The Effect of Neutral Oligosaccharides on Reducing the Incidence of Necrotizing Enterocolitis in Preterm infants: A Randomized Clinical Trial

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Original Article

ABSTRACT

Background: Necrotizing enterocolitis (NEC) is one of the most destructive diseases associated with conditions of neonatal prematurity. Supplementation with enteral prebiotics may reduce the incidence of NEC, especially in infants who fed exclusively with breast-milk. Therefore, we compared the efficacy and safety of enteral supplementation of a prebiotic mixture (short chain galacto-oligosaccharides/long chain fructo-oligosaccharides [$_{SC}$GOS/$_{LC}$FOS]) versus no intervention on incidence of NEC in preterm infants.

Methods: In a single-center randomized control trial 75 preterm infants (birth weight [BW] ≤1500 g, gestational age ≤34 weeks and were not fed with formula) on 30 ml/kg/day volume of breast-milk were randomly allocated to have enteral supplementation with a prebiotic mixture ($_{SC}$GOS/$_{LC}$FOS; 9:1) or not receive any prebiotic. The incidence of suspected NEC, feeding intolerance, time to full enteral feeds, duration of hospitalization were investigated.

Results: Differences in demographic characteristics were not statistically important. $_{SC}$GOS/$_{LC}$FOS mixture significantly reduced the incidence of suspected NEC, (1 [4.0%] vs. 11 [22.0%]; hazard ratio: 0.49 [95% confidence interval: 0.29-0.84]; P = 0.002), and time to full enteral feeds (11 [7-21] vs. 14 [8-36] days; P - 0.02]. Also duration of hospitalization was meaningfully shorter in the prebiotic group (16 [9-45] vs. 25 [11-80]; P - 0.004]. Prebiotic oligosaccharides were well tolerated by very low BW (VLBW) infants.

Conclusions: Enteral supplementation with prebiotic significantly reduced the incidence of NEC in VLBW infants who were fed exclusively breast-milk. This finding suggests that it might have been the complete removal of formula which caused a synergistic effect between nonhuman neutral oligosaccharides (prebiotic) and human oligosaccharides.

Keywords: Exclusive breast feeding, necrotizing enterocolitis, oligosaccharides, prebiotic, preterm neonates

INTRODUCTION

Necrotizing enterocolitis (NEC) is one of the most destructive diseases associated with conditions of neonatal prematurity.[1] Despite advances in the science of newborn care, the incidence of the disease has increased, particularly in very premature infants.[2] Large numbers of cases are occurring in very low birth weight (VLBW) neonates,[3] with an incidence of 5-10%.[4] The disease's mortality and morbidity rates are also high.[1] In fact, the more premature the infants, the greater will be the incidence of NEC.

Various lengths and depths of intestinal wall necrosis are associated with NEC.[5] Intestinal perforation or colon strictures can also occur in approximately one-third of the affected neonates.[5,6] Furthermore, its mortality is 10-50%.[7] Pathogenesis of NEC is still not well defined.[8] However, NEC appears to be a multifactorial disease.[9] "Intestinal ischemia, pathologic bacterial colonization, and high protein substrate in the intestinal lumen" seem to be the main factors.[10-13] Extensive research has been conducted to determine the risk factors, ways of prevention and treatment of NEC.

However despite several years of research, the most favorable strategy remains unclear.[1] Different researchers have attempted to find "a way, which has a serious preventative impact on the incidence of NEC." Some examples are mentioned in the following:

Due to the presence of many protective factors in breast-milk, its effect on the incidence of NEC has been investigated by several researchers.[14,15] McGuire and Anthony in a meta-analysis of four small randomized controlled trials (RCTs) found that NEC were 4 times more common in infants who were fed formula milk than in those receiving breast-milk.[15]

Others have evaluated the effect of different feeding strategies on the incidence of NEC. Armanian *et al*. in a RCT showed that prolonged time of low volume milk (i.e. slow feed advancement) in VLBW infants reduced the incidence of NEC.[16] However, Kennedy and Tyson in a Cochrane review of three RCTs demonstrated that rapid versus slow advancement of feeds in preterm neonates had no significant effect on NEC.[17]

Probiotics are described as "live enteral micro-organisms supplementations which have a potential health benefit on the host."[8] Therefore, studies on probiotics have been in the center of interest. *Lactobacillus* and *Bifidobacterium* being the most commonly used.[8]

Caplan and Jilling concluded that enteral augmentation with probiotics in preterm neonates can change the course of intestinal inflammation and necrosis and reduce the incidence of NEC.[18]

In several other clinical trials, researchers found that incidence of neonatal NEC was reduced by probiotic preparations.[8,19-22] On the other hand, the prebiotics are "nondigestible food components that affect the host beneficially by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thereby improving host health."[23] Oligosaccharides are considered to be the archetype of prebiotics. They have antibacterial adhesion effect and immune-regulatory effect.[24,25]

Oligosaccharides that are contained in breast-milk sit on the position of microbial receptors and prevent pathogens from binding with epithelial cell walls of infant's gastrointestine (GI).[26] They have also been found to protect the growth of lactobacilli and bifidobacteria in the GI of breast-fed infants, and therefore, supplementation with enteral oligosaccharides stimulated a bifidogenic intestinal micro-flora with a decrease of pathogens.[26-30]

But based on our web search, the effect of prebiotics on incidence of NEC was investigated by only a few researchers.

Mihatsch *et al*. and Indrio *et al*. in their studies on the use of prebiotics in preterm infants showed that none of the neonates in their studies was affected by NEC.[31,32] They observed that prebiotic supplementation increased bifidobacteria and lactobacilli colony counts in the stool of preterm neonates. Furthermore, sepsis were not reported in any of the infants.[31,32] The majority of other studies have examined the different effects of prebiotics except the effect on incidence of NEC.

Given the importance of the NEC and the expected properties of prebiotics, in this study we decided to investigate our hypothesis, that is, the evaluation the effect of milk supplementation with prebiotics on the reduction of incidence of neonatal NEC in VLBW premature infants.

METHODS

Study design and participants

This single-center RCT was conducted between December 2012 and November 2013 at the Isfahan University of Medical Sciences in our tertiary neonatal intensive care units (NICUs) (Alzahra and Shahid Beheshti Hospital NICUs). Preterm neonates were eligible for participation if they had a BW of ≤1500 g, gestational age (GA) ≤34 weeks and were not fed with formula. When the volume of breast-milk reached 30 ml/kg/day, VLBW infants were enrolled in a case-controlled study within our trial. Newborns with: (a) Asphyxia, (b) major congenital anomalies, (c) congenital cyanotic heart disease, (d) GI system anomalies, (e) proven sepsis or infection immediately before starting the study, (f) refusing to participate and (g) transmission to other department were not included.

The effects of short chain galacto-oligosaccharides/long chain fructo-oligosaccharides ($_{SC}$GOS/$_{LC}$FOS) (9:1) mixture on the incidence of NEC was investigated in two groups of prebiotic (group P) and control (group C). We used unequal randomization as 2:1 in which 2 controls were considered against a case in this trial. The neonates included were randomly allocated to two groups who received either a diet of breast-milk with a supplement of prebiotics ($_{SC}$GOS/$_{LC}$FOS mixture) (prebiotic group [P]; included 25 subjects) or breast-milk with no supplements (control group [C]; included 50 neonates). An independent employee divided the infants into two groups based on their file number. In order to select the neonates, randomly, those with an even digit at the end of their file numbers were placed in group P and neonates with their file numbers ending in an odd digit were assigned to group C. Care providers were not blinded to an infant's protocol. Group assignment and enrolment of participants was supervised by the primary study author. The $_{SC}$GOS/$_{LC}$FOS mixture was prepared and sterilized by Nutricia MMP Company (Nutricia MMP, Mashhad, Iran).

Intervention

In both groups, after considering the inclusion and exclusion criteria, the infants were entered in the study. The subjects were given parenteral nutritional support during the advances in the milk volume. In the prebiotics group (P), 0.5 g/kg/day of $_{SC}$GOS/$_{LC}$FOS mixture was initially administered, and was gradually increased until the milk volume reached 70 ml/kg/day. With the volume at 70-110 ml/kg/day, the dose of the $_{SC}$GOS/$_{LC}$FOS mixture was increased to 1 g/kg/day and with the milk volume at 110-150 ml/kg/day, the mixture was increased to 1.5 g/kg/day. Next, the $_{SC}$GOS/$_{LC}$FOS mixture was added to their diet for one-two additional days. An independent nurse in the experimental group added the supplement to breast-milk. But in control group, no supplements of prebiotics were added to the breast-milk during hospitalization.

In both groups, the infants were fed with an initial dose of 20 cc/kg/day when the attending neonatologist decided to initiate enteral feeding. On day 2, feeding volumes were increased to 40 cc/kg/d; on the 3rd day of the study, volumes were increased to 60 cc/kg/d, and so forth, until a volume of 150 cc/kg/day was achieved. In both groups, the infants were entered in the study when the milk volume reached 30 cc/kg/day. Parenteral nutrition was gradually tapered as enteral feeding volumes were increased.

Outcome measures

The primary outcome of the study in both groups was the effect of the $_{SC}$GOS/$_{LC}$FOS mixture on the incidence of suspected NEC. Diagnosis of NEC was made as shown in Figure 1.[16,33] Secondary outcomes were feeding characteristics such as milk volumes, feeding intolerance (gastric residue, e.g. the presence of milk in the stomach 2 h after completion of a feeding), abdominal distension, postnatal age when full enteral feeds was achieved, and death of each neonate, which were recorded daily. Furthermore, age at the time of discharge from hospital, weight at day 30 and the associations of patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) were also determined. Full feeds were defined as feeds that reached 150 mL/kg/day. PDA and IVH[34] were confirmed by echocardiography and brain ultrasonography, respectively.

Ethics statement

This paper is derived from a residency thesis No. 392237 in Isfahan University of Medical Sciences, Isfahan, Iran. The study was approved by

the regional Ethical Review Board at the university. Written informed consent was taken from parents. This trial was registered at IRCT.ir with a reference number as IRCT2013090710026N2.

Data analyses

The sample size of infants was based on the sample size design for an outcome other than incidence of NEC (no studies were initially designed to assess the effect of prebiotics on the incidence of NEC), that is, stool colony counts of bifidobacteria and pathogenic bacteria after 7 days of supplementation of a previous study.[35] Normally distributed and nonparametric quantitative data were presented as means (± standard deviation [SD]) and median (range), respectively. The numeric variables were compared with the independent *t*-test or Mann–Whitney as appropriate. The qualitative variables were presented as frequency (percent). To examine the effect of the intervention on incidence rate of qualitative primary and secondary outcomes, the Kaplan–Meier with log-rank test was used and hazard ratio (HR) was calculated. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA)

Stage	Classification	Clinical Signs	Radiologic Signs
I	Suspected NEC	Abdominal distention Bloody stools Emesis/gastric residuals Apnea/lethargy	Ileus/dilation
II	Proven NEC	As in stage I, plus: Abdominal tenderness ±Metabolic acidosis Thrombocytopenia	Pneumatosis intestinalis and/or portal venous gas
III	Advanced NEC	As in stage II, plus: Hypotension Significant acidosis Thrombocytopenia/disseminated intravascular coagulation Neutropenia	As in stage II, with pneumoperitoneum

Modified from Walsh MC, Kliegman RM: Necrotizing enterocolitis: treatment based on staging criteria, *Pediatr Clin North Am* 33:179, 1986.

Figure 1: Modified bell staging criteria for necrotizing enterocolitis

RESULTS

Throughout the trial, a total of 131 infants with a BW < 1500 gr and GA ≤ 34 weeks were studied. Twenty-four infants were excluded because of major congenital anomalies, GI system anomalies, asphyxia and sepsis prior to the start of the study [Figure 2]. There were a total of 107 infants who met the eligibility criteria, and 19 infants who refused to participate or were transmitted to other wards. Of the 88 neonates enrolled, 34 were assigned to the "prebiotic" group (P) and 54 to the "control" group (C). Nine and four neonates were transmitted to other departments in group P and C, respectively [Figure 2]. In our study, 75 neonates were randomized and completed the study [Figure 2]. Differences in demographic characteristics were not statistically important [Table 1]. Average GAs in group P and C were 30.48 ± 2.31 weeks and 30.38 ± 2.53, respectively (*P* - 0.76). Average BW in group P was 1262.80 ± 213.35 g. and in group C, 1205.60 ± 177.23 (*P* - 0.10). The mean age at the start of the feeding was 4.24 ± 2.00 days and 3.90 ± 1.99 days in group P and C, respectively (*P* - 0.48). Prebiotic oligosaccharides were well tolerated by VLBW infants. Adverse

Table 1: Basic and clinical characteristics of study infants*

Characteristic/outcome	Prebiotic group (*n*=25)	Control group (*n*=50)	*P*
Gestational age (week) (mean±SD)	30.48±2.31	30.38±2.53	0.76‡
Birth weight (g) (mean±SD)	1262.80±213.35	1205.60±177.23	0.10‡
Age at beginning enteral feeds (day) (mean±SD)	4.24±2.00	3.90±1.99	0.48‡
Body weight at 30 days (g) (mean±SD)	1702.80±325.42	1542.40±270.67	0.06‡
Time to full enteral feeds (days) (median [range])	11 (7-21)	14 (8-36)	0.02†
Duration of hospitalization (median [range])	16 (9-45)	25 (11-80)	0.004†
Milk intolerance (lavage) (%)	10 (40)	20 (40)	0.19$
PDA (%)	1 (4)	4 (8)	0.60$
IVH (%)	4 (16)	11 (22)	0.08$
Side effects (%)	0 (0)	0 (0)	>0.999~
Death (%)	1 (4.0)	1 (2.0)	0.668~

‡Independent *t*-test, †Mann-Whitney test, $Chi-square test, ~Fisher's exact test. SD=Standard deviation, IVH=Intraventricular hemorrhage, PDA=Patent ductus arteriosus

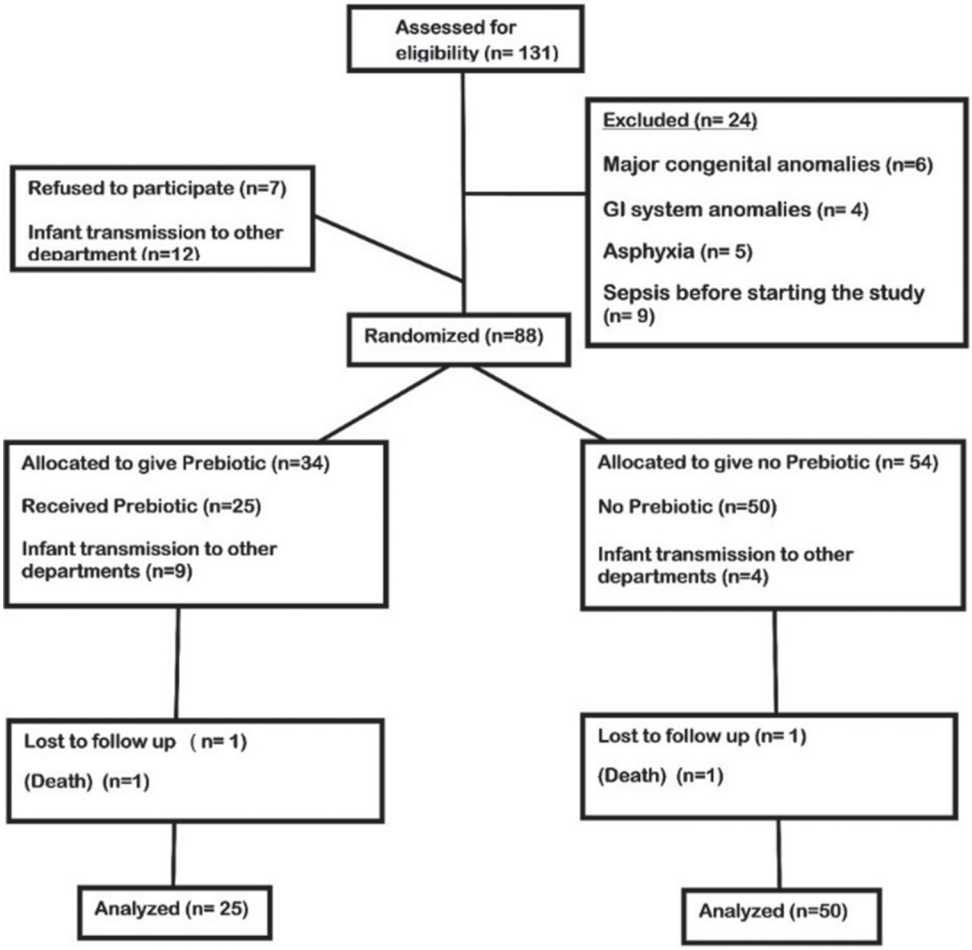

Figure 2: Flowchart of the participants

events such as weight loss, constipation or diarrhea were not observed ($P > 0.999$).

The primary outcomes of the two groups were clearly different in terms of the incidence of. NEC. Only one neonate (4.0%), in the prebiotic group developed NEC, as compared to 11 infants (22.0%) who had not received $_{SC}$GOS/$_{LC}$FOS mixture [HR: 0.49 (95% confidence interval [CI]: 0.29-0.84); *P* - 0.002; Table 2]. Except one neonate who developed proven NEC in group C, others developed suspected NEC in both groups. The median time of the incidence of NEC in the control group was 15th (12th-21st) day of life.

Secondary outcomes such as hospitalization, sepsis and time of reaching full volume of milk were also investigated. Hospitalization time in the prebiotic group was shorter than the control group. The median (range) of hospital stay time was 16 (9-45) days (95% CI: 15.34-24.09) and 25 (11-80) days (95% CI: 25.52-34.39) in group P and C respectively (*P* - 0.004; Table 1]. Four neonates (16%) in the prebiotic group suffered from sepsis, but 17 infants (34%) who had not received $_{SC}$GOS/$_{LC}$FOS mixture developed sepsis, although the difference was not statistically significant [HR: 0.60 (95% CI: 0.27-2.72); *P* = 0.79; Table 2]. Time to establish a total milk intake was significantly shorter in the prebiotic group than the control group. The median (range) time of reaching full enteral feeds (150 ml/kg/day) in group P was 11 (7-21) days (95% CI: 10.69-13.14) and in group C, 14 (8-36) days (95% CI: 13.17-16.50) [*P* - 0.02; Table 1].

The occurrence of milk intolerance (lavage) was investigated in both groups. 10 (40%) and 20 (40%) infants had lavage in prebiotic group and control group respectively [*P* - 0.19; Table 1]. Nine neonates (36%) were required cutting off milk (nonprofit organization) in prebiotic group as compared to 28 neonates (56%) in control group [HR: 0.63 (95% CI: 0.29-1.38); *P* - 0.26;

Table 2: Primary and secondary outcomes in our study groups*

Outcome (%)	Prebiotic group (*n*=25) (%)	Control group (*n*=50) (%)	HR (95% CI for HR)	*P**
NEC	1 (4.0)	11 (22.0)	0.49 (0.29-0.84)	0.009
Sepsis	4 (16.0)	17 (34.0)	0.60 (0.27-2.72)	0.793
Requiring to cut-off milk (NPO)	9 (36.0)	28 (56.0)	0.63 (0.29-1.38)	0.262

*Resulted from Kaplan-Meir with log-rank test. HR=Hazard ratio, CI=Confidence interval NEC=Necrotizing enterocolitis, NPO=Nonprofit organization

Table 2]. Average body weights at 30 days of life in the prebiotic group was 1702.80 ± 325.42 g and in control group, 1542.40 ± 270.67 g. It appears that, the weight average at 30 days of life, in prebiotic group was marginally slightly greater, although this was not statistically meaningful [*P* - 0.06; Table 1].

The incidence of IVH and PDA in prebiotic group was 4 (16%) and 1 (11.1%) respectively, while in the control group it was 11 (22%) and 4 (8%) [*P* - 0.08 and *P* - 0.60, respectively; Table 1]. Mortality was similar between the two groups; only one neonate died in each group [*P* - 0.66; Table 1].

DISCUSSION

In VLBW neonates, interestingly, we observed that enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides ($_{SC}$GOS/$_{LC}$FOS mixture) was effective in the reduction of NEC in VLBW neonates. The present result is in line with the recent studies which show that increasing the beneficial gut flora by supplementation with probiotic bacteria induces protection against NEC[2,36] in preterm infants. For example in a meta-analysis study, NEC in preterm infants was shown to be reduced by the consumption of probiotics.[8]

However, few studies have investigated the relationship between enteral prebiotics supplementation in preterm infants and NEC. Mihatsch *et al*. and Indrio *et al*. investigated the effect of prebiotics on stool viscosity, GI transport and gastric motility in preterm infants. Although these trials were not initially designed to assess the effect of prebiotics on the incidence of NEC. They found that NEC did not occur in any of the neonates in their studies.[31,32] In two other studies conducted by Westerbeek and Modi *et al*. to verify outcomes other than NEC, the difference in the occurrence of NEC was not statistically significant in the prebiotic versus control groups.[37,38]

In the intervention group of infants, could be the complete removal of formula from the infants diet. Using of formula, in other studies (e.g. 24, 32, 35-40) may had a negative impact on the study results; since, it could be rolled as confounding factor.

The reason that in the present study supplementation with $_{SC}$GOS/$_{LC}$FOS mixture significantly reduced the incidence of NEC, may be the existence of formula in other studies, and that its complete removal in our study, had a negative effect, as a confounding factor, on statistically meaningful difference in the incidence of NEC between the study groups. Therefore, more extensive studies are required to evaluate this factor and its effects on preterm infants.

Inspecting the various recent articles, indicates that given the positive effects of oligosaccharides on gut flora, there was an idea that prebiotic supplementation can confer protection against neonatal sepsis. However, to our knowledge, none of studies have found a significant relationship. Sepsis was defined, across the studies, as blood culture being positive, regardless of clinical conditions. Westerbeek *et al*. found that in the prebiotic-supplemented group, 6 of 55 (11%) infants had >2 serious infectious episodes compared with 12 of 58 (21%) in the placebo group (odds ratio: 0.47; 95% CI: 0.16-1.40; *P* = 0.16).[38] Niele *et al*. in a 1-year follow-up found that the incidence of sepsis/meningitis was similar among infants in prebiotic mixture and placebo groups.[39]

Srinivasjois *et al*.,[40] in a systematic review and meta-analysis of three trials involving prebiotic versus control groups, have found that late onset sepsis happened with an relative risk (RR) of 1.05 (95% CI: 0.45-2.44) in Modi *et al*.,[37] 0.78 (95% CI: 0.53-1.16) in Westerbeek *et al*.[38] and 0.43 (95% CI: 0.09-1.99) in Riskin *et al*.,[35] respectively. Despite the lack of statistical significance, Westerbeek *et al*.[38] observed that the incidence of sepsis was lower in the prebiotic than compared with the placebo group (9 of 55 [16%] infants vs. 17 of 58 [29%] infants [RR: 0.4, 95% CI: 0.17-1.16,

$P = 0.10$]). Also, Modi *et al.*[37] and Riskin *et al.*,[35] found that neonatal sepsis was lower in the prebiotic in comparison with the placebo group ([SF: 10; $_{SC}GOS/_{LC}FOS$: 9; $P = 0.180$]) and (L: 2 [13%]; DP: 4 [31%]; $P > 0.05$), although the difference was not statistically significant.

In our study, there was a trend towards lower neonatal sepsis in the prebiotic group (P - 4 [16%]; C: 17 [34%]; 1.66 [95% CI: 0.37-3.67]; $P = 0.79$). Accordingly, a larger cohort study, with the large sample size, is required to evaluate the actual relationship between prebiotic and neonatal sepsis in preterm infants.

Two other interesting results observed in the present study were shorter time to establish a total milk intake and shorter hospitalization period.

Perhaps, complete removal of formula in our study, was the reason that the time of reaching full feeding volume was meaningfully different between the two groups (in prebiotic group vs. control group was 11.92 ± 2.97 vs. 14.84 ± 5.85 with a HR 1.24 [95% CI: 5.4-–0.44]; P - 0.02). However, in other studies, attendance of formula was serious or semi-serious.

Modi *et al.* and Riskin *et al.* defined full enteral feeding volume as 150 ml/kg/day, while Westerbeek *et al.* defined full feeds as 120 ml/kg/day. Time to full enteral feeding volume (median [range]) in prebiotic versus control group was 6 (5-8) versus 7 (6-9) days, $P = 0.10$ in Modi *et al.*[37] while Westerbeek *et al.*[24] observed that the time required was 10 (4-48) versus 11 (7-50) days, $P = 0.47$. Riskin *et al.*[35] stated that the average time of full enteral feeding (mean ± SD) was 41.0 ± 32.0 versus 54.2 ± 31.9 days, in prebiotic versus control group respectively.

With reviewing the literatures, we found only two studies that examined the relationship between prebiotics and length of hospital stay, where no difference was found between the two study groups.[24,35]

Another feature which many studies have examined was weight gain or weight at discharge. Indrio *et al.* found a weight gain per day of 34.90 ± 6.90 versus 34.60 ± 9.46 g in prebiotic versus control group.[41] Kapiki *et al.* reported 22.8 ± 6.0 versus 27.4 ± 7.0 g/day in prebiotic versus control group.[42] Moreover, Riskin *et al.* shown that weight at discharge had a mean ± SD of 2567 ± 355 versus 2846 ± 667 g.[35] None of the above studies showed any statistically meaningful difference between the prebiotic and control groups. Also in other studies, such as Mihatsch *et al.*,[31] Modi *et al.*[37] and Westerbeek *et al.*,[38] no significant difference between the two groups was reported.

In our study, average body weights at 30 days of life in prebiotic and control group was 1702.80 ± 325.42 g. and 1542.40 ± 270.67 g, respectively (P - 0.06). The result is in line with the all those of all the above studies.

The strengths of the study include the RCT design in high risk neonates (i.e., VLBW infants) and the complete removal of formula. This study has some limitations. The major limitation of this study could be the rather small number of the infants included (75 premature neonates), even though the results clearly indicated a significant difference between the prebiotic and control groups. Another limitation of this study was that Supply of Prebiotics in our country was very difficult.

CONCLUSIONS

Enteral supplementation with prebiotic significantly reduced the incidence of NEC in VLBW infants who were fed exclusively breast-milk. This finding suggests that it might have been the complete removal of formula which caused a synergistic effect between nonhuman neutral oligosaccharides (prebiotic) and human oligosaccharides, which, in turn, reduced the incidence of NEC and decreased the time to full enteral feed as well as the hospitalization time. Therefore, further studies with larger sample sizes are recommended to investigate the issue.

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