COMPARATIVE FREQUENCY OF NECROTIZING ENTEROCOLITIS IN PRETERM NEONATES ON PROBIOTICS VS STANDARD CARE ALONE

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ABSTRACT

Background: Preterm infants show increased pathogenic microbes and reduced normal flora, raising their risk of Necrotizing Enterocolitis (NEC). Probiotics, as non-pathogenic microbes, help prevent NEC and sepsis by enhancing intestinal barriers and modulating host response.

Objectives: To compare frequency of NEC in preterm neonates on probiotics versus standard care only.

Methods: It is Randomized Clinical trial conducted at Department of Neonatology, Children Hospital Multan from 25th April 2022 to 24th October 2022. A total of 188 (94 in each group) preterm infants of gestational age ≤35 weeks were included. Patients with congenital anomalies, requiring inotropic support, respiratory distress needing oxygen support within 72 hours of life and severe perinatal asphyxia were excluded. Group A (Probiotic group) - was given Immutec - Lactobacillus rhamnosus and Bifidobacterium BB-12 species 2.5×109 cfu (colony forming units) per day with feed. Group B (standard care group) – received enteral feeding and the remaining standard care (thermo neutral environment, fluid electrolyte balance, antibiotics when indicated). During this period the infant was investigated for NEC (blood counts, abdominal radiograph and stool for blood).

Results: Necrotizing enterocolitis was found in 05 (5.32%) in group A (probiotics group) and 21 (22.34%) in group B (standard care only group) with p-value of 0.0007.

Conclusion: This study concluded that probiotics are better in terms of preventing necrotizing enterocolitis in preterm neonates than standard care only.

Keywords: preterm neonates, necrotizing enterocolitis, probiotics.

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INTRODUCTION

Necrotizing enterocolitis (NEC) is the most prevalent gastrointestinal (GI) medical and surgical emergency in neonates. It is an acute inflammatory condition with a multifactorial and still-debated etiology, characterized by a spectrum of intestinal damage that can range from superficial mucosal injury to full-thickness necrosis and intestinal perforation.[1] NEC poses a major clinical challenge, affecting nearly 10% of preterm neonates with a birth weight under 1500 grams, and is associated with high mortality rates. Risk factors for NEC include chorioamnionitis, formula feed, broad spectrum antibiotic and a sibling with NEC.[2] Its pathogenesis involves a marked influx of pathogenic immune cells and elevated inflammatory cytokine signaling. Early bacterial colonization can directly disrupt the intestinal epithelial barrier. Toll-like receptor 4 (TLR4), which is uniquely upregulated in the premature intestine and recognizes Gram-negative LPS, plays a central role in

initiating NEC.[3] It results from bacterial invasion of the intestinal wall, leading to inflammation and destruction of intestinal tissue. If not promptly identified and managed, it can progress to intestinal perforation, allowing bowel contents to leak into the peritoneal cavity and cause peritonitis.[4] The most crucial diagnostic tool for NEC is a series of plain abdominal radiographs, including anterior-posterior and left lateral decubitus views. Diagnostic indicators on imaging include dilated bowel loops, pneumatosis intestinalis, and portal venous gas. Laboratory tests have limited specificity, though certain findings may support the diagnosis.[5] Laboratory studies have limited utility and are non-specific. Leukopenia with a white blood cell count below 1500 per microliter is a strong indicator of established sepsis. Hyponatremia and low serum bicarbonate also showed some association with the NEC.[6]

Standard management for neonates with NEC involves stopping enteral feedings, gastric decompression using a nasogastric tube, administration of intravenous antibiotics, and provision of total parenteral nutrition. Despite this approach, mortality rates remain high.[7]

Probiotics are living non-pathogenic microbes that colonize the intestinal wall and may protect preterm neonates from developing sepsis and NEC by increasing barrier for transfer of bacteria across the intestine, and by modifying host response.[8] There is a reported increase in pathogenic microorganisms in preterm neonates along with a decrease in normal flora that render these babies at increased risk of developing NEC. The growth of pathogens might be prevented by inducing the colonization of the intestine with non-pathogenic bacteria (probiotics) of species that are normal residents in the gut of preterm and term preterm neonates.[9]

A knowledge gap exists regarding the use of probiotics in the management of NEC, and standard care remains the primary treatment in many clinical settings. This study aims to compare probiotic therapy with standard care in preterm neonates with NEC to evaluate the potential benefits of probiotic use.

METHODS

This randomized clinical trial, conducted in the Department of Neonatology at Children's Hospital, Multan, from April 25 to October 24, 2022, was carried out in accordance with the CONSORT 2010 guidelines. Ethical review certificate was sought from Research unit of College of Physicians and Surgeons (CPSP), Pakistan (reference no: CPSP/REU/PED/-2021-100-6183). A total of 188 neonates were enrolled using a non-probability, consecutive sampling technique.[10] Preterm neonates (≤35 weeks' gestation), of either gender, admitted within 72 hours of life, and who had not started oral feeding were included. Exclusion criteria comprised neonates with congenital anomalies and chromosome abnormalities or genetic metabolic errors.[11] Informed written consent was obtained from parents after explaining the study objectives. Baseline characteristics such as age, gender, gestational age, birth weight, type of feeding (breast milk or formula), and mode of delivery (cesarean or vaginal) were documented. All neonates received enteral feeding according to the same institutional protocol. Participants were randomly divided into two groups (n=94 each) through draw randomization. Folded papers containing group labels were placed in a jar, and the on-duty staff nurse selected one at random for each neonate. Group A (probiotic group) received Immutec containing Lactobacillus rhamnosus and Bifidobacterium BB-12 at a daily dose of 2.5×10^9 CFU with feeds, while Group B (standard care group) received standard enteral feeding and supportive care, including thermoneutral environment, fluid and electrolyte management, and antibiotics when indicated.[12,13] Abdominal girth was measured before feeds. An increase in girth >2 cm prompted gastric aspiration. If aspirates measured 30–50% of pre-feed volume or ≥33 ml/kg, feed advancement was withheld for 24 hours. Aspirates exceeding 50% led to temporary discontinuation of feeds and further investigation for NEC, including blood tests, abdominal radiographs, and stool testing. If results were negative, feeds were restarted at half the previous volume.[14] Confirmed NEC cases were managed according to standard protocols. Neonates were discharged once they tolerated full enteral feeding for 48 consecutive hours and had no other indication for hospitalization. Duration of hospital stay and all study-relevant data were recorded on a predesigned proforma. Data were analyzed using SPSS v23. Mean ± SD was calculated for birth weight and gestational age, while frequencies and percentages were used for gender, mode of delivery, type of milk, and incidence of NEC. The chi-square test was applied to compare NEC frequency between groups, with a p-value ≤ 0.05 considered significant.

RESULTS

In Group A, 76.60% of neonates (n=72) were born at less than 32 weeks of gestation compared to 68.09% (n=64) in Group B, while 23.40% (n=22) in Group A and 31.91% (n=30) in Group B were between 32–35 weeks. Mean gestational ages were similar between Group A (30.32 ± 1.58) and Group B (30.50 ± 1.86). A total of 5 neonates in Group A and 21 in Group B developed NEC, demonstrating a statistically significant difference ($p = 0.0007$). Intergroup comparisons of NEC occurrence in relation to gestational age, gender, birth weight, mode of delivery, and type of feeding are detailed in Table 01.

The comparative analysis between Group A and Group B, each comprising 94 neonates, revealed significant associations between several clinical parameters and the incidence of necrotizing enterocolitis (NEC). In the

gestational age category of less than 32 weeks, Group B had a significantly higher proportion of NEC cases (31.25%) compared to Group A (5.56%) with a highly significant p-value of 0.0001. However, in the 32–35 weeks category, NEC incidence was comparable between both groups (4.55% vs. 3.33%), with no significant difference (p = 0.822). Gender-based analysis showed a higher occurrence of NEC among males in Group B (20%) than Group A (8.33%), though this did not reach statistical significance (p = 0.067). Among females, however, NEC was reported only in Group B (26.47%), whereas none occurred in Group A, yielding a statistically significant p-value of 0.0013.

Table 01

		Group A (n=94)		Group B (n=94)		P-value
		Necrotizing enterocolitis		Necrotizing enterocolitis		
		Yes	No	Yes	No	
Gestational age (weeks)	<32	04 (5.56%)	68 (94.44%)	20 (31.25%)	44 (68.75%)	0.0001
	32-35	01 (4.55%)	21 (95.45%)	01 (3.33%)	29 (96.67%)	0.822
Gender	Male	05 (8.33%)	55 (91.67%)	12 (20.0%)	48 (80.0%)	0.067
	Female	00 (0.0%)	34 (100.0%)	09 (26.47%)	25 (73.53%)	0.0013
Birth weight (kg)	≤1.5	01 (4.76%)	20 (95.24%)	12 (60.0%)	08 (40.0%)	0.0001
	>1.5	04 (5.48%)	69 (94.52%)	09 (12.16%)	65 (87.84%)	0.154
Mode of delivery	SVD	03 (6.52%)	43 (93.48%)	11 (22.92%)	37 (77.08%)	0.026
	CS	02 (4.17%)	46 (95.83%)	10 (21.74%)	36 (78.26%)	0.011
Type of feeding	Breast	02	50	08	43	0.042

Birth weight also showed a significant impact: neonates with weight ≤1.5 kg had a markedly higher NEC rate in Group B (60%) compared to Group A (4.76%), with a p-value of 0.0001. In those weighing more than 1.5 kg, the NEC incidence was higher in Group B (12.16%) than in Group A (5.48%), though the difference was not statistically significant (p = 0.154). Regarding mode of delivery, both spontaneous vaginal delivery (SVD) and cesarean section (CS) were associated with significantly more NEC cases in Group B (22.92% for SVD and 21.74% for CS) than in Group A (6.52% and 4.17%, respectively), with p-values of 0.026 and 0.011. Lastly, type of feeding showed a significant difference, with breastfed neonates in Group B showing a higher NEC incidence (15.69%) compared to those in Group A (3.85%) (p = 0.042).

DISCUSSION

Necrotizing enterocolitis (NEC) is a serious gastrointestinal emergency in preterm infants, associated with high mortality and long-term complications, and believed to result from a combination of pathogenic bacteria, immature gut barrier, and enteral feeding. Probiotics, which support healthy gut colonization, are considered a promising preventive strategy. In this study, NEC occurred in 5.32% of neonates receiving probiotics (Group A) compared to 22.34% in those receiving standard care only (Group B), showing a statistically significant reduction (p = 0.0007). Our findings are comparable with several international studies that support the preventive role of probiotics against NEC in preterm infants. Hunter C conducted a retrospective cohort study on 311 preterm neonates and found a significantly lower incidence of NEC in those who received Lactobacillus reuteri (2.5%) compared to untreated neonates (15.1%), with a p-value of 0.04, supporting the findings of our study.[15] A 2017 British study similarly concluded that neonates who received probiotics had significantly lower mortality rates (p = 0.01) compared to those who received standard care, further supporting the findings of our research.[16] Marwyn Sowden divided 200 preterm neonates into two equal groups and observed that 5 neonates in the placebo group developed NEC, compared to only 1 in the probiotic group. Additionally, there was a statistically significant improvement in feeding among neonates receiving probiotics (p < 0.001).[17] Similarly, a meta-analysis of RCTs conducted by Yu Dai reported that the use of probiotics in preterm infants not only shortens the length of hospital stay but also significantly reduces mortality.[18] These findings specify that probiotics reduce gastrointestinal complications that finally improve overall neonatal outcomes. However, the study by Belal N on Canadian preterm neonates concluded that while

probiotics reduced the mortality rate, they had no effect on the development of NEC or sepsis. This finding differs from ours and raises questions about the credibility of probiotics in preventing NEC.[19] The consistency of these studies strengthens the evidence supporting probiotic role in neonatal intensive care units. Meanwhile, contrary findings by Valeria Melendez Hebi on preterm-delivered infants concluded that human milk offers greater protection against the development of NEC, and that probiotics alone are insufficient to reduce this risk—particularly in neonates fed with formula.[20] The discrepancy between his findings and our results opens the door for further researches that evaluate effect of probiotic strains on NEC development while keeping in mind the dosage protocols and feeding practices.

Hence there are multiple researches who favor use of probiotics in preterm neonates for the prevention of development of NEC but few studies conflict the authenticity of probiotics and highlight the need for further researches to validate the efficacy of probiotics in preterm neonates. Inshort despite of some conflicting evidence, the overall literature largely supports the beneficial role of probiotics in reducing adverse outcomes among preterm infants. Furthermore multi-centric studies with larger sample size are recommended to authenticate the corresponding findings.

CONCLUSION

This study concluded that probiotics are better in terms of preventing necrotizing enterocolitis in preterm neonates than standard care only.

ETHICAL APPROVAL

Approval of article was granted by the Research Evaluation Unit of CPSP, Lahore Reference No: CPSP/REU/PED-2021-100-6183, Dated: April 18, 2024.

AUTHOR'S CONTRIBUTIONS

MFT: Study design, data collection, manuscript writing
FM: Study design, biostatistics
NI: Study design, result compilation
HR: Manuscript writing, biostatistics
FB: Result compilation, data analysis
MSB: Biostatistics, literature review
All Authors: Approval of the final version of the manuscript to be published

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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