IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

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Original Article

Effects of Probiotic Lactobacillus Reuteri (DSM 17938) on the Incidence of Necrotizing Enterocolitis in Very Low Birth Weight Premature Infants

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ABSTRACT

***Background*:** Feeding intolerance is a common problem among premature infants. There is limited information on the safety and effects of oral probiotic supplements, especially products containing Lactobacillus reuteri, and the incidence of necrotizing enterocolitis (NEC) in low birth weight preterm infants. This study aimed to evaluate the effects of Lactobacillus reuteri on the gastrointestinal complications and feeding tolerance in premature infants.

***Methods*:** This randomized triple-blind clinical trial was conducted on 60 premature infants divided into two groups of intervention and placebo. Subjects in the intervention group received one drop/kg of supplementary oral probiotic with 0.5 ml of distilled water, and infants in the placebo group only received 0.5 ml of distilled water. Probiotic administration continued to reach full enteral feeding.

***Results*:** In this study, mean time to reach full enteral feeding was 12.83 and 16.75 days in the intervention and placebo groups, respectively, which was indicative of a significant difference (P=0.01). However, mean of neonatal weight at discharge had no significant difference between the two groups. In addition, 6.7% and 36.7% of infants in the intervention and placebo groups were diagnosed with NEC, respectively, which showed a significant difference (P=0.005). Also, prevalence of jaundice and sepsis was not significantly different between the study groups.

Conclusion: According to the results of this study, Lactobacillus reuteri could reduce the time to reach full enteral feeding while diminishing the incidence of NEC in very low birth weight premature infants.

Keywords: Lactobacillus Reuteri, Necrotizing Enterocolitis, Probiotic, Very Low Birth Weight Infants

Introduction

Feeding intolerance is one of the most common problems in preterm infants and is normally caused by necrotizing enterocolitis (NEC) (1). NEC is considered as the most frequent and life-threatening disorder of the gastrointestinal (GI) tract among very low birth weight (VLBW) neonates (2). Regarding the advances in the treatment of hyaline membrane disease, as well as the increasing number of infants diagnosed with NEC, it is anticipated that NEC will be the principal cause of morbidity and mortality among preterm infants (3).

NEC is a multifactorial disease primarily associated with intestinal immaturity; however, the main risk factors related to NEC remain controversial (4). According to the literature, newborns who are exclusively breastfed are exposed to a lower risk of NEC. In this regard, there have been concerns about the role of early and aggressive increase in feeding volumes in raising the risk of NEC among VLBW infants, and a safe feeding regimen for these infants is a matter of debate (2).

Stimulation protocols consisting of minimal enteral feeds followed by judicious volume advancement have been reported to reduce the incidence of NEC in smaller study cohorts; however, no significant benefits have been reported in a meta-analysis of all randomized studies in this regard (4). Since NEC could lead to nosocomial infections, evaluation of the incidence of this disorder plays a pivotal role in the primary prevention of NEC among premature infants (2). Nosocomial infections are associated with high mortality and morbidity, neuro-developmental disorders and prolonged hospital stay (5).

GI tract is a major source of potential pathogens, which may contribute to the

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development of nosocomial infections. Probiotics are able to normalize the intestinal flora in preterm infants while reducing the incidence of NEC and nosocomial sepsis (5, 6).

In 1965, Lilly and Stillwell first described probiotics in the literature as "live microorganism, which when administered in adequate amounts; confer a health benefit on the host" (7, 8). Microorganisms are able to colonize in the GI tract by adhering to the intestinal epithelium, producing antimicrobial substances and modulating the immune response and host metabolism (7). Probiotics are known to have beneficial effects on the health of premature neonates (5, 7).

Intestinal microbial communities are acquired from the birth canal, as well as close parental contact after birth (8). In premature infants, microbial colonization of bacteria is frequently transferred from intensive care units rather than the vaginal canal or mother's skin surface. Therefore, these infants often receive prenatal antibiotic treatment in order to prevent acute sepsis, which may further alter the composition of intestinal bacteria. On the other hand, preterm infants have delayed colonization with healthy bacteria, such as Lactobacillus and Bifidobacterium species, which may diminish the function of gut microbial communities and the immune system.

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 (8).

Prophylactic enteral antibiotics could reduce the risk of NEC; however, there are still concerns about the adverse treatment outcomes, such as the development of resistant bacteria. According to previous studies, oral administration of supplementary probiotics could prevent NEC and decrease the time to reach full enteral feeding (4). Therefore, changing the intestinal pathogenic microflora via probiotic administration plays a pivotal role in increasing bowel movements and feeding tolerance, and eventually, reducing the incidence of NEC (9-11). Reliable evidence needs to be obtained in order to confirm this hypothesis.

According to recent reports, probiotic supplementation in preterm neonates may improve intestinal function; however, the exact mechanism remains unknown. In this regard, further investigation is required as to determine the safety and efficacy of probiotics in extremely low birth weight infants (≤1000 g) (6).

These concerns have resulted in the limited use of probiotics for premature infants (12). This study aimed to evaluate different factors associated with feeding intolerance, including the mean starting time of feeding, neonatal weight at discharge and time to reach full enteral feeding, as well as GI complications (e.g., jaundice, NEC and sepsis), among VLBW premature neonates.

Materials and Methods

This randomized triple-blind clinical trial was conducted on 63 premature infants admitted at the neonatal intensive care unit (NICU) during October 2012-March 2013. Gestational age of infants was estimated at 28-34 weeks using the Dubowitz method, and birth weight of infants was calculated to be 1000-1800 grams.

Premature neonates who met the inclusion criteria were divided into two groups of probiotic administration (intervention) and placebo using the random allocation software. Inclusion criteria of the study were prematurity and birth weight of 1000-1800 grams.

Exclusion criteria were the presence of disorders such as digestive obstruction, GI bleeding, gastroschisis, omphalocele, withdrawal syndrome, neonatal proven or clinical sepsis, congenital heart defect and asphyxia (degree II or III). Study protocol was approved by the Ethics Committee (code: P2883), and the study was registered at clinical trial websites (code: IRCT2014030716870N1).

Prior to participation, effects of the administered probiotic drop were explained to the parents of selected neonates, and written informed consent was obtained as well. In addition, information such as gestational age, gender, birth weight and cause of hospitalization were recorded for all the neonates. After stabilizing the infants in both groups, breastfeeding was initiated. On the fourth day of feeding, volume of feeds reached 40 cc/kg/day, and afterwards, infants in the intervention group were administered with probiotic drops. After stabilizing the condition of premature infants, subjects in the intervention group were prescribed with one drop/kg of supplementary oral probiotic, which was administered with 0.5 ml of distilled water every 12 hours.

Subjects in the placebo group received 0.5 ml of distilled water every 12 hours. Probiotic treatment continued until the volume of milk intake by the infant reached 120 ml/kg per day,

which was defined as full enteral feeding. In each group, breastfeeding began at 10 cc/kg/day, which was given to infants every three hours in equal volumes. If tolerated, 10 cc/kg/day was added to the previous volume of feed on the next day. Criteria for infant discharge in this study were as follows: 1) complete treatment of the main disease in infants; 2) ability of infant to maintain body temperature at 25-28°C in regular clothing; 3) established coordination of sucking and swallowing in the neonate and 4) ability of infant to feed through breasts or a pacifier.

Intervention was instructed by a head nurse and run by the nursing staff of the hospital. During the study, factors such as the incidence of NEC, sepsis, jaundice, weight at discharge and mortality were recorded in both groups. Neonatal weight at discharge was measured using Seca weight gauge (model: 3341321008, Germany). In this study, NEC was defined as the presence of abdominal distension and gastric residual volume along with one or more of the following symptoms: apnea, lethargy, occult blood in the stool and pneumatosis intestinalis on the abdominal film.

Neonatal sepsis was diagnosed through the evaluation of clinical symptoms and positive cultures, and all infants were examined by a pediatrician on a daily basis, while a matron recorded the data in questionnaires. This was a triple-blind clinical trial; due to the age of premature infants, the intervention was implemented by nurses, and the physician was not aware of the condition of neonates in detail. In addition, statistical analysts were not informed of the study groups.

In this study, probiotic treatment was performed using the Biogaia®Drop (BioGaia ProTectic, Stockholm, Sweden), which is composed of 5 ml of a mixture containing Lactobacillus reuteri DSM 17938. One drop of this product holds a minimum of 20 million live Lactobacillus reuteri protectis.

Data analysis was performed in SPSS V.16 using the Chi-square test, independent T-test and Fisher's exact test, and $P<0.05$ was considered as significant.

Results

In total, 63 eligible infants were evaluated in this study, and three neonates (two in placebo group and one in intervention group) were excluded due to the lack of parental consent to complete the course of treatment and early discharge. Moreover, three infants (two in placebo group and one in intervention group) died during the time of study. Among the patients, 14 infants (46.7%) in the intervention group and 16 infants (53.3%) in the placebo group were male, which had no significant difference between the groups (P=0.39).

Mean gestational age of the studied neonates was 30.87±1.90 and 30.97±1.94 weeks in the intervention and placebo groups, respectively, which was indicative of no significant difference (P=0.841). Mean of birth weight in the intervention and placebo groups was 1396.33±234.55 and 1418.67 ± 328.47 grams, respectively, which had no significant difference between the study groups (P=0.712).

According to our findings, mean of the time to reach full enteral feeding was 12.83±4.26 and 16.78±6.66 days in the intervention and placebo groups, respectively, which was indicative of no significant difference (P=0.01). Additionally, mean of neonatal weight at discharge was 1756.55±146.39 and 1747.32±159.51 grams in the intervention and placebo groups, respectively, which showed no significant difference between the study groups (P=0.821). Mean time for starting trophic feeding was estimated at 2.3±0.99 and 3.12±1.22 days in the intervention and placebo groups, respectively, which was indicative of no significant difference (P=0818) (Table 1).

In the present study, two infants (6.7%) in the intervention group and 11 infants (36.7%) in the placebo group were diagnosed with NEC, and there was a significant difference between the groups in this regard (P=0.005). In addition, 29 neonates (96.6%) in the intervention group and 26 neonates (86.7%) in the placebo group were diagnosed with jaundice; however, the difference was not significant (P=0.353). Also, four infants (13.3%) in the intervention group and 10 infants (33.4%) in the placebo group were diagnosed with sepsis, and there was no significant difference between the groups in this regard (P=0.109). It is also noteworthy that one infant in the intervention group and two infants in the placebo group died during the course of study, and no significant difference was observed between the groups in this regard (P=0.5) (Table 2).

Discussion

The present study aimed to evaluate the effects of oral Lactobacillus reuteri probiotic supplementation on the time to reach full enteral

feeding and NEC incidence in premature infants with birth weight of 1000-1800 grams.

Table 1. Comparison of Nutritional Parameters in Intervention and Placebo Groups

Variables	Group	Number of Cases	Mean	Standard Deviation	Minimum	Maximum	T-test
Supplementary Feeding Time (Day)	Intervention	30	3.2	0.997	2	5	t=0.34 P=0.81
	Control	30	3.13	1.224	2	7	
	Total	60	3.17	1.107	2	7	
Neonatal Weight at Discharge (g)	Intervention	29	1756.55	146.392	1520	2120	t=0.23 P=0.82
	Control	28	1747.32	159.516	1490	2150	
	Total	57	1771.08	145.681	1490	2150	
Time to Reach Full Enteral Feeding (Day)	Intervention	29	12.83	4.268	6	22	t=2.75 P=0.01
	Control	28	16.75	6.592	7	36	
	Total	57	14.75	5.829	6	36	

Table 2. Comparison of Neonatal Complications in Intervention and Placebo Groups

Complications	Intervention Group		Placebo Group		Total		Fisher's Exact Test
	N	%	N	%	N	%	
Jaundice	29	96.6	26	86.7	55	91.7	P=0.35
Necrotizing Enterocolitis	2	6.7	11	36.7	13	21.7	P=0.005
Sepsis	4	13.3	10	33.4	14	23.3	P=0.01
Mortality	1	3.3	2	6.7	33	5	P=0.5

According to our findings, the time to reach full enteral feeding was shorter in infants administered with probiotics compared to the placebo group, and this difference was significant. In one study, Rojas et al. (2012) reported that use of supplementary Lactobacillus reuteri probiotic could reduce feeding intolerance and length of hospital stay in premature infants weighing <1500 grams (13).

In another study, Braga et al. (2011) observed that the mean time to reach full enteral feeding in premature infants with birth weight of 750-1499 grams was 15.2 days in the group receiving probiotic treatment (breast milk containing Bifidobacterium breve and Lactobacillus casei) and 17.4 days in the control group (only breastfeeding). They concluded that the time to reach full enteral feeding was shorter in the probiotic group compared to the control group, and this difference was considered to be significant (P=0.02) (14).

On the other hand, Rougé et al. (2009) reported that the time to reach full enteral feeding in infants receiving supplementary Bifidobacterium longum BB536GG and Lactobacillus rhamnosus probiotics was shorter in neonates with birth weight of >1000 grams (P=0.04) (6). In another research, Indrio et al. (2008) indicated that prescription of supplementary Lactobacillus reuteri and formula could improve feeding tolerance in the treatment group compared to the infants who were exclusively breastfed or used formula feeding only (P<0.01) (15).

According to the findings of the present study, incidence of NEC in infants administered with probiotics was lower than the placebo group, and this difference was considered to be significant. In their research, Lin et al. (2008) reported that administration of probiotics (bifidobacterium and lactobacillus) to VLBW infants for six consecutive weeks could significantly reduce the incidence of NEC (P=0.002) (2).

In another study, Kitayime et al. (1997) observed that Bifidobacterium breve could effectively colonize the immature bowel and was associated with fewer abnormal abdominal signs, such as severe abdominal distention, intestinal perforation and NEC (P<0.05) (16). According to the findings of Bin-Nun et al. (2005), feeding with milk containing probiotics such as Bifidobacteria infantis, Streptococcus thermophilus and Bifidobacteria bifidus could effectively reduce the incidence and severity of NEC in premature neonates with birth weight of <1500 grams (17).

In their study, Benor et al. (2013) estimated the incidence of NEC at 4% among VLBW infants whose mothers received probiotic supplementation with Lactobacillus acidophilus and Bifidobacterium lactis. This rate was calculated to be 18.2% among the neonates of the

placebo group, and this difference was reported to be significant (P=0.12); however, rate of mortality and neonatal sepsis was similar between the two groups (13).

In another study, Li et al. (2013) suggested that use of probiotics could be a safe option in the prevention of NEC among VLBW neonates (18). According to their findings, infants who were fed with formula supplemented with Lactobacillus reuteri or Bifidobacterium lactis had fewer and shorter episodes of diarrhea, and these effects were more prominent in infants prescribed with Lactobacillus reuteri (19).

In the current study, we observed no significant differences between the two groups in terms of infections and mortality. As such, the results obtained by Braga et al., Lin et al., Rougé et al. and Bin-Nun et al. were indicative of no significant differences between the infants receiving probiotics and placebo group regarding the incidence of neonatal sepsis (2, 12, 14, 17). It is also noteworthy that the aforementioned studies used different probiotics for the treatment of premature infants. On the other hand, Rojas et al. (2012) claimed that rates of neonatal mortality and nosocomial infections were similar in infants administered with probiotics and the placebo group (13).

Conclusion

According to the results of this study, oral administration of probiotics containing Lactobacillus reuteri could reduce the incidence of NEC and the time to reach full enteral feeding in premature infants. Therefore, it is recommended that this probiotic compound be used as an adjuvant for the treatment of feeding intolerance in preterm infants. Future studies are required as to investigate the correct use of routine probiotic supplementation and Lactobacillus reuteri in high-risk neonates with NEC.

Acknowledgments

Hereby, we extend our gratitude to Shahid Sadughi University for the financial support of this study. We would also like to thank the nursing staff, especially Ms. Sotodehnia, and the NICU head nurse for assisting us in this research project.

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