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ORIGINAL ARTICLE

Role of Probiotics in Prevention of Necrotizing Enterocolitis in Preterm Infants

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ABSTRACT

Objective: To compare the efficacy of prophylactic enteral probiotic administration versus placebo in the prevention of necrotizing enterocolitis in preterm infants.

Patients and Methods: This randomized control trial was carried out at Paediatric Department of Holy Family Hospital, Rawalpindi. A total of 154 preterm infants, 28-33 weeks' gestational age and <2500 grams at birth were enrolled after informed parental consent a donation of for 6 months. They were randomized in to 2 groups with 77 in each group. The infants in the study group were given BILUS (B.bifidus & L.acidophilus) with breast milk twice a day. The infants in the control group were given breast milk alone. Physical and radiological signs of Necrotizing Enterocolitis (NEC) were observed and NEC was staged according to Modified Bell's criterion.

Results: The frequency of Necrotizing Enterocolitis was lower in the study group as compared to the control group; 4 of 77 (5.2%) versus 6 of 77(7.8%) but the results were not statistically significant (p value = 0.25)

Key words: Necrotizing enterocolitis, Preterm Infants, Probiotics.

Author`s Contribution
[1] Conception, Synthesis and Planning of the research,[2] Active participation in active methodology, [3]Active participation in active methodology, *Interpretation, analysis and discussion*

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Introduction

Necrotizing enterocolitis (NEC) is one of the most common gastrointestinal emergencies in the newborn infants. It is one of the most devastating life-threatening diseases affecting 7 to 14% of preterm infants.[1] Not only is it associated with high mortality but also with long-term adverse outcomes such as neurodevelopmental impairment in the survivors.[2] The precise pathogenesis is unknown and it is considered as multifactorial disease. Three major factors are thought to contribute i.e. presence of pathogenic organisms, altered enteric mucosal integrity and the challenge of enteral feeding.[3] A better understanding of the intestinal ecosystem in preterm babies may hold the key to prevention of NEC. The microflora in the gut of preterm baby lacks the biodiversity that is seen within days in a healthy baby born at term.[4] One factor may be the late introduction of breast milk, that contains bifidobacteria which promote healthy microbiota development.[5] Probiotics are live microbial agents that are delivered enterally, colonize the gut and provide benefits to the host. As it takes time for the gut of preterm babies to be colonized by beneficial bacteria that contribute to healthy gut function, early prophylactic treatment with probiotics seems a very logical strategy.[6]

Probiotics may assist in establishing a normal non-pathologic flora by preventing the binding of pathogenic bacteria to the enterocyte, local production of antimicrobial products or by altering the intestinal luminal

pH, by producing potentially microbicidal short chain volatile fatty acids.[7] Genomic studies of the probiotic lactobacillus rhamnosus have revealed that it contains pilus fibers with mucus binding predisposition by which it displaces pathogenic bacteria.[8] These data suggest that probiotics by modifying the occurrence of cascade of events may play a major role in reducing the incidence of NEC. Various probiotics have been used for this purpose but the most commonly employed are species of bifidobacteria and lactobacillus.[9]

In this study, we evaluated the role of probiotics (bifidobacteria and lactobacillus) in the prevention of necrotizing enterocolitis in preterm infants. The results of some previous studies are quite encouraging but more studies are needed so that probiotic administration can be made a routine protocol in the care of preterm infants.

Patients and Methods

This randomized control trial was carried out in the nursery of Paediatric Department of Holy Family Hospital, Rawalpindi from 10th September 2010 to 9th March 2011. Premature infants with gestational age between 28 and 33 weeks, weighing <2500 grams who started to feed enterally and survived beyond the seventh day after birth were included in the study and those with necrotizing enterocolitis developed within 7 days of birth were excluded. Approval of this study was taken by the ethical committee of Rawalpindi Medical College and allied hospitals. Informed parental consents were taken. About 154 preterm infants were randomized into the study group and control group with 77 in each group. Randomization was done by consecutive non-probability sampling. The study group was given Bilus which contains Bifidobacterium Bifidus and lactobacillus acidophilus 1 x 10^6 CFU each. This was mixed with expressed breast milk and given twice a day. The control group received breast milk alone. This was administered by the nursing staff via nasogastric tube.

Feeding was started when the infant had stable vital signs, active bowel sounds, visibly normal abdomen and with no dirty or bloody aspirate. Strict feeding protocol was followed. Depending on the birth weight and gestational age feeding was started. The feeding was advanced slowly with daily increment of 15-20ml/kg. The probiotic was added when feeding was tolerated usually by fifth or sixth day of life. During this time infants' vitals, i.e. heart rate, respiratory rate, the temperature was taken daily and were examined for feeding intolerance, abdominal distension, bloody stools, and emesis. Abdominal distension and any residual feed were checked by nursing staff before each feed. If present, was reported to doctor and feeding was stopped if residual feed was more than 30% of last feed. Laboratory parameters that were done in all cases were complete blood count, electrolytes, and stool routine examination. Blood and stool cultures along with x-rays were done in suspected cases of NEC. NEC was categorized according to modified Bell's criterion.

Patients were monitored for a period of two weeks or until discharge/death. A proforma was filled for all of the study participants. SPSS version 14.0 was used to enter and analyze the data. Chi-Square test was used on categorical variables like efficacy in both groups. The significance level was assumed to be any value less than 0.05.

Results

In probiotic group, the mean age of patients was 5.29 days $\pm$ 0.51 SD and was 5.30 days $\pm$ 0.54 SD in the control group. The average birth weight was 1498.7 grams $\pm$ 214.3 SD in the probiotic group and 1461.0 grams $\pm$ 206.6 SD in the control group. When birth weight was compared in categories, 42 (54.5%) in probiotic group had low birth weight while in control group 32(41.6%) had low birth weight. Similarly, in probiotic group 35 (45.5%) had VLBW while in the control group 45 (58.4%) had VLBW. (Table 1).

Table 1. Baseline characteristics of patients in the two study groups

	Probiotic group (n = 77)	Control group (n = 77)
Age (days)		
Mean + SD	5.29 + 0.51	5.30 + 0.54
Birth weight (grams)		
Mean + SD	1498.7 + 214.3	1461.0 + 206.6
Weight in categories		
LBW n (%)	42 (54.5)	32 (41.6)
VLBW n (%)	35 (45.5)	45 (58.4)

The mean gestational age in the probiotic group was 31.5 + 1.5, weeks compared to 31.3 + 1.5 weeks in the control

group. The categories according to gestational age were also compared. (Table 2)

Table 2. Gestational age of preterm babies in the two study groups		
	Probiotic group (n = 77)	**Control group (n = 77)**
Gestational age (weeks) Mean ± SD	31.5 + 1.5	31.3 + 1.5
Gestational age in categories (n%)		
< 30 weeks	11 (14.2)	11 (14.2)
30 – 31 weeks	16 (20.8)	29 (37.7)
32 – 33 weeks	50 (65.0)	37 (48.1)

The clinical signs like temperature, heart rate and respiratory rate were compared in both study groups, and were found to be statistically insignificant. Similarly, lethargy, feeding intolerance and abdominal distension were compared. Emesis and bowel sounds were also noted (Table 3).

Table 3. Comparison of clinical signs of patients in the two study groups			
Clinical signs	**Probiotic group (n = 77)**	**Control group (n = 77)**	**p-value**
Temperature	n (%)	n (%)	
Normal	74 (96.1)	71 (92.2)	
Hyperthermia	2 (2.6)	0 (0.0)	0.21
Hypothermia	0 (0.0)	2 (2.6)	
Unstable	1 (1.3)	4 (5.2)	
Respiratory rate n(%)			
Normal	73 (94.8)	72 (93.5)	0.45
Apnea	1 (1.3)	0 (0.0)	
Tachypnea	3 (3.9)	5 (6.5)	
Heart rate n(%)			
Normal	74 (96.1)	71 (92.2)	0.58
Bradycardia	2 (2.6)	4 (5.2)	
Tachycardia	1 (1.3)	2 (2.6)	
Lethargy n(%)	5 (6.5)	6 (8.0)	0.72
Feeding intolerance	5 (6.5)	6 (8.0)	0.72
Abdominal distension	5 (6.5)	6 (8.0)	0.72
Emesis	4 (5.2)	6 (8.0)	0.48
Bowel sounds	3 (3.9)	2 (2.7)	0.67
Bleeding P/R	5 (6.5)	5 (6.5)	0.96

Comparison of haemoglobin, serum electrolyte and total leukocyte count in both groups, was also statistically insignificant. (Table 4) Investigations on stool and abdominal x-rays were done in both study groups; though the difference in proportions of stool R/E results between both the study groups was evident, still it could not be proven statistically. On stool culture, growth of Klebsiella and E-coli were noted. X-ray abdomen findings were almost similar among study groups (Table 4).

Table 4. Comparison of pathological investigations in the two study groups			
Investigations	**Probiotic group (n=77) n (%)**	**Control group (n=77) n (%)**	**p-value**
Hemoglobin			
Normal	75 (97.4)	72 (93.5)	0.51
Abnormal	2 (2.6)	5 (6.5)	
Serum electrolytes			
Normal	73 (94.8)	71 (89.6)	0.28
Abnormal	4 (5.2)	6 (7.8)	
TLC			
Normal	73 (94.8)	71 (92.2)	0.49
Decreased	3 (3.9)	6 (7.8)	
Increased	1 (1.3)	0 (00)	
Stool R/E			
Normal	73 (94.8)	69 (89.6)	0.27
Abnormal	4 (5.2)	8 (10.4)	
Stool Culture			
No Growth	76 (98.7)	73 (94.8)	
E-coli	0 (0.0)	2 (2.6)	0.4
Klebsiella	1 (1.3)	2 (2.6)	
Abdominal X-ray			
Normal	72 (93.5)	71 (92.2)	0.43
Distended bowel loops	3 (3.9)	3 (3.9)	
Pneumatosis intestinalis	2 (2.6)	2 (2.6)	
Ascites	0 (0.0)	1 (1.3)	

In group study 4 (5.2%) patients developed NEC, while in the control group 6(7.8%) patients developed NEC and all of these were in 2nd stage of necrotizing enterocolitis. The difference in the proportions was evident, however, was statistically insignificant. (Table 5)

Table 5. Comparison of stages of Necrotizing enterocolitis between the two study groups			
	Probiotic group (n=77) n(%)	**Control group (n=77) n(%)**	**p-value**
Without NEC	73 (94.8)	71 (92.2)	0.74
NEC stages*			
Stage 1	1 (1.3)	0 (0.0)	
Stage 2	2 (2.6)	6 (7.8)	0.25
Stage 3	1 (1.3)	0 (0.0)	

* *Probiotics decreases the risk of NEC RR (95% CI) 0.67 (0.20 – 2.27)*

Discussion

Our study shows that prophylactic probiotics (bifidobacterium bifidus and lactobacillus acidophilus) are not effective in reducing the frequency of NEC. Although the difference in cases of NEC in both groups is evident (5.2% in the study group as compared to 7.8% in control group) but this was not statistically significant. A total of 10 patients developed NEC out of which 8 had stage 2 NEC, 1 had stage 1 NEC and 1 had stage 3. The results imply that larger sample size may be required to prove that probiotics reduce the incidence of NEC and achieve significant results. However, there could be other factors to account for these results.

Firstly, the study was not powered to detect stool colonization by probiotic bacteria. Stool colonization by probiotic bacteria is the means of assessing the viability of probiotic species. This can be equated for checking the bioavailability of a drug. The viability of probioic bacteria was checked by this method by Lin et al in their pilot study.[10] Secondly, there are many variables associated with the development of NEC. The consistent ones are prematurity and low birth weight.[11] The other risk factors that are associated with increased risk of NEC are vaginal delivery, need for ventilatory support, low APGAR score at 5 minutes.[12] The study was randomized but these risk factors were not accounted for, as all preterm infants in the study were not hospital born and thus the complete data was not available in those cases.

Thirdly, the difference in results could be because of the dose used. The dose used in our study was 1x 10^6 CFU. Although there is no fixed dose of the probiotics used in various studies nor is the dosing interval determined but it seems that increasing the dose to 10^9 CFU has better results. In his first study Lin et al used probiotics in a dose of 1 x 10^6 CFU, however in the second study by Lin et al the dose used was 2 x 10^9 CFU and Samanta et al also gave a probiotic mixture in a dose of 2.5 x 10^9 CFU but the probiotics used were also different.[10,13] The results of our study are in accordance with the study done by Dani et al in which although NEC was found less frequent in the probiotic group i.e. 1.4% in the probiotic group versus 2.7% in the study group, the results were not significant.[14] Another study was done in USA in 2009, in which the effect of two probiotic and prebiotic products on weight gain, stool microbiota and stool short chain fatty acids was assessed. NEC was a secondary outcome. There were 3 groups, 2 of which received probiotic preparation but the probiotic did not decrease the incidence of NEC.[15]

The results of our study are also in accordance with the study in Turkey in 2011, in which lactobacillus sporogenes was given to 110 infants until discharge. It was seen that there was no difference in the incidence of NEC in the probiotic supplemented as compared to the control group.[16] In our study, it was seen that most cases of NEC i.e. 5 out of 10 (50%) occurred in one month, the month of November. It has been reported in different studies that many cases of NEC occur sporadically, however, NEC epidemics have also been reported in literature. In a retrospective study carried out at two neonatal intensive care units in Cincinatti USA, data regarding the occurrence of NEC was analyzed over an 8-year period. It was found that there were 12 temporal clusters of NEC which comprised 18% of total 203 cases.[17] Our study did not focus on improvement in feeding tolerance with probiotics, but a number of studies have focussed on this aspect and provide encouraging results. A study done by Samanta et al showed that the number of days required to reach full feeds was significantly lower in babies who received probiotics (13.76 vs. 19.2).[13] Therefore feeding tolerance was better. Another study was carried out in China, which focused on the same subject. The incidence of feeding intolerance in the probiotics treatment group was lower than that in the conventional treatment group (4% vs 14%; $P < 0.01$) in their study.[18] The time to regain birth weight (6.8±1.2 days vs 7.7±1.6 days; $P<0.05$) and the time to reach full enteral nutrition (8.0±1.4 days vs 9.0±2.0 days; $P<0.05$) in the probiotics treatment group were shorter than those in the conventional treatment group.

The extensive and safe use of commercial probiotics worldwide over several decades alongside clinical trials to assess potential adverse effects, provide the most compelling evidence for the safety of probiotics for the general population.[19] To date over 2,000 premature neonates have been exposed to prophylactic probiotics in different prospective studies and no adverse short term effects have been noted.[20] This history of safe consumption of some probiotics has generally been considered proof of short term safety for this vulnerable

population of infants but the proof of safety of a specific strain requires study of that strain rather than extrapolation from related strains. The trials mentioned earlier did not report any cases of sepsis secondary to probiotic use; however, they were not powered to detect such cases. The European society for Paediatric gastroenterology, hepatology and nutrition's committee on nutrition found limited high-quality data on the safety of probiotics added to infant formulas and no long-term studies on benefits and adverse effects of such supplementation.[21] Furthermore, the potential for sepsis from probiotics is unknown because the culture media used in most diagnostic laboratories do not support the fastidious anaerobic probiotic growth.[22]

It is not known that which probiotic or probiotic combination is best to use. It seems that double or triple probiotic strains provide the greatest effect. The appropriate dose and the frequency of dosing also need to be addressed. The quality of probiotics available is also unknown. Each probiotic strain is a unique component itself and each strain has specific properties that cannot be extrapolated from other, even closely related strains. The specific properties of probiotic bacteria need to be characterized.[22] Thus there are still a lot of things unknown about probiotics and concerns regarding its safety. Some of the trials done so far, have shown definite benefits but other trials like ours have shown inconclusive results. Currently, there are 16 randomized controlled trials studying 12 different probiotic preparations in preterm infants which report data on clinically important outcomes such as NEC, mortality, sepsis, or feeding advancement. Although the results from these trials are encouraging, There is no evidence to recommend that all preterm infants should be fed probiotics routinely.[23] The ability to manipulate enteric microbial flora in preterm, very low birth weight infants towards a normal non-pathogenic microenvironment addresses one of the fundamental issues in the pathogenesis of NEC.[8] The current study is, therefore, an important one and it is recommended that further studies are done with a larger sample size and enhanced scientific methods.

Conclusion

Prophylactic probiotics Bilus (Bifidobacterium bifidus and lactobacillus acidophilus) given twice a day in a dose of $1x10^6$ with breast milk for two weeks is not effective in reducing the frequency of NEC in preterm infants. To unequivocally prove the clinical efficacy of probiotics in NEC large multicenter trials are needed. Our results suggest for more research regarding appropriate bacterial strain, dose, and timing of administration to achieve clinically robust effects and also include analysis of possible adverse effects of probiotic administration, such as probiotic-associated sepsis and tolerance of milk feeding.

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