. doi: 10.1186/1471-2431-12-142. PMID: 22947597; PMCID: PMC3472183. 10.7717/peerj.2429. PMID: 27761306; PMCID: PMC5068355.
17. Dilli D, Aydin B, Fettah ND, Özyazıcı E, Beken S, Zenciroğlu A, Okumuş N, Özyurt BM, İpek MŞ, Akdağ A, Turan Ö, Bozdağ Ş. The pro-pre-save study: effects of probiotics and prebiotics alone or combined on necrotizing enterocolitis in very low birth weight infants. *J Pediatr.* 2015 Mar;166(3):545-51.e1. doi: 10.1016/j.jpeds.2014.12.004. Epub 2015 Jan 13. PMID: 25596096.
18. Janvier A, Malo J, Barrington KJ. Cohort study of probiotics in a North American neonatal intensive care unit. *J Pediatr.* 2014 May;164(5):980-5. doi: 10.1016/j.jpeds.2013.11.025. Epub 2014 Jan 7. Erratum in: *J Pediatr.* 2014 Jun;164(6):1503. PMID: 24411521.
19. Härtel C, Pagel J, Rupp J, Bendiks M, Guthmann F, Rieger-Fackeldey E, Heckmann M, Franz A, Schiffmann JH, Zimmermann B, Hepping N, von der Wense A, Wieg C, Herting E, Göpel W; German Neonatal Network. Prophylactic use of *Lactobacillus acidophilus/Bifidobacterium infantis* probiotics and outcome in very low birth weight infants. *J Pediatr.* 2014 Aug;165(2):285-289.e1. doi: 10.1016/j.jpeds.2014.04.029. Epub 2014 May 29. PMID: 24880888.
20. Sawh SC, Deshpande S, Jansen S, Reynaert CJ, Jones PM. Prevention of necrotizing enterocolitis with probiotics: a systematic review and meta-analysis. *PeerJ.* 2016 Oct 5;4:e2429. doi: