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Original Article

The Effect of Probiotics on Milk Tolerance and Prevention of Necrotizing Enterocolitis in Premature Infants Admitted to the Neonatal Intensive Care Unit of Bentolhoda Hospital in Bojnurd, Iran, in 2021-2022

Ghasem Bayani, Hojatollah Ehteshammanesh, Shahin Mafinejad, Fateme Kamali, Mona Akbarian Sanavi*

1. North Khorasan University of Medical Sciences, Bojnurd, Iran

ABSTRACT

Background: Necrotizing enterocolitis is the most common gastrointestinal emergency in infants. If not treated promptly, it can progress to necrosis, rupture, peritonitis, sepsis, and death. Therefore, the use of probiotics can have health benefits.

Methods: This double-blind clinical trial was conducted on 86 premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd, Iran, in 2021-2022. In this study, premature infants were randomly assigned to control and intervention groups. In the intervention group, after the infant's feeding volume reached 5 cc per kilogram of body weight per day, oral probiotics at a dose of one drop per kilogram of body weight, diluted with normal saline to a volume of 0.5 cc, were administered every 12 hours for 3 weeks.

Results: There was no statistically significant difference between the two groups in terms of variables such as weight at admission, weight at discharge, length at discharge, and head circumference at discharge. However, a statistically significant difference was observed in terms of the difference in weight between admission and discharge, as well as the duration of hospital stay. The average difference in weight between admission and discharge in the intervention group was approximately 90 grams greater than that in the control group. Also, the average duration of hospital stay in the intervention group was approximately 8 days less than that in the control group.

Conclusion: The use of probiotics in premature infants prevents necrotizing enterocolitis and shortens the time to reach full enteral feeding and the duration of hospital stay.

Keywords: Enterocolitis, Infant, Milk tolerance, Necrotizing, Premature, Probiotics

Introduction

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in infants (1). The risk of NEC increases with decreasing gestational age. Approximately 5% of premature infants less than 32 weeks of gestation and weighing less than 1500 grams, and 10% of very premature infants (less than 28 weeks) weighing less than 1000 grams, develop necrotizing enterocolitis. The mortality rate in premature infants with this complication varies between 20% and 30% (2). The etiology of NEC is multifactorial, and abnormal intestinal microbes have been increasingly reported as a major cause (3). Premature rupture of membranes, assisted

* *Corresponding author*: Mona Akbarian Sanavi, North Khorasan University of Medical Sciences, Bojnurd, Iran. Email: mona.akbarian@yahoo.com

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ventilation, sepsis, hypotension (4), exposure to acid-suppressing drugs, long-term treatment with antibiotics (5), feeding with cow's milk (6), defects in intestinal epithelial barrier function, impaired intestinal motility, impaired regulation of microvascular blood flow (7), intestinal immaturity, genetic and metabolic predisposition, and changes in intestinal microbes (8), gestational age, birth weight, formula feeding in infancy (which in turn is related to immaturity of the gastrointestinal and immune systems) (9, 10), and inflammatory and vasoactive mediators have been recognized as clinical risk factors (11).

Bacteria in human milk colonize the intestine and prevent the development and proliferation of pathogenic bacteria and enhance innate immunity, and thus reduce the risk of NEC. In special conditions, such as premature infants with very low birth weight admitted to intensive care units, the rate of intestinal colonization by beneficial bacteria Bifidobacterium and Lactobacillus is reduced, and the intestinal flora changes to higher levels of pathogenic bacteria such as Klebsiella, Enterobacter, Citrobacter, and Pseudomonas, which are commonly observed in hospitals (12). This debilitating disease can cause mucosal inflammation, death of intestinal epithelial cells, gas accumulation in the submucosa of the intestinal wall, and transmural perforation, which leads to leakage of intestinal contents into the peritoneal cavity and ultimately multiple organ failure (3). If not treated promptly, it can progress to necrosis, rupture, peritonitis, sepsis, and death (4).

Various approaches have been used to prevent NEC, including early enteral feeding, breast milk feeding, intestinal antibiotics, IgA supplements, anti-cytokine agents, growth factors, antenatal steroids, and probiotic products (13). Therefore, it seems that preventing NEC is more important and effective than treating it. It has recently been reported in the literature that probiotics are useful for preventing necrotizing enterocolitis and reducing its complications, including improved milk tolerance (14). Probiotics are live microorganisms (bacteria) that, when administered in sufficient quantities, provide health benefits to the host (15). Probiotics protect the premature intestine from inflammation and damage through several mechanisms. These mechanisms include re-regulation of cytoprotective genes; re-regulation of inflammatory gene expression; production of butyrate and other short-chain fatty acids that nourish colonocytes and reduce pH and oxygen in the intestinal lumen, thereby reducing the growth of pathogenic Enterobacteriaceae; competition with other microbes; regulation of cellular and humoral immunity such as the production of immunoglobulin A and anti-inflammatory cytokines such as interleukin 10 and TGF-β; balancing the Th1 to Th2 ratio (6); increasing the breakdown of carbohydrates and proteins for better intestinal absorption; inhibiting the growth of gastrointestinal pathogenic bacteria; and increasing beneficial intestinal microbes (16). The mechanisms by which probiotics protect the host against intestinal and urinary tract infections include: (1) increasing the resistance of the mucosal membrane to bacterial migration and toxin penetration by enhancing intestinal cell adhesion, (2) altering the host response to microbial products, (3) enhancing the mucosal immunoglobulin A (IgA) response, (4) enhancing intestinal defenses by inhibiting the growth of pathogens, (5) producing bacteriocins (small proteins that kill bacteria), and (6) eliminating potential pathogens through competitive proliferation. Theoretically, it has been suggested that very premature infants, who have a low diversity of microorganisms in their intestines, may benefit from probiotic administration (17). There is significant evidence for the beneficial effect of probiotic supplements in premature infants to prevent necrotizing enterocolitis. A meta-analysis of 29 clinical trials on 4,000 premature infants who received probiotic supplements showed a 43% reduction in necrotizing enterocolitis with a hazard ratio of 0.57. Sepsis and mortality were also significantly reduced (18). The results of a cohort study of 2,178 premature infants showed that probiotic administration was associated with a reduced risk of necrotizing enterocolitis (OR 0.62, 95% CI 0.48-0.80) and mortality (OR 0.52, 95% CI 0.39-0.70) (16). The results of a meta-analysis of 34 studies and 9,161 premature infants showed that among the 5 probiotic administration strategies that included Bacillus, Bifidobacterium, Lactobacillus, Saccharomyces, and probiotic mixtures, two probiotic administration strategies (including the probiotic mixture administration strategy and the Bifidobacterium administration strategy alone) are the best strategies for the prevention and treatment of necrotizing enterocolitis in premature infants (14). The results of Lee et al. study showed that the incidence of necrotizing enterocolitis was similar between the control group (2.8%) and the probiotic group (2.4%) (hazard ratio, 1.15). Necrotizing enterocolitis mortality was also not statistically different (19).

Randomized controlled trials and quasi-randomized trials conducted in VLBW infants or preterm infants with a gestational age of less than 32 weeks were included in the meta-analysis. As a result, a total of 148 subjects were analyzed in four trials, and no statistically significant difference in the incidence of NEC was observed. The authors concluded that the current evidence is insufficient to recommend probiotic administration as a routine clinical procedure in the prevention of NEC (20).

Considering the above studies and the contradictory results, this study was conducted to determine the effect of probiotics on milk tolerance and prevention of necrotizing enterocolitis in premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd during the years 2021-2022.

Methods

Study Design

This study was a double-blind clinical trial (neither the patient nor the evaluator knew which treatment was being administered to which patient) conducted on 86 premature infants admitted to the neonatal intensive care units of Bentolhoda Hospital in Bojnurd from November 1, 2021, to March 20, 2022 (Figure 1).

Study Population

In this study, a 30% difference in milk tolerance between the intervention and control groups was expected. Considering a milk tolerance of 10% in the control group, a type I error (α) of 0.05, a study power (1-β) of 80%, and one-sided tests to assess differences and relationships between groups, and assuming a 30% attrition

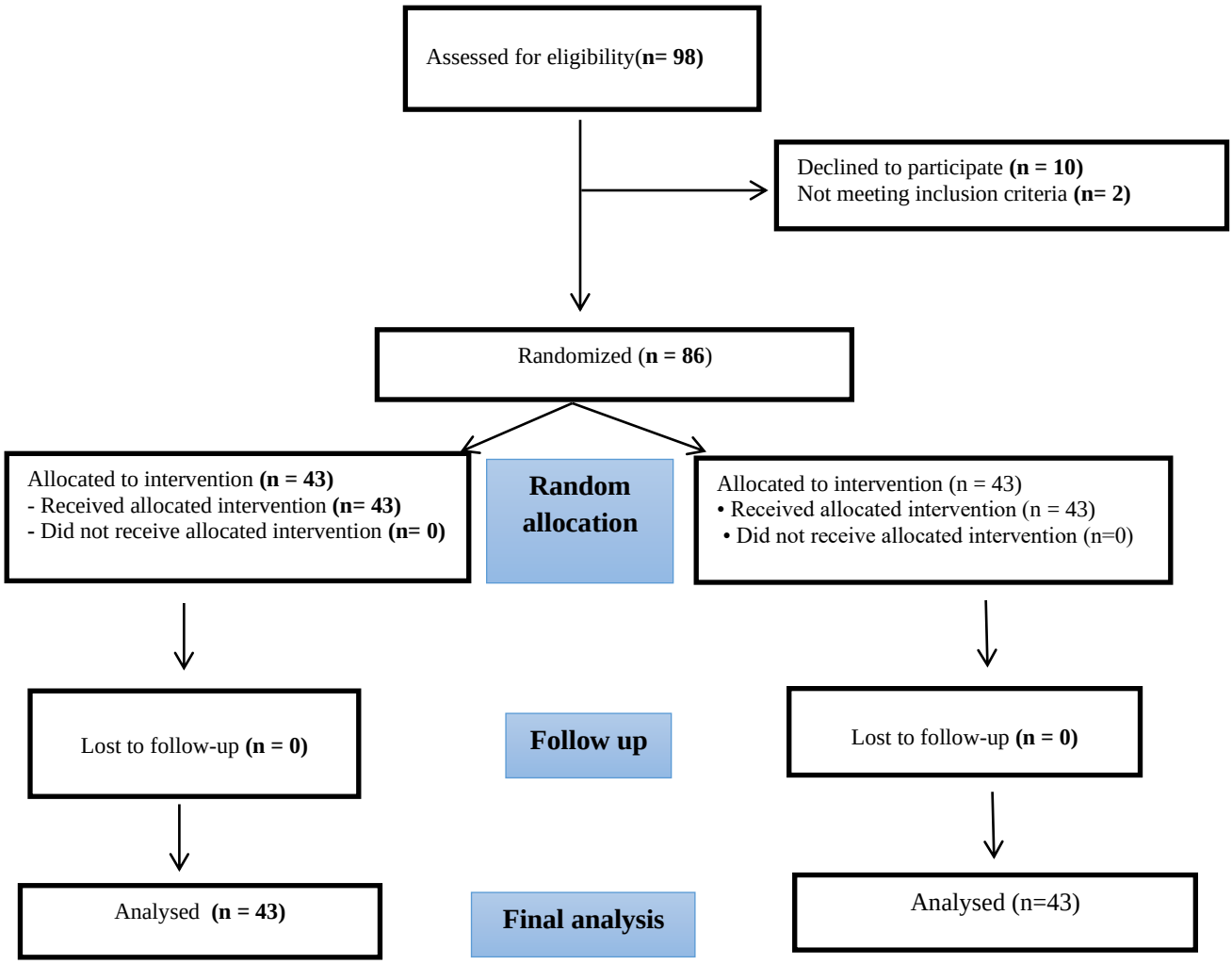

Figure 1. Consort flow chart

rate, the sample size was set to 43 infants per group.

In this study, all premature infants admitted to the hospital were included based on the inclusion and exclusion criteria. Eighty-six infants were randomly assigned to two groups: a control group (receiving placebo) and an intervention group (receiving probiotics) using a computer program for block randomization. It is worth mentioning that the manufacturer for both the placebo and the probiotics was Zist Takhmir. By providing two solutions, A and B, the nature of the solutions (drug and placebo) remained unknown until the end of the study, even to the investigator. In this study, the involved physicians (neonatal subspecialists working at Bentolhoda Hospital who were responsible for treatment from the beginning to the end) and the statistical analyst were not aware of the type of milk given to the infants.

Inclusion criteria were gestational age less than 30 weeks and birth weight less than 1500 grams.

Diagnostic factors for NEC included increased residual, discoloration of gastric secretions, blood in stool, abdominal distension, poor feeding, thrombocytopenia, neutropenia, gas in the portal vein on ultrasound, and pneumatosis intestinalis and ileus on plain radiography.

Exclusion criteria were infants with gastrointestinal obstruction, congenital heart disease, omphalocele, gastroschisis, grade 2 and 3 asphyxia, infants born to addicted mothers (due to the fact that one of the problems of infants of addicted mothers is gastrointestinal problems such as feeding intolerance, abdominal distension, and diarrhea, which can overlap with the symptoms of necrotizing enterocolitis), lack of enteral feeding in the first 48 hours after birth, death of premature infant, congenital malformation in the gastrointestinal system of the premature infant, and active gastrointestinal bleeding in the first week after birth.

Instruments

The first checklist included demographic information such as gender, gestational age, birth weight, 5th-minute Apgar score, growth restriction, antenatal steroid administration, mode of delivery, maternal diagnosis of pre-eclampsia or chorioamnionitis, and reason for maternal admission.

The second checklist was used to assess information on the diagnosis of early- or late-onset necrotizing enterocolitis (after 7 days of birth), types of necrotizing enterocolitis (grade 1, 2, and 3), length of hospital stay, time to reach oral feeding, weight at discharge, and milk tolerance.

Due to the objectivity of the questionnaire questions, the validity and reliability of the data collection tools were considered to be 100%. Checklists were completed before the start of the study and after its completion.

Intervention

Enteral feeding was started within the first 24 hours of birth. For infants born at or before 28 weeks and 6 days, feeding was started at a rate of 0.5 cc per kilogram of body weight every two hours, then increased to 15 cc per kilogram of body weight (daily or twice daily), and finally reached a daily intake of 150-180 cc per kilogram. For infants born earlier than 30 weeks, feeding was started at 15 cc per kilogram of body weight in 24 hours and increased to a maximum of 25 cc per kilogram of body weight in 24 hours on the second day, and then increased to 15 cc per kilogram of body weight twice a day (depending on the patient's clinical signs, it could be increased up to 25 cc per day).

The Baby Care drops used in this study are a synbiotic combination (probiotic + prebiotic) manufactured by Zist Takhim Company, Tehran, Iran, which contains large amounts of an effective and safe probiotic strain called Bifidobacterium lactis. This strain has a significant advantage in improving immune system disorders due to its high ability to bind to human mucus and faster and better colonization among other probiotics. The prebiotic in this product is fructooligosaccharide, which helps the growth and activity of probiotics. Baby Care also does not contain any flavoring, artificial color, or gluten.

In the treatment group, after the infant's feeding volume reached 5 cc per kilogram of body weight per day, oral probiotics were administered at a dose of one drop per kilogram of body weight, diluted with normal saline to a volume of 0.5 cc, every 12 hours for 3 weeks. In the control group, only 0.5 cc of normal saline was administered every 12 hours for 3 weeks.

Infants who had less than 20% residue during the study were fed as usual. In the case of residue of 20-50%, feeding was reduced or the breastfeeding interval was increased. In the case of a baby with more than 50% residue, treatment was initiated with suspicion of necrotizing

enterocolitis.

Also, no action was taken for infants who had less than 20% of the residual volume before the next feeding. For infants who had between 20 and 50% of the residual volume, the milk volume was increased or decreased depending on their clinical condition, or their feeding interval was increased. Infants who had more than 50% of the residual volume were suspected of NEC and entered the treatment for necrotizing enterocolitis.

Infant feeding tolerance factors included residue control, color change in gastric secretions, and abdominal control.

Feeding Method

All infants under 1200 grams and under 32 weeks were fed via gavage. Infants 32-34 weeks or weighing 1200-1500 grams received gavage or oral (finger) feeding in both groups.

If the infant had no contraindications to enteral feeding, trophic feeding was started at a rate of 10-25 cc/kg during the first 3-5 days. After tolerating trophic feeding, the amount of milk was increased by a maximum of 20-25 cc/kg per day using a slow method. Depending on the patient's general condition, 2/3 of the milk volume was reduced from the serum volume. Preferably, breast milk or standard formula was used at the beginning of feeding. In the absence of breast milk, standard formula was used. Serum therapy was started in infants weighing less than 1500 grams at a rate of 80 cc/kg and increased to 150-180 cc/kg over the next 7 days, and 2/3 of the milk volume tolerated by the infant was reduced from the serum volume. After tolerating 100 cc/kg of breast milk, the volume of milk was not increased and fortifier (FMS) was added to the milk (at the rate of one scoop per 25 cc of breast milk). After tolerating the milk with the fortifier, the milk was increased again to 150-180 cc/kg. Given that Intralipid was causing damage to the patient's blood vessels, we did not use Intralipid, and amino acids were started at 2 gr/kg from the first day and increased to 3 gr/kg depending on the patient's general condition.

In this study, an attempt was made to use breast milk for all infants, but given the possibility of the mother not being present at the infant's bedside or the insufficiency of breast milk, the lack of a milk bank in this center, and the fact that the family might not regularly bring expressed breast milk for their hospitalized infant, in the absence of breast milk, standard formula was used for the infant in both study groups.

Statistical Analysis

In this study, using SPSS statistical software, descriptive statistics and frequency distribution tables were used to calculate demographic information for all participants and then separately for the two groups. Analytical statistics were employed, considering the assumptions of normality and variance equality. To test study hypotheses regarding quantitative variables, independent samples t-tests were used to compare groups, and paired t-tests assessed within-group changes. Non-parametric equivalents (Mann-Whitney U and Wilcoxon tests) were used when assumptions were violated. Chi-square tests compared qualitative variables between groups, and McNemar's test was used for within-group comparisons of qualitative variables. The significance level was set at 0.05 for all analyses.

Ethical approval

This study was approved by the Ethics Committee of Bojnord University of Medical Sciences (IR.NKUMS.REC.1401.021). Ethical considerations were observed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The aim and method of the study were explained to the premature infant's mothers, and their questions were answered by the first researcher. They could withdraw from the study at any time.

Results

The frequency distribution of infant gender was examined based on chi-square test in study groups. In the control group, 50% were female, and in the intervention group, 50% were female. There was no statistically significant difference in gender between the two groups, meaning the two groups were equal in terms of the frequency of males and females (Table 1).

The frequency distribution of infant gender was examined based on chi-square test in study groups. In the control group, 77% had cesarean deliveries, and in the intervention group, 63% had cesarean deliveries. There was no statistically significant difference in the mode of delivery between the two groups using the chi-square test in the studied samples (Table 2).

The frequency distribution of infant gender was examined based on chi-square test in study groups. In the control group, 23% were prescribed steroids, and in the intervention group, 10% were prescribed steroids. There was no statistically significant difference in steroid use between the two groups using the chi-square test in the studied

Table 1. Frequency distribution of the gender of premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd

Groups	Gender			Statistic	P-value
	Male	Female	Total		
Control	Number	15	15	30	X^2=0	1.00
	Percentage	50	50	100		
Intervention	Number	15	15	30		
	Percentage	50	50	100		
Total	Number	30	30	60		
	Percentage	50	50	100		

Table 2. Frequency distribution of the mode of delivery of mothers of premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd

Groups	Mode of delivery			Statistic	P-value
	Vaginal	C-section	Total		
Control (30 infants)	Number	7	23	30	X^2=1.27	0.26
	Percentage	23	77	100		
Intervention (30 infants)	Number	11	19	30		
	Percentage	37	63	100		
Total	Number	18	42	60		
	Percentage	30	70	100		

samples (Table 3).

In our study, the frequency distribution of necrotizing enterocolitis (NEC) was examined based on the study group. In the control group, 33% had stage 1 NEC, 11% had stage 2 NEC, and 6% had stage 3 NEC, while in the intervention group, only two cases had stage 1 NEC. There was a statistically significant difference in the incidence of NEC between the two groups, with the rate being higher in the control group (Table 4).

The frequency distribution of milk tolerance was examined based on the study group. In the control group, 50% had milk tolerance, and in the intervention group, 76% had milk tolerance. There was a statistically significant difference, with the rate of milk tolerance being higher in the intervention group (Table 5).

The variables of gestational age, birth weight, birth length, head circumference, Apgar score, and time to start oral feeding were examined in the two study groups. Based on statistical tests, no significant difference was observed between the two groups in all cases except for the time to start oral feeding. Specifically, the time to start feeding was significantly longer in the control group,

Table 3. Frequency distribution of steroid use in premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd

Groups	Steroid use			Statistic	P-value
	No	Yes	Total		
Control (30 infants)	Number	23	7	30	X^2=1.92	0.166
	Percentage	77	23	100		
Intervention (30 infants)	Number	27	3	30		
	Percentage	90	10	100		
Total	Number	50	10	60		
	Percentage	83	17	100		

Table 4. Frequency distribution of the incidence of necrotizing enterocolitis in premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd

Study groups	Necrotizing enterocolitis			Statistic	Significance level	95% Confidence Interval and Relative Risk
	No	Yes	Total			
Control (30 infants)	20	10	30	X^2=4.812	0.028	RR=0.609; 95% CI=0.397-0.195
Intervention (30 infants)	28	2	30	30		

Table 5. Milk tolerance status

Study groups	Milk tolerance			Statistic	Significance level	95% Confidence Interval and Relative Risk
	No	Yes	Total			
Control (30 infants)	15	15	30	X^2=4.214	0.03	RR=0.579; 95% CI=0.992-0.1
Intervention (30 infants)	7	23	30			

Table 6. Comparison of gestational age, birth weight, head circumference at birth, 5-minute Apgar score, and time to start oral feeding in premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital

Variable	Groups	Standard deviation	Median	Min	Max	Statistic	P-value
Gestational age	Control	1.725	27.70	22	30	3.422	0.754
	Intervention	1.189	28.03	26	30		
Birth weight	Control	185.893	1018.83	680	1500	48.67	0.406
	Intervention	195.202	1006.50	580	1460		
Birth length	Control	4.768	37.43	20	42	24.71	0.101
	Intervention	5.049	36.60	20	46		
Head circumference at birth	Control	2.446	26.15	22	35	13.918	0.238
	Intervention	2.152	26.30	21	33		
5th-minute Apgar	Control	1.724	8.17	2	10	5	0.660
	Intervention	1.479	8.13	3	10		
Time to start oral feeding	Control	1.106	7.13	1	5	7.53	0.0362
	Intervention	0.740	3.27	2	5		

Table 7. Comparison of weight at admission, weight at discharge, length at discharge, head circumference at discharge, difference in weight between admission and discharge, and length of hospital stay in premature infants admitted to the neonatal intensive care unit of Bentolhoda Hospital in Bojnurd

Variable	Groups	Standard deviation	Median	Min	Max	Statistic	P-value
Weight at admission	Control	185.893	1008.83	680	1500	5.72	0.352
	Intervention	195.202	1006.50	580	1460		
Weight at discharge	Control	406.296	1413.50	640	2865	3.26	0.862
	Intervention	256.700	1481.33	550	1700		
Length at discharge	Control	5.774	39.80	20	47	9.68	0.357
	Intervention	4.268	39.17	30	48		
Head circumference at discharge	Control	2.828	28.07	22	35	3.82	0.902
	Intervention	3.002	28.43	21	37		
Difference in weight between admission and discharge	Control	315.903	404.6667	-75.00	1495.00	6.85	0.0325
	Intervention	209.472	494.8333	-30.00	810.00		
Length of hospital stay	Control	17.533	48.80	7	90	7.35	0.0185
	Intervention	18.933	36.13	4	90		

meaning oral feeding was started earlier in the intervention group (Table 6).

The variables of weight at admission, weight at discharge, length at discharge, head circumference at discharge, the difference between weight at admission and discharge, and length of hospital stay were examined in the two study groups. Statistically significant differences were found only for the difference in weight between admission and discharge, and for length of hospital stay. The average weight difference between admission and discharge in the intervention group was approximately 90 grams greater than in the control group. Also, the average length of hospital stay in the intervention group was approximately 12 days less than that in the control group (Table 7).

Discussion

This randomized clinical trial investigated the effect of oral probiotics on the prevention of necrotizing enterocolitis in very low birth weight

(VLBW) infants. The results of the study showed that the incidence of necrotizing enterocolitis in the intervention group was lower than that in the control group, which is in line with the results of studies conducted in this field. Sharif et al. (2020), for example, showed that giving probiotics to premature and very low weight infants may reduce the risk of necrotizing enterocolitis, mortality, and serious infection (21). Bayani et al. (2021) reported that the incidence of NEC was significantly reduced in the probiotic group (13). In addition, the results of Bonsante et al. showed that the routine use of probiotics reduced the incidence of necrotizing enterocolitis (hazard ratio 0.20), the risk of mortality (hazard ratio 0.46), and rectal bleeding (hazard ratio 0.60) (22). Norishadkam et al.'s study on infants weighing 1000 to 2500 grams showed that probiotic administration prevents necrotizing enterocolitis in premature infants (20% versus 66.7% in the control group), but its greatest effect was in reducing type 1 necrotizing enterocolitis (16% versus 45.8%), and it had less effect on type 2 necrotizing enterocolitis (0% versus 4.2%). The results of our study are in line with theirs, with the difference that our study was performed on VLBW infants, while Norishadkam et al. involved LBW infants (23). One of the possible mechanisms for this protective effect is that probiotic substances in human milk promote the growth of non-pathogenic probiotic microorganisms, mainly lactobacilli and bifidobacteria, which modulate the intestinal microbiome and promote mucosal barrier functions (24).

The growth of the intestinal microbiota in premature infants is influenced by several factors, including gestational age, mode of delivery, diet, and exposure to antibiotics. All of these factors are likely important confounders in the relationship between probiotics and NEC. In fact, it is well documented that infants who are breastfed have a lower risk of NEC compared to infants who are formula-fed, and that cesarean section is associated with disruption of the intestinal microbiota. Given the definite protective role of human milk feeding and the symbiotic properties of human milk, it is essential to understand whether the use of probiotics should also be encouraged in infants who are breastfed, or whether this intervention should be exclusively directed towards formula feeding (25). Previous reports have shown that Bifidobacteria and Lactobacillus, when combined with other probiotics, can enhance microbiome maturity and immune regulation in premature infants (26, 27). Bifidobacterium produces organic acids, antibacterial proteins, and H_2O_2 that help form the microbial environment. On the other hand, Lactobacillus first strengthens the bowel barrier by inducing the secretion of adhesion and inhibition of cellular apoptosis. Enterococcus faecalis has features such as easy adhesion and rapid growth that makes it an ideal probiotic for effective intestinal function (28). Probiotics also improve the production of anti-inflammatory cytokines, increase antioxidant activity, regulate cell death, increase mucosal responses to IgA immunoglobulin, inhibit pathogenic colonization, produce antimicrobial peptides, secrete short-chain fatty acids, and improve the body's immune response (13).

Hospital Stay

The results of our study also showed that probiotic consumption led to increased weight at discharge and a reduction in the length of hospital stay, which is consistent with the results of previous studies. In this regard, a meta-analysis conducted by Jin et al. showed that the use of probiotics in premature infants is a safe option and can reduce the incidence of sepsis, mortality, length of hospital stay, and time to full enteral feeding (29). Bayani et al. (2021) showed that the probiotic group experienced greater weight gain during hospitalization compared to the placebo group (P = 0.034). Also, the length of hospital stay in infants exposed to probiotics was significantly shorter compared to the placebo group (P = 0.019). Therefore, probiotic administration can significantly reduce the incidence and severity of NEC and improve feeding tolerance. In addition, probiotic agents contribute to the rapid establishment of full enteral feeding and reduce the length of stay (13). Samanta et al. suggested that probiotic administration reduces the length of hospital stay in VLBW infants (30). Consistent with the results of the present study, Norishadkam et al. (23) reported that reaching full enteral feeding in the Lactobacillus reuteri probiotic group was significantly shorter compared to the control group. In line with the present study, Braga et al. observed that the time to reach full enteral feeding in infants exposed to the combined use of Lactobacillus casei and Bifidobacterium breve was significantly shorter (P = 0.02) (31). The results of a systematic review (2013) showed that patients who received probiotics stayed in the hospital for an average of 6 days less than those who did not (32).

Milk Tolerance

The first limitation of the study was that the sample size was small. A larger sample size would strengthen the findings and can detect smaller differences in outcomes, especially for rare events like NEC. The second limitation of the study was that it focused on short-term outcomes, so it is recommended that long-term follow-up be included in future studies to assess developmental outcomes, growth, and potential adverse effects of probiotics would provide a more comprehensive understanding of the intervention's impact. The third limitation of the study was that potential side effects of probiotic administration, such as sepsis or gastrointestinal disorders, were not investigated. The fourth limitation was that this study did not analyze the microbiome or inflammatory markers, and it is recommended that this be investigated in future studies. The fifth limitation was that the effect of probiotics on the degree of necrotizing enterocolitis was not investigated and it is recommended to be investigated in future studies. The sixth limitation was that the difference in weight gain between the two study groups was not measured and the weight index is affected by many variables. The seventh limitation of the study was that the difference in weight gain between the two groups was not measured, and it is recommended that this issue be measured in future studies.

Conclusion

Our findings suggest that oral administration of probiotics may reduce the incidence of NEC, increase feeding tolerance, and reduce hospitalization in premature infants. Therefore, the use of probiotics can be recommended as an adjunctive therapy to overcome feeding intolerance in premature infants. However, further studies are needed to evaluate the best methods and doses of preparation, as well as the types of probiotics used.

Clinical Implications

According to these results, the use of probiotics in neonatal intensive care units would be highly beneficial for very low birth weight (VLBW) infants to reduce the risk of medical complications such as NEC and sepsis. By using probiotics at appropriate doses, in various forms (e.g., mixed with breast milk, premature formula, or standard formula), and with multiple strains, VLBW infants have an opportunity to strengthen their immune systems and optimize microbial activity in their large intestine. Finally, the results of this study show that probiotics provide positive outcomes for very low birth weight neonates, and gestational age is an important factor. However, due to the multifaceted nature of probiotics, it is recommended that probiotic administration be integrated into routine VLBW neonatal care, with continuous monitoring and support.

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None.

Conflicts of interest

The authors declare that they have no competing interests.

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