INTRODUCTION

Necrotizing enterocolitis (NEC) is a serious condition related to the digestive tract in preterm infants. NEC is a worldwide problem in very low birth weight infants, with highly variable incidence affecting 2.6% to 28%, and causing 1% to 5% of admissions to the neonatal intensive care unit (NICU). The most frequent risk factors associated with NEC include prematurity and low birth weight. Over 90% of neonates who develop NEC are preterm. NEC is reported to be 1 - 5% of the live births globally. In Pakistan, the incidence of NEC is around 14% with a mortality rate of 39.28%. The precise pathogenesis of NEC is unknown but is widely considered as a multifactorial disease. Gastrointestinal immaturity, enteral feeding (especially formula feeding), presence of bacteria, and inflammation in the gastrointestinal tract may all contribute to the development of NEC. Host-pathogen interactions trigger inflammation in the gut that result in bowel injury, which may contribute to the pathogenesis of the disease.

It significantly increases mortality (attributable mortality of 15% to 30%) and morbidity (including surgery in 20% to 40% of infants and delayed neurodevelopment), despite the use of potent antimicrobial agents. Effective preventive strategies are lacking. Hence, the agents that modulate inflammation and enhance host defences may improve the outcome of infants with neonatal sepsis or NEC. Researchers in different settings are trying number of preventive strategies like probiotics, exclusive breast milk feedings, steroids, platelet activating factor and their receptor antagonist etc. Probiotics are live exogenous micro-organism delivered enterally that improve mucosal defences of the gastrointestinal tract and potentially provide benefit to the host. The most frequently used probiotics are *Lactobacillus* and *Bifidobacterium*. There is increasing interest in the potential health benefits of protective colonization of the gastrointestinal tract of preterm infants. However, the limited number of clinical trials limits the definition of optimal strains, timing, dosage, and duration of probiotics administered to VLBW preterm infants; and these issues need to be evaluated.
in small and large trials. Two previous studies done in Bangladesh showed significant efficacy of probiotics in the prevention of NEC.15,16 But those studies did not specify the dose, count of organism, and exact duration of treatment. Regional studies on this issue is also lacking. So the primary objective of this study was to determine the efficacy of oral probiotics in the prevention of necrotizing enterocolitis in preterm VLBW infants.

**METHODOLOGY**

This randomized double-blind control trial was carried out in the neonatal unit of Sylhet MAG Osmani Medical College Hospital (SOMCH), Sylhet, Bangladesh, from July 2012 to June 2015. Neonates with diagnosis of preterm (<33 weeks gestation) VLBW (birth weight <1500g) and fulfilling the inclusion criteria (able to tolerate oral feed and survive beyond 48 hours) were enrolled in this study. The ethical permission for this study was taken from SOMCH ethical committee (reference number: 2773). Babies with suspicion of clinical sepsis, presence of perinatal asphyxia, major congenital anomaly, and babies who expired due to other neonatal illness were excluded. Gestation was assessed from history of last menstrual period and after birth by new Ballard scores. Sample was calculated by using Guilford and Frucher's formula, considering 5% level of significance, and 5% precision level (marginal error). Infants were selected by convenient sampling, i.e. every second case was enrolled. Informed written consent was taken from the parents or guardians after detailed explanation of the purpose of study. Group allocation was done to Group A and Group B (Figure I). Coding was done by a faculty of another department who was not related to this study. Group A was the case group (preterm VLBW) which received probiotics along with regular breast feeding and standard care; and Group B was the control group (preterm VLBW) which only received regular breast feeding and standard care. First case was selected to one group by lottery method and subsequent group was continued accordingly. Participants and investigators were not known group allocation.

The study group was fed with probiotics at a dose of 3x10^9 CFU/day. (Cap TS6 probiotic + containing *Bifidobacterium* spp., *Lactobacillus* at 6 x 10^9 CFU = 6 billion CFU, manufactured by Tensall Bio-tech Co. Ltd. Taiwan, distributed by Century Health Care, Bangladesh) dissolved with 6 ml of breast milk then given 3 ml (3 billion probiotics) once daily from first feeding (after trophic feeding) through nasogastric tube until discharged (continued for at least 10 days). Probiotics was added to breast milk by registrar or assistant registrar of the corresponding unit before feeding. Probiotics was given after two hours of giving intravenous antibiotic.

Feeding was started when the infants have stable vital signs, active bowel sound without abdominal distension, no bile or blood from the nasogastric tube. A strict feeding protocol was followed for all study infants. If trophic feedings were tolerated for 24 hours, gradual advancement of feeding volume was continued. Trophic feedings were not mixed with probiotics. The amount of feeding was advanced slowly at approximately 10 - 20 ml/kg/day. Feeding was stopped if there was any sign of feeding intolerance (defined as the presence of gastric aspirate in the amount more than half of the previous feeding, with abdominal distension). An oral intake of 150 ml/kg/day was defined as complete enteral feeding.

The research assistant and the principal investigators were in charge of the care of the infants during their hospital stay. There was no modifications in the hospital management protocols, clinical practices, equipment, and infrastructure in the unit during the study period except the addition of probiotics to the study group. NEC (stage II or more) was diagnosed clinically in any infant presenting with the triad of feeding intolerance, abdominal distension, and grossly bloody stool. NEC was categorized according to the modified Bell's classification. To exclude mild and doubtful cases, only cases of NEC (stage II and III) were considered.

Abdominal X-rays and ultrasound were performed to diagnose and determine progression of NEC. Laboratory values such as complete blood count with differential, arterial blood gas measurements, and electrolytes were measured. Thrombocytopenia, persistent
metabolic acidosis and hyponatremia constitute the most common triad of signs and help to confirm the diagnosis. The primary outcome was the occurrence of NEC (stage II and III) by modified Bell's classification. Secondary outcome measures were days to achieve full enteral feeding and length of hospital stay. After data collection decoding was done by guide.

Data were analysed with SPSS (statistical package for social sciences) version 21.0. Quantitative data were expressed as mean and standard deviation and comparison was done by unpaired 't' test. Qualitative data was expressed as frequency and percentage and comparison was carried by Chi-square (X²) test. A probability value (p) of less than 0.05 was considered statistically significant.

RESULTS

Fifty-two (86.6%) patients were finally enrolled to the Group-A (Study group) and 50 (83.3%) patients in Group-B (Control group). There were total 69 (67.6%) male and 33 (32.3%) female neonates (Table I). Male: female ratio was 2.1:1. There were no significant differences in mean age, sex, gestational age, weight, and age at initiation of feeding of the patients between the study group and control group. Baseline characteristics between the study group and control group showed age in median and IQR which is 2.0 (1.0 - 11.0) hours vs. 2.0 (1.0 - 8.25) hours; Mann Whitney U test (p=0.970); gender [33 (63.5%) male vs. 36 (72.0%) female; x²=0.849; p=0.357]; gestational age (31.38 ±0.93 weeks vs. 31.68 ±0.84 weeks; t=1.676; p=0.097); weight (1310.6 ±110.4 gm vs. 1338.0 ± 97.71 gm; t=1.326; p=0.188) and age at initiation of feeding (3.33 ±0.58 days vs. 3.42 ±0.88 days; t=0.630; p=0.530).

Table II showed the distribution of the patients by development of NEC (stage I or III). NEC developed in 1 (1.9%) neonates in study group and 6 (11.5%) neonates in control group. Development of NEC was significantly less in study group than that of control group (χ²=4.050; p=0.044).

Table III showed the distribution of the patients by age of achievement of full oral feeding (an oral intake of 150 ml/kg/day) and total duration of hospital stay in days. The mean age of achievement of full feeding in study and control groups were 14.88 ±3.15 days and 18.80 ±4.32 days, respectively. Age of achievement of full feeding was significantly earlier in study group than that of control group (t=-5.090; p=0.001). The mean duration of hospital stays in study and control groups were 15.82 ±2.94 days and 19.57 ±4.26 days, respectively (Table II). Duration of hospital stay was significantly shorter in study group than that of control group (t=-5.037; p<0.001).

**DISCUSSION**

Probiotics may protect high-risk neonates and infants from developing necrotizing enterocolitis in many ways. There may be an increased barrier for migration of bacteria and their products across the mucosa, competitive exclusion of potential pathogens, modification of host response to microbial products, augmentation of IgA mucosal responses, and/or enhancement of enteral nutrition that inhibits the growth of pathogens and upregulation of immune responses. Development of NEC was significantly less in number in our study group (4% vs. 16.6%; p=0.031). Development of NEC in the probiotics group (4% vs. 16.6%; p=0.031). Development of NEC in the probiotics group was associated with a significantly decreased risk of NEC in preterm VLBW infants (RR=0.33; 95% confidence interval [CI],
In another meta-analysis of 11 randomized, controlled trials Deshpande et al. found a higher proportion of neonates in the no probiotics (control group) developed definite NEC compared to the probiotics group (71 [6.56%] vs. 26 [2.37%]) and estimated a lower risk (RR: 0.35 [CI: 0.23-0.55]; p<0.001) of NEC in the probiotic group. Mihatsch et al. found only two cases of NEC (Bell >2) in the probiotics group and 4 in the placebo group of their study which was statistically insignificant. Sari et al. also did not find any significant difference in the incidence of NEC between the study and control groups (5.8 vs. 9%, respectively; p=0.447). The possible explanation of both the findings are: they used single strain of probiotics whereas we used multiple strain.21,22

In this study, the mean age of achievement of full oral feeding was significantly earlier in probiotics treated group than that of control group (p<0.001). This result was similar to that of the study by Samanta et al. where the time to full oral feeds was significantly shorter in the probiotic group (weighted mean difference -2.74 days, 95% CI -4.98 to -0.51) than in controls. Bin Nun et al. did not find any difference between the study and control group in terms of early oral feeding (p=0.13). Several other studies also reported no significant difference in reaching full oral feeds.18,21

In this study, the mean duration of hospital stays was significantly shorter in study group compared to control group (p<0.001). This result was similar to that of the study by Sari et al. where the duration of hospital stay was significantly shorter in probiotic group than that of control group (17.17 ±3.23 days versus 24.07 ±4.00 days). Though, Lin et al. did not find any significant difference in the hospital stay between case (46.7 ±27.1 days) and control (46.5 ±26.1 days) in his study. Lin et al. set 34 weeks (not less than that) as preterm and 1500 grams as low birth weight which might produce the probable result.18 In this study, infants (both case and control) need to stay in the hospital for longer period than usual, probably because of the prematurity and sepsis (in some cases). It requires extended research to find out the exact reasons behind this. A very recent study also showed that probiotics can reduce the incidence of sepsis in LBW infants. This might be another area of further research.

CONCLUSION

Probiotic supplementation reduced the frequency of necrotising enterocolitis (NEC) in preterm neonates with very low birth weight. It was also associated with faster achievement of full oral feeding and shorter duration of hospital stay.

REFERENCES


