ORIGINAL ARTICLE

Probiotic Prophylaxis in Prevention of Necrotizing Enterocolitis - A Case Control Study

SHEHLA USMAN[1], ANEELA ZAREEN[2], MOHSIN ALI[3], *HUSSAN ALI SARWAR[4]. JAMIL AZHAR[5], SABEEN JAMAL[5]*

ABSTRACT

Background: Necrotizing enterocolitis (NEC) is thought to be a result of the combination of loss of integrity of the intestinal mucosa and the host response, it is a multifactorial disease.
Aim: To determine the efficacy of probiotic use prophylactically in prevention of Necrotizing enterocolitis in preterm neonates as compared to preterm neonates without probiotics. Study design was Randomized controlled trial with duration from: November 6, 2014 to May 6 , 2015.
Methods: A total of 278 cases (139 in both groups) of preterm neonates (<37 weeks of gestation) who have not been given enteral feed yet of both genders were included in the study from neonatal department, fulfilling the inclusion criteria, in Sheikh zayed hospital, Lahore .Parents were informed and written consent was taken. Neonates were randomly assigned to group A and group B each containing 139 preterm neonates by lottery method. Group A receiving probiotics combination, Lactobacillus rhamnosus and Bifidobacterium BB-12 species 2.5×10^9 cfu (colony forming units) per day with feed as Expressed breast milk(EBM) or formula milk for 05days and group B receiving only feed as EBM or formula milk. Neonates in both groups were observed for signs and symptoms of NEC during their stay in the hospital at day 5 of prophylactic treatment.
Results: Comparison of efficacy in both groups was recorded as 127(91.37%) in Group-A and 79.86%(n=111) in Group-B while remaining 8.63%(n=12) in Group-A and 20.14%(n=28) in Group-B no findings of efficacy were found, p value was calculated as 0.006 showing a significant difference.
Conclusion: This study concluded that the efficacy of probiotics in prevention of Necrotizing enterocolitis in preterm neonates is higher, significantly.
Keywords: Preterm neonates, Necrotizing enterocolitis, prevention, efficacy

INTRODUCTION

Necrotizing enterocolitis (NEC) is described a disease that results from a combination of the loss of intestinal mucosa integrity and the host response. The determining factors are ischemia of intestine, mucosa damage edema of intestinal wall, passage of air or bacteria and ulceration of intestine, with the necrosis[1]. Statistics show that 5% of very preterm or very low birth weight infants and about 10% of the extremely preterm or extremely low birth weight babies are affected by NEC. It is reported in literature that necrotizing enterocolitis associated mortality is more than 10%, with more than 25% for infants with severe NEC that need surgery. Although NEC has more incidence in preterm infants it can occur in term and near term babies[2]. Probiotics may protect infants from developing sepsis and NEC by increasing barrier for transfer of bacteria across the intestine, and by modifying host response[3] .According to a review article by Nair V et al[3] there is evidence based upon clinical studies regarding usefulness of probiotics in prevention of NEC in preterm neonates. According to a systemic review of randomized controlled trials by Bernardo WM et al[1] the NEC incidence was 3.2% in neonates receiving probiotics and 7.2% in the controls (p value <0.001). In a study by Samanta et al, efficacy in probiotics group was 94.6% and in control group was 84.3%[1] (P value 0.02). However in another study by Li D et al, the difference in NEC incidence was insignifant between the control group (2.8%) and probiotics group(2.4%).(p value 0.38)[4]. In a population-based Canadian study of 16,669 preterm infants (gestational age <33 weeks) admitted in 25 NICUs, the incidence of NEC was 5.1 percent.[5]Reported mortality rates range from 15 to 30 percent and also are inversely related to gestational age and birth weight. While reviewing the pathology of necrotizing enterocolitis (NEC), it is due to changes from infarction in intestine.[7] The progression of the disease and the underlying pathogenic factors may vary further specific findings.

The factors that have been implicated in the pathogenesis of NEC include many points that include Prematurity along with Microbial bowel overgrowth, Milk feeding, altered mucosal defense, instability in the intestinal circulation as well as certain medications causing intestinal injury or lead to microbial overgrowth. Prematurity and feeding are consistent risk factors for NEC.[8] The clinical presentation of NEC consists of systemic signs and abdominal signs. Systemic signs may be nonspecific including lethargy, poor feeding, apnea, respiratory failure, or temperature instability. Hypotension resulting from septic shock may be present in the most severe cases, bacteremia was found in 20 to 30 percent of infants with NEC, which may contribute to these findings.[9] Abdominal signs are distention, vomiting, tender abdomen, per rectal bleed (hematochezia), diarrhea and bilious drainage from nasogastric tubes. The Bell staging criteria provide a uniform definition of NEC, based upon the systemic signs, intestinal signs, and radiographic features, this staging is most commonly used. **Stage I,** is suspected NEC, characterized by nonspecific systemic signs, like altered temperature, apnea, lethargy. Abdominal signs are

[1,3]Senior registrar, Paediatric Medicine Avicenna Medical College Lahore
[2]Associate Professor Paediatric Medicine Avicenna Medical College Lahore.
Correspondence to Dr. Shehla Usman, H 149B T, Aabpara Cooperative Housing Scheme Raiwind Road Lahore ***Email:*** *flik2035@gmail.com, Cell: 03314086766*

presence of more gastric residuals, distended abdomen, vomiting, and blood in stool.[18] Abdominal radiological features may show dilation of the bowel as with mild ileus. Stage I cases are further divided by the absence (stage IA) or presence (stage IB) of gross blood in stools. There is, however, a disagreement stated in literature whether Bells stage I is necrotizing enterocolitis, or other disease like feeding intolerance/ileus in the preterm babies[10.] **Stage II** is proven NEC that includes features of stage I along with absent bowel sounds with or without tender abdomen. Some infants, in stage II, along with these signs have cellulitis of the abdominal wall or a mass right lower quadrant. Infants having stage IIA are mildly ill, whereas in those with stage IIB NEC there is also mild metabolic acidosis and thrombocytopenia. Findings of abdominal radiographs include ileus, dilated intestine pneumatosis intestinalis, as well as ascites. **Stage III** or advanced NEC is the most severe form. In stage IIIA, the bowel is intact, whereas in stage IIIB, there is bowel perforation visualized as a pneumoperitoneum on the abdominal radiograph. Infants with advanced NEC are critically ill. In addition to the signs of less severe stages, they typically have hypotension, bradycardia, multiple apneas with signs of peritonitis (eg, abdominal distention, tenderness). Laboratory findings include a combination of respiratory and metabolic acidosis, low neutrophil count, and disseminated intravascular coagulation (DIC). Probiotics living non-pathogenic microbes that colonise the intestine wall and are beneficial to the host[11]. Ideally speaking, a probiotic agent should be healthy, non-pathogenic and non-invasive, it should resist degradation specially by acids and bile salts, modulate immune responses, be sensitive to usual antibiotics, in addition to that they should have origin from human microflora and remain un altered from technological processing. In our intestine there are micro flora which have an important role in our defense mechanisms against bacterial infections. There is a reported increase in pathogenic microorganisms in the preterm infants along with a decrease in normal flora that render these babies at increased risk of developing NEC.[12] It has been proposed in literature that the growth of pathogens might be prevented by inducing the colonization of the intestine non-pathogenic bacteria (probiotics) of species that are normal residents in the gut of preterm and term infants In our study preterm was defined as Neonates born before 37 complete weeks of gestation from last menstrual period(from history). Necrotizing enterocolitis was defined as per the modified Bell's criteria (Table 7). Presence of any stage was considered positive. In terms of efficacy, probiotic was defined to be effective when the preterm does not develop NEC (as given in operational definition) within 5 days of prophylactic treatment.

MATERIALS AND METHODS

Study design was Randomized study, a controlled trial. This study was carried out in the neonatal department Sheikh Zayed Hospital, Lahore..From November 6, 2014 to May 6, 2015. Sample size was estimated by using 95% confidence interval, Power 80% .The determined sample size was 278 (139 in each group) from www.openepi.com, Fleiss, Statistical Methods for Rates and Proportions with efficacy in probiotics group 94.6%, and control group 84.3%.[1] from Non-probability consecutive sampling**.** Cases included were Preterm neonates (<37 weeks of gestation) who have not been given enteral feed yet of both genders. Preterm neonates with signs and symptoms of sepsis (fever>100F, hypothermia<98F vomiting, rash) positive blood culture, raised CRP (greater than 6) or raised or decreased total leukocyte count in complete blood count(greater than 16,000/uL or less than 4,000/uL)**,** Preterm neonates with congenital heart defects (on echocardiography), limb defects (absent or fused limbs), gastrointestinal defects (on ultrasound abdomen, barium studies) were excluded from the study.

Data collection: 278 preterm neonates admitted in neonatal department, fulfilling the inclusion criteria, in Sheikh zayed hospital, Lahore were enrolled in the study. Parents were informed and written consent was taken. Neonates were randomly assigned to group A and group B each containing 139 preterm neonates by lottery method. Group A receiving probiotics combination, Lactobacillus rhamnosus and Bifidobacterium BB-12 species 2.5×10^9 cfu (colony forming units) per day with feed as Expressed breast milk(EBM) or formula milk for 05days and group B receiving only feed as EBM or formula milk. Neonates in both groups were observed for signs and symptoms of NEC during their stay in the hospital at day 5 of prophylactic treatment. Efficacy was labelled as per operational definition.

Data analysis: Statistical package for Social Sciences (SPSS) version 17.0 was used. Quantitative data like gestational age, weight was described by using mean and standard deviation. Qualitative data like, presence of NEC, gender was presented by using frequency and percentages. The development of NEC in 2 groups was interpreted by chi square test. Level of significance was determined by p value which if less than 0.05, was considered significant. Data was stratified for gestational age, weight and gender to deal with effect modifiers. Post stratification chi-square test was used and P value $\leq$0.05 was considered significant.

RESULTS

A total number of 278 neonates (139 cases in each group) that fulfilled the inclusion criteria were enrolled to determine the efficacy of prophylactic probiotics in preventionting Necrotizing enterocolitis in preterm neonates as compared to preterm neonates without probiotics. Gestational age of the patients was recorded as 60(43.17%) in Group-A and 45.32%(63) in Group-B were between 34-35 weeks of gestation while 56.83%(79) in Group-A and 54.68%(76) in Group-B were between 36-37 weeks of gestation, mean±sd was calculated as 35.46$\pm$1.01 and 35.39±0.99 weeks respectively. (Table 1)Mean birth weight (in grams) was calculated as 2453.0±212.44 in Group-A and 2438.23±209.62 in Group-B (Table 2). Patients were distributed according to gender showing that 65(46.76%) in Group-A and 68(48.92%) in Group-B were male while 74(53.24%) in Group-A and 71(51.08%) in Group-B were females (Table 3). Comparison of efficacy in both groups was recorded as 127(91.37%) in Group-A and 111(79.86%) in Group-B while remaining 12(8.63%) in

Group-A and 28(20.14%) in Group-B had no findings of efficacy, p value was calculated as 0.006 showing a significant difference (Table 4). Stratification for gestational age and gender was recorded and given in Table 5 & 6 respectively.

Table 1: Gestational age (n=278)

Gestational age (in weeks)	Group 1 (n=139)	Group 2 (n=139)
34-35	60(43.13%)	63(45.32%)
36-37	79(56.83%)	76(54.68%)
Total	139(100%)	139(100%
Mean±SD	35.46±1.01	35.39±0.99

Table 2: Birth weight(gms)(n=278)

Mean Birth weight (in grm)	Group 1 (n=139)		Group 2 (n=139)	
	Mean	SD	Mean	SD
	2453.09	212.44	2438.23	209.62

Table 3: Gender (n=278)

Gender	Group I	Group II
Male	65(46.76%)	68(48.92%)
Female	74(53.24%	71(51.08%)
Total	139(100%)	139(100%)

Table 4: **Comparison of efficacy in both groups (n=278)**

Efficacy	Group I	Group II
Yes	127(91.37%)	111(79.86%)
No	12(8.63%)	28(20.14%)
Total	139(100%)	139(100%)

P value: 0.006

Table 5: Stratification for efficacy with gestational age

Group	Efficacy	
	Yes	No
34-35 weeks (P value 0.92)		
A	30	30
B	31	32
36-37 weeks (P value:0.01)		
A	97	18
B	80	4

Table 6: Stratification for efficacy with gender

Group	Efficacy	
	Yes	No
Male (P value 0.30)		
A	56	9
B	54	14
Female (P value:0.003)		
A	71	3
B	57	14

DISCUSSION

In the above study, our aim was to determine the effectiveness of probiotics in prevention of NEC in preterm neonates, although there is literature available on this subject but the results of studies are controversial and do not match with each other. In our study, 60(43.17%) in Group-A and 63(45.32%) in Group-B were between 34-35 weeks of gestation while 79(56.83%) in Group-A and 54.68%(76) in Group-B were between 36-37 weeks of gestation, mean±sd was calculated as 35.46±1.01 and 35.39±0.99 weeks respectively, 46.76%(65) in Group-A and 48.92%(68) in Group-B were male while 74(53.24%) in Group-A and 51.08%(71) in Group-B were females, comparison of efficacy in both groups was recorded as 127(91.37%) in Group-A and 111(79.86%) in Group-B while remaining 8.63%(12) in Group-A and 20.14%(28) in Group-B had no findings of efficacy, p value was calculated as 0.006 showing a significant difference. According to a study by Caplan et al[13] Bifidobacteria supplementation showed a reduction in NEC-like lesions in neonatal rat models. Several studies are being performed that have assessed the pattern of colonization and NEC incidence in preterm infants supplemented with different probiotics. In A randomized controlled trial, infants whose feed was supplemented with Bifidobacterium breve were shown to have increased rates of stool levels of bifidobacteria at 2 weeks age. (73 vs. 12%),weight gain was better and feed tolerant. However, this study did not comment on incidence of NEC or severity[14]. It was reported in a multicenter double-blind study, that the probiotics and placebo group did not reveal any statistically significant difference in probiotics and placebo groups with regards to their outcome regarding NEC.[15] . According to another study by Bin-Nun et al[16], supplementation with probiotics resulted in a significant lowering of NEC incidence in very low birth weight (VLBW) infants. Adding to that, in another study performed by Lin et al[17] significant decrease in NEC incidence was reported,. However, in accordance with other studies and our results, the hypothesis "frequency of NEC in preterm neonates is less if they are given probiotics as compared to preterm neonates without probiotics" is justified and it recommends that the use of probiotics in preterm neonates should be used for prevention of NEC.

CONCLUSION

We concluded that the efficacy of probiotics in prevention of Necrotizing enterocolitis in preterm neonates is significantly higher when compared to preterm neonates without probiotics.

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Table 7) Modified bell's criteria for diagnosis of necrotizing enterocolitis

	Systemic findings	**Radiographic findings**	**Gastrointestinal findings**
Stage I	Apnea(cessation of breathing for >20sec) and bradycardia(heart rate less than 100/min), temperature instability (fever >100F, hypothermia <98F)	Normal or mild ileus	Gastric residuals, occult blood in stool, mild abdominal distention
Stage II A	Apnea (cessation of breathing for >20sec) and bradycardia (heart rate <100/min), temperature instability (fever >100F, hypothermia <98F).	Ileus present with dilatation of gut loops and focal pneumatosis	Gross blood in stools, prominent abdominal distention, absent bowel sounds
Stage II B	Thrombocytopenia(platelet count <150,000/uL) and mild metabolic acidosis (pH <7.3)	Widespread pneumatosis, ascites, portal-venous gas	Abdominal wall edema with palpable loops and tenderness
Stage III A	Mixed acidosis(pH<7.3), oliguria (urine output <0.5ml/kg/hour), hypotension (mean arterial blood pressure less than 35mmHg), coagulopathy (Prothrombin time>15sec and Activated partial thromboplastin time >40sec).	Prominent gut loops, increased ascites.	Worsening wall edema, erythema and induration
Stage III B	Shock(mean blood pressure <30mmHg, capillary refill time >5sec,poor/impalpable peripheral pulses) , deterioration in laboratory values(complete blood count, renal functions, liver functions, coagulation profile, serum electrolytes) and vital signs.	Pneumoperitoneum	Perforated bowel