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Effectiveness of Prebiotics and Probiotics with Breast Milk Vs Breast Milk Alone in Prevention of Sepsis in Neonates - A Randomised Controlled Trial

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Prebiotics, Probiotics, Sepsis, Neonates, NICU

ABSTRACT:

Introduction: Neonatal sepsis is not an uncommon phenomenon in neonates, and it has been noted that India has high incidence of neonatal sepsis with high case fatality rates as well. Therefore, it is thus of utmost importance to discover ways and preventive measures to prevent sepsis in these neonates. A combination of breast milk with prebiotics plus probiotic agents seems to be promising modality in preventing neonatal sepsis according to few scientific publications.

Objective: This study was undertaken to evaluate the effectiveness of the prebiotics and probiotics with breast milk versus breast milk alone in prevention of sepsis in neonates.

Method: This prospective, randomized control trial was conducted in NICU of tertiary care hospital in Maharashtra, India from 1st November 2019 to 31st October 2021.

Results: A total of 280 neonates were enrolled in study. 140 neonates who received prebiotic and probiotics with breast milk were categorized in group A, while 140 neonates who received only breast milk were categorized in group B. Majority of the enrolled neonates were male, term, AGA neonates in both the groups. Of the 140 neonates in group A, 136 neonates had no sepsis (97.14%), while in group B 76 neonates (54.28%) had no sepsis. In group A, 2 neonates had probable sepsis while 2 neonates had proven sepsis. In group B, 15 neonates had probable sepsis while 49 neonates had proven sepsis. This difference in between study groups was noted to be statistically significant ($p<0.05$ by chi-square test).

Conclusion: Administration of prebiotics and probiotics with breast milk was found to be significantly effective in preventing sepsis in neonates, in comparison to breast milk alone. However more research in this field will help in validating our study findings.

1. Introduction

Neonatal Sepsis is found to be the second-commonest reason for death among neonates, leading to deaths of more than 10 lakh neonates per year globally. It is estimated that of the 30 lakhs annual neonatal sepsis cases, India contributes maximum to clinical sepsis.[1-5]

The immune system of neonates is not developed and therefore, exposed to higher infection risk. The different defense characteristics of the mother's milk consist of heavy quantity of secretory antibodies of IgA subtype, formed by white blood cells (specifically lymphocyte) that have migrated to mammary glands from gut of

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mother. Therefore, breast-feeding moderates the primary exposure of the neonate's intestine to micro-organisms and confines translocation of bacteria via mucosa of the gut.[6]

A food preparation known as a probiotic is made up of viable, defined microorganisms in reasonable quantities that modify the microbiota of the host by imbedding or colonizing it and provide health benefits in the host.[7] On the other hand, a prebiotic is a food ingredient that is not digestible, which positively impacts the host by precisely encouraging the growth as well as activity of one or a few colonic bacteria.[8]

A synbiotic comprises of both pro- as well as prebiotics and allows an augmented function of exogenously given probiotic type of microorganisms along with substrate for commercial bacteria. According to preliminary findings, a combination of prebiotics and probiotics can both provide commercial bacteria with a substrate and enhance the effects of exogenously administered probiotics. According to low-quality research publications, lactoferrin, a prebiotic component of human milk, can lower neonatal late-onset sepsis and necrotizing enterocolitis (NEC).[9]

Addition of prebiotics plus probiotic agents to breast milk seems to be promising modality in preventing neonatal sepsis according to few scientific publications. However, there is not enough substantial scientific evidence published which can point out the beneficial effectiveness of the combination. Hence, this study was planned to evaluate the effectiveness of a combination of prebiotics and probiotics with breast milk in prevention of sepsis in neonates as compared to breast milk alone. This study can help in addition of crucial scientific evidence which can help the Pediatricians in understanding the utility of this novel combination modality in preventing sepsis in the Indian neonatal population.

2. Objectives

OBJECTIVES: The purpose of this study was to compare the efficacy of breast milk alone versus breast milk plus prebiotics and probiotics in preventing neonatal sepsis.

Method: This prospective, randomized control trial was carried out in NICU of tertiary care hospital in Maharashtra, India from 1st November 2019 to 31st October 2021.

Inclusion and Exclusion Criteria: The study included newborns admitted to the NICU who were receiving enteral feedings. However, it excluded the newborns with a diagnosis of sepsis, those who were not able to receive enteral feeds, and those whose feedings had been stopped.

Sample Size: Calculation of sample size was done as per the formula from the study by Samanta et al. (2014). A total of 280 subjects were included in the study and equally randomized into two study groups. 140 were administered probiotic and prebiotic in expressed breast milk in Intervention group (group A), while the second group of 140 neonates were administered only breast milk (Control group B). The neonates were randomly allocated into two groups using a simple randomization technique.

Intervention (Group A) group received formulation containing various components in powdered sachets of 1 gram each (brand name was *Darolac plus*). The prebiotic and probiotic sachet were added to mother's milk from the day of starting of feeding to the baby. The frequency was once a day. Staff nurses individually prepared the fresh suspensions in the pantry under aseptic precaution. The formulation was given to all neonates till discharge. The baby received the feed in two divided doses if the feed volume was less than 2 mL per feed, and once a day if it was 2 mL or more. If the feeding was stopped, the preparation was discontinued.

Control group (Group B) constituted a control group of neonates who were exclusively fed breast milk. Placebo was not used.

Demographic data collection including age, gender, maternal history, clinical features were noted. Detailed clinical examination of the neonate was done.

The data was collected and entered in case record form. Data collection comprised of clinical features suggestive of sepsis and laboratory investigation. In both the groups of babies, those

babies who developed clinical features of sepsis were further subjected for laboratory investigations for confirmation of sepsis.

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Complete Blood Count (CBC) was measured by a 5-part cell counter machine of SIMENS ADVIA 2120 hematology system. C-reactive protein method (CRP) was measured by turbidimetric method of SIMENS fully automated analyzer. Blood culture was conducted by BACTEC method.

Statistical Analysis: After data was noted, entry was done in a Microsoft Excel. Analysis of data was done with statistical software SPSS version 25. Quantitative data was displayed in study in form of Mean and Standard deviation, wherever applicable. Descriptive statistics were used to note down the distribution of patients based on age, gender, etc. To compare the quantitative mean values between the study groups, the unpaired T test was used when the data was uniformly distributed, while the Mann-Whitney test was used when the data was non-uniformly distributed. The chi-square test was used to compare the discrete values between the two study groups. P values below 0.05 was regarded as significant.

Operative Definitions:

Neonatal sepsis[10,11,12] is defined by the occurrence of two clinical symptoms along with minimum two laboratory signs in presence of or proven infection.

Proven sepsis: Neonate is found to have clinical features of sepsis along with isolation of organism from blood, CSF, and urine.

Probable sepsis: with positive septic screen (any 2)

- TLC < 5000/mm3
- ANC < 1500/mm^3
- Immature total neutrophil count ratio (I/T) > 0.2
- C- reactive protein (CRP) positivity

No sepsis:

- When the clinical picture of sepsis and laboratory signs are negative.
- The Enrolment flow chart of neonates in the study is mentioned below in figure 4.1.

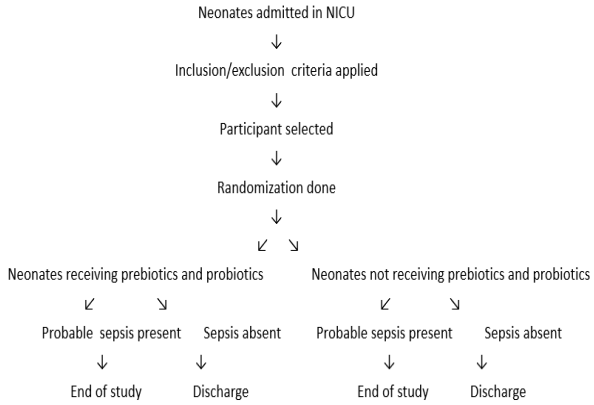

Figure 1: Enrolment flow chart of neonates in the study

Ethical Issues: The study was initiated after getting permission from the institutional ethics committee. Written consent of the parents was taken before enrolling the neonates in the study. In accordance with the inclusion and exclusion criteria, study participants were enrolled.

3. Results

Table 1: Demographic, baseline details in study groups:

	Group A (n=140)	**Group B (n=140)**
Number of Males	76 (54.29%)	85 (60.71%)
Number of Females	64 (45.71%)	55 (39.29%)
Chi-square (χ2) = 1.18, dF = 1 **P=0.27 considered NOT significant by Chi-square test**		

Table 1 shows demographic details in study groups. A total of 280 neonates were enrolled in the study. 140 neonates who received prebiotic and probiotics with breast milk were categorized in group A, while 140 neonates who received only breast milk were categorized in group B. Majority of the enrolled neonates were males in both the groups [group A having 76 males (54.28%), while group B having 85 males (60.71%)]. The gender

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distribution in both groups were statistically comparable (p=0.27, by chi-square test).

Table 2: Gestational status of enrolled neonates:

	Group A (n=140)	**Group B (n=140)**
Term	96 (68.57%)	98 (70%)
Pre-term	44 (31.43%)	42 (30%)
Chi-square (χ2) = 0.067, dF = 1 P=0.79 considered NOT significant by Chi-square test		

Table 2 shows gestational status in study groups. Most of the enrolled neonates in both group A and group B were term neonates (n=96 and n=98 respectively). 44 neonates in group A (31.42%) and 42 neonates in group B (30%) were pre-term neonates. No statistical difference was noted between the two groups in the gestational status (p=0.79)

Table 3: Distribution of neonates according to gestational age in study groups:

	Group A (n=140)	**Group B (n=140)**
AGA	109 (77.86%)	92 (65.71%)
SGA	31 (22.14%)	48 (34.29%)
Chi-square (χ2) = 4.096, dF = 1 P=0.07 considered NOT significant by Chi-square test		

Most neonates (n = 109 in group A and n = 92 in group B) in both research groups were appropriate for gestational age (AGA). Small for gestational age (SGA) newborns comprised 31 of group A neonates and 48 of group B neonates. No statistical difference was noted between the two groups in the weight by gestational age status (p=0.07).

Table 4 : frequency of sepsis amongst both groups

	Group A (n=140)	**Group B (n=140)**
No sepsis	136 (97.14%)	76 (54.28%)
Probable sepsis	2 (1.43%)	15 (10.72%)
Proven sepsis	2 (1.43%)	49 (35%)
Chi-square (χ2) = 70.24, dF = 2 P values <0.001 considered significant by chi-square test		

Of the 140 neonates in group A, 136 neonates had no sepsis (97.14%), while in group B 76 neonates (54.28%) had no sepsis. 2 neonates in group A had probable sepsis while 2 neonates had proven sepsis. In group B, 15 neonates had probable sepsis while 49 neonates had proven sepsis. Statistical analysis using the chi-square test revealed that there was a significant difference in the sepsis state across the study groups (p<0.05). In group A, one case was early onset sepsis (EOS) while three cases were late onset sepsis (LOS). 33 of the 49 cases in group B were LOS while 16 cases were EOS.

Table 5: Organisms isolated from infections in study groups

	Group A (n=140)	**Group B (n=140)**
Pseudomonas	2 (1.42%)	6 (4.28%)
Candida	0	14 (10%)
E.coli	0	7 (5%)
B.cepacia	0	7 (5%)
MRSA	0	6 (4.28%)
Klebsiella	0	6 (4.28%)
E.coli + Candida	0	2 (1.42%)
Pseudomonas + MRSA	0	1 (0.71%)
TOTAL	**2**	**49**
Chi-square (χ2) = 50.73, dF = 1 P values <0.001 considered significant by chi-square test		

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2 of the proven sepsis cases in group A had pseudomonas species isolated from blood. In group B, the commonest organism isolated from the 49 proven cases was Candida (n=14, 28.57%), followed by *E. coli* and *B. cepacia* (n=7 each, 14.29%).

4. Discussion

Neonatal sepsis is not an uncommon phenomenon in neonates, and it has been noted that India has high incidence of neonatal sepsis with high case fatality rates as well. Initially the sepsis can present either with mild or non-specific clinical features, but it can quickly give rise to septic shock, and even death in few cases.[6] Therefore, it is thus of utmost importance to discover ways and preventive measures to prevent sepsis in these neonates. Breastfeeding moderates the primary exposure of the neonate's intestinal mucosa to the microorganisms and confines translocation via the mucosa of the gut.[7] Probiotic as well as prebiotic have gained a lot of attention lately amongst Pediatricians with regards to their possible ability in providing better protection against sepsis in neonates.

In present study, majority of the enrolled neonates were males in both the groups, with group A having 76 males (54.28%), while group B having 85 males (60.71%). The gender distribution in both groups were statistically comparable (p>0.05 by chi-square test). The majority of the enrolled neonates in both group A and group B were term neonates (n=96 and n=98 respectively). 44 neonates in group A (31.42%) and 42 neonates in group B (30%) were pre-term neonates. No statistical difference was noted between the two groups in the gestational status (p=0.55, by chi-square test). Majority neonates in both study groups were appropriate for gestational age (AGA) by weight (n=109 in group A and n=92 in group B). 31 neonates in group A and 48 neonates in group B were small for gestational age (SGA). No statistical difference was noted between the two groups in the weight by gestational age status (p=0.07, by chi-square test).[13]

Identical studies had similar baseline findings as in the present study. Kukkonen et al., studied 939 infants in total (which included synbiotic, group of 468; placebo group of 471) and were followed up for 6-months, and 925 infants (461 in synbiotic; 464 in placebo) were followed up till 2 years. The baseline details were comparable between groups, like our study.[1]

In the study by Samanta et al.,186 preterm VLBW neonates underwent randomization, of which 91 neonates were given probiotics and 95 were not given the same. Demographic characteristics along with clinical variables of both mother as well as infant were similar, and no statistical difference was noted between the two groups (p>0.05).[14]

In the study by Janvier et al., 294 infants were given probiotics, and 317 infants were in the comparison group. The baseline parameters were again comparable between the study groups.[15]

In the present study, of the 140 neonates in group A, 136 neonates had no sepsis (97.14%), while in group B 76 neonates (54.28%) had no sepsis. 2 neonates in group A had probable sepsis while 2 neonates had proven sepsis. In group B, 15 neonates (10.71%) had probable sepsis while 49 neonates (35%) had proven sepsis. This difference in sepsis status was found to be statistically significant (p<0.05). In group A, one case was early onset sepsis (EOS) while three cases were late onset sepsis (LOS). In group B, 16 (32.65%) of the 49 cases were EOS while 33 cases (67.35%) were LOS.
2 of the proven sepsis cases in group A had pseudomonas species isolated from them. In group B, the commonest organism isolated from the 49 proven cases was Candida (n=14, 28.57%), followed by *E. coli* and *B. cepacia* (n=7 each, 14.29%). These findings clearly indicate that addition of probiotic and prebiotic supplementation to breast milk feeding led to a clear and significant advantage in preventing sepsis in these vulnerable and NICU-admitted neonates. Various studies from western studies have shown positive impact of giving probiotic, prebiotic or synbiotic formulations to neonates, in preventing sepsis.

In research by Kukkonen et al., the incidence (at least one time) of respiratory infections (66% vs. 68%), gastroenteritis (13% vs. 14%), or middle ear infections (15% vs. 19%) did not show a statistically significant difference between the synbiotic and placebo groups during intervention. However, a noteworthy finding was that fewer neonates in the synbiotic group received antibiotics than in the placebo group (23% vs. 28%; Odds ratio: 0.74; P=0.049).[1]

Samanta et al.'s study found that the probiotic-treated group's incidence of NEC was considerably lower than

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that of the non-exposed group (5.5% vs. 15.8%], respectively; P<0.05). Sepsis occurrences were observed to be lower in the study group (14.3% vs. 29.5%; p<0.05). The exposed group had a lower death rate (4.4% vs.14.7%; P<0.05) than the control group. These findings suggest the advantages of feeding prebiotics to the neonates, just like that noted in our study.[14]

In the study by Janvier et al., administration of probiotics was related with lower NEC (from 9.8% versus 5.4%, P<0.02), but a reduced mortality was noted which was not statistically significant (9.8% to 6.8%). However, a significant decrease in the combined outcome of NEC or death was noted (from 17% versus 10.5%, P <0.05). Effect of probiotics on health care–associated infection was not noted.[15]

Probiotic medication was associated with a lower NEC (from 9.8% compared 5.4%, P <0.02) in the study by Janvier et al., there was also a decreased death rate (from 9.8% to 6.8%), although this difference was not statistically significant. However, there was a significant decrease in the combined outcome of NEC or death was noted (from 17% compared 10.5%, P <0.05). Probiotics impact on infections linked to healthcare was not observed.

The outcomes of meta-experiments of potential clinical trials were randomly collected and connected with the probiotics contribution in averting NEC also aid the important role of the microbial floral of the gut in the causation of disease.[16] Recent studies points towards preserving environmental homeostasis (microbial, immune balance) that have beneficial effect in prevention of NEC in preterm neonates.[17]

Pediatricians should be careful while prescribing prebiotic and/or probiotics for neonates, for home usage. Some parents may encounter problems in giving the powder to newborns. Parents should be instructed regarding mixing the powder with milk as it can cause some kind of choking event, as was noted in the study by Kukkonen et al.[1] As a result, if the preparation is provided in the form of a powder, parents need to have specific instructions on how to use it in conjunction with appropriate amounts of formula or breast milk. But in present study, we used the combination of prebiotic and probiotic powder only during NICU stay.

This study can help in addition of crucial scientific evidence which can help the Pediatricians in understanding the utility of this novel combination modality in preventing sepsis in the Indian neonatal population.

Conclusion: Administration of prebiotics and probiotics with breast milk was found to be significantly effective in preventing sepsis in neonates, in comparison to breast milk alone. However more research in this field will help in validating our study findings.

Limitations: The study was carried out at only one study center and sample size was very small. In addition, our follow-up period was short and ended with discharge of neonate from hospital.

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