ORIGINAL ARTICLE

Comparison of Probiotics Versus Zinc in The Prevention of Necrotizing Enterocolitis in Preterm Neonates

Zabit Khan[1], Gulbin Shahid[2], Iqtada Haider Shirazi[3] Sadia Riaz[4], Syeda Shireen Gul[5]

Author`s Affiliation

Postgraduate Trainee[1]
Assistant Professor[2,5]
Professor of Neonatology[3]
Senior Registrar[4]
The Children's Hospital, Pakistan Institute of Medical Sciences, Islamabad.

Author`s Contribution

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Address of Correspondence

Zabit Khan
dr.zabit@gmail.com

ABSTRACT

Objective: To compare the effect of probiotics versus zinc in the prevention of necrotizing enterocolitis (NEC) in preterm neonates.

Study design: Randomized controlled trial

Setting: Department of Paediatric Medicine, Pakistan Institute of Medical Sciences, Islamabad, from 29 March 2016 to 15 August 2016.

Methodology: A total of one hundred and sixty-four (n=164) preterm infants with gestational age < 37 weeks and birth weight < 2500 g were enrolled in the study. All the neonates were randomly divided into two Groups. Group A was given probiotics, (lactobacillus acidophilus 1 ml BD), and group B were given Zinc (Zinc sulphate 2 mg/Kg body weight) both starting within the first 3 days of life and continued for two weeks. All the babies were followed for two weeks for assessment of NEC. The significance of the difference in frequency between both groups was assessed by employing chi-square test.

Results: Both groups were similar in terms of gender, age, and gestational age. NEC was diagnosed in 2.4% (n=2) babies in group A and in 4.9% (n=4) of patients in group B as per our operational definition. Chi-square test was employed and the difference was not found to be statistically significant (P=0.405).

Conclusion: In this study, NEC was observed less frequently in preterm neonates taking probiotics as compared to preterms taking Zinc, both started within the first 3 days of life. However, the difference was not statically significant. We recommend further studies with larger sample size.

Key Words: Necrotizing enterocolitis, Probiotics, Zinc sulphate.

Introduction

Necrotizing enterocolitis (NEC) is a multifactorial disease that results from the interaction between the loss of integrity of the intestinal mucosa and the host response to this damage. NEC is the most prevalent emergency of the gastrointestinal tract in the neonatal period.[1,2,3] Its incidence is variable, affecting 7-11% of newborns with very low birth weight (< 1,500 g).[4,5,6,7] Occurrence of NEC is inversely related to gestational age at birth pointing to the role of physiological intestinal immaturity in its etiology.

NEC is a devastating disease with a mortality of up to 20-40% in VLBW infants.[5,8,9] Twenty-seven to thirty-six

percent of infants with NEC need some sort of surgical intervention[10] and more than one-third of affected infants are left with strictures primarily in the colon.[11]

Pathogenesis of NEC is multifactorial and likely results from a complex interaction of factors resulting in loss of integrity of intestinal mucosa and the host response to the damage. Contributing events include intestinal ischemia, mucosal damage, edema, ulceration, and passage of air or bacteria through the wall, resulting in necrosis of the mucosa and intestinal wall. The main preexisting factors that cause increased risk for developing NEC are prematurity, enteral feeding, and colonization by pathogenic microorganisms such as Escherichia coli, Klebsiella, Clostridium perfringens, Staphylococcus epidermidis and Rotavirus. [12]

When compared to term infants, VLBW infants at risk of NEC have abnormal fecal colonization, demonstrate a paucity of normal enteric bacterial species and have delayed onset of bacterial colonization. [13, 14]

Treatment of infants with NEC generally includes a regimen of bowel rest, gastric decompression, systemic antibiotics and parenteral nutrition. Infants with perforation are generally operated upon; however, there has been a recent interest in primary peritoneal drainage as an alternative. Prevention of NEC still remains elusive. Avoidance of preterm birth, use of antenatal steroids and breast-milk feeding are practices that offer the greatest potential benefits. [10, 15]

The quest for preventive strategies for development of NEC in premature infants led to the interest in the use of probiotics. Probiotics were first described in the literature by Lilly and Stillwell in 1965, as growth-promoting factors produced by certain microorganisms. Recently, they have been described as living organisms which, when included in the diet in adequate amounts, can bring health benefits to the host.[16,17] As microorganisms able to colonize the digestive tract by adhering to the intestinal epithelium, producing antimicrobial substances, and modulating the immune response and host metabolism, probiotics have been discussed regarding their usefulness for preterm infants.[18,19] Potential mechanisms by which probiotics may protect high-risk infants from developing NEC or sepsis, or both, include an increased barrier to migration bacteria and their products across the mucosa[20,21], competitive exclusion of potential pathogens[22], modification of host response to microbial products[23], augmentation of immunoglobulin A (IgA) mucosal responses, enhancement of enteral nutrition that inhibits the growth of pathogens, and up-regulation of immune response.[24]

Similarly, the role of Zinc in many intestinal functions and clinical conditions of children has been widely studied, however, the clinical effects of Zinc on the neonatal intestine remain largely unexplored. Several experimental models and clinical evidence suggest a role for zinc in the pathogenesis of NEC.[26] Zinc modulates the expression of important inflammatory cytokines and their receptors in the intestine in models of colitis.[(26)]Subjects deficient in zinc showed a reduced immune response against pathogens.[27] Zinc also has trophic effects on intestinal mucosa and modulates intestinal permeability.[28] Finally, zinc deficiency worsens the extent of damage from asphyxiation due to the reduced enzymatic antioxidant activities. However, no systematic study has been carried out so far on the possible role of zinc in the development of NEC. A multicenter trial on premature babies reported a significant reduction in NEC incidence in subjects receiving high doses of zinc. [29] Based on the above-mentioned evidence, it is reasonable to imagine a role for zinc in the prevention of NEC, and design specific studies in this field.

The purpose of our study was to determine the best possible approach to the prevention of NEC in premature infants by comparing the efficacy of probiotics with Zinc.

Methodology

The study was conducted at the Neonatal Department of Paediatric Medicine, Pakistan Institute of Medical Sciences, Islamabad, Pakistan. A total of 164 preterm infants weighing <2500g were enrolled and randomly divided into two groups. Babies who were very sick, were on ventilatory support, who had already developed NEC and babies with dysmorphic features, gut malformations and with inborn errors of metabolism were excluded from the study. Out of the study groups, Group A was given probiotics (lactobacillus acidophilus 1 ml twice a day) and Group B was given Zinc (Zinc Sulphate 2mg/kg of body weight once daily) both given for two weeks starting within the first 3 days of life. All the babies were followed up for two weeks and assessed for development of NEC. Diagnosis of NEC was made by clinical evaluation, by checking stools for occult blood, abdominal ultrasound, and abdominal X-rays. The outcome was measured in the two groups and the difference in the incidence of NEC was documented.

Data was analyzed on SPSS version 17.0. Frequency and percentage were computed for qualitative variables like gender and final outcome among two groups. Age was

presented by mean. Chi square test was used to compare the impact of probiotics and Zinc group in term of development of NEC and mortality. P value ≤ 0.05 was considered significant.

Results

The gender and age distribution of the two assigned groups A and B were similar as was the gestational age (Group A mean gestational age 31.41 weeks, Group B mean gestational age 31.82 weeks). The demographic features of the selected population are represented in table I.

Table I: Demography of study population (Group A-probiotic group vs Group B-Zinc group)

	Group A (probiotic)	Group B (Zinc)	Total
Gender			
Males	47(57.3%)	42(51.2%)	89(54.3%)
Females	35(42.7%)	40(48.8%)	75(45.7%)
Mean Age (days)			
Males	1.68	2.14	
Females	2.37	1.83	
Mean Gestational Age (weeks)	31.41	31.62	

NEC was diagnosed in 2.4% (n=2) babies in group A and was diagnosed in 4.9% (n=4) of patients in group B as per our operational definition. Chi-square test was employed and the difference was not found to be statistically significant (*P*=0.405). Results are presented in table II.

Table II: Frequency of NEC in Group A & B

NEC	Group A (probiotics)	Group B (Zinc)	P-value
Present	2(2.4%)	4(4.9%)	
Absent	80(97.6%)	78(95.1%)	
Total	82(100%)	82(100%)	0.405

Discussion

NEC is one of the leading causes of morbidity and mortality in premature neonates. The incidence in very low birth weight infants is 7-11 %[4, 5, 6, 7] with a mortality as high as 20-40 %. [5, 8, 9]

Infants who survive have various complications, such as nosocomial infection, malnutrition, growth failure, bronchopulmonary dysplasia, retinopathy of prematurity, and neurodevelopmental delays. The most important etiology in the pathogenesis of NEC is structural and immunological intestinal immaturity. In preterm infants with immature gastrointestinal tracts, development of NEC may be associated with a variety of factors, such as colonization with pathogenic bacteria, secondary ischemia, genetic polymorphisms conferring NEC susceptibility, anemia with red blood cell transfusion, and sensitization to cow milk proteins. To date, a variety of preventive strategies has been accepted or attempted in clinical practice with regard to the pathogenesis of NEC. These strategies include the use of breastfeeding, various other feeding strategies, probiotics, glutamine, arginine, lactoferrin and Zinc. [29, 30]

Despite advancements in the knowledge and understanding of the pathophysiology of NEC, there is currently no universal measure for the prevention of this serious and often fatal disease.

The aim of our study was to compare the effect of two strategies namely probiotics and Zinc in the prevention of NEC in premature infants. Probiotics alone have been fairly extensively studied in this regard[31, 32, 33, 34] whereas Zinc has also recently been evaluated for this purpose.[29] To our knowledge no study has yet been reported comparing the effect of the two in the prevention of NEC. Our study population included preterm neonates with gestational age <37weeks and a birth weight of <2500gms. This compares to a recent Cochrane Database Systematic Review and meta-analysis on use of probiotics for prevention of NEC in which 24 randomized or quasi-randomized controlled trials were included.(33) They enrolled preterm infants also <37 weeks gestational age and/or with a birth weight <2500gms.Though the included studies had a highly variable enrollment criteria (i.e. birth weight and gestational age, baseline risk of NEC in the control groups, timing, dose, formulation of the probiotics and feeding regimen), still the authors concluded that probiotics significantly decreased the incidence of severe NEC and all-cause mortality in infants weighing <1500gms at birth. Though not yet approved for use in premature infants by FDA this review supports the routine use of probiotics in premature neonates.

As regards efficacy of Zinc in the prevention of NEC, Hoyle B, et al enrolled 193 newborns (gestational age <32 weeks) weighing <1500 grams.[35] The very low birth weight neonates were randomized at day 7 after birth to receive oral zinc 10 mg/day as part of a multivitamin (n = 97; 95 completed the study) or placebo that comprised a multivitamin without zinc (n = 96; 93 completed the study). The multivitamin preparation did not contain copper. All neonates received parenteral nutrition that included a daily dose of zinc 1 mg. No probiotic was

administered. The 2 regimens were maintained until hospital discharge. The primary endpoint was the rate of neonates presenting with NEC as judged according to Modified Bell Staging Criteria. The secondary outcome was mortality rate. Their results showed that occurrence of NEC was significantly higher in the placebo group and mortality risk was significantly reduced in the zinc group (odds ratio, 0.275; P = .021). No adverse effects were significantly evident in the neonates receiving oral supplementation of zinc.

Terrin G et al[29] aimed to investigate the efficacy of zinc supplementation in reducing morbidity and mortality in preterm neonates and to promote growth. This was a prospective, double-blind, randomized controlled study of very-low-birth-weight preterm neonates randomly allocated on the seventh day of life to receive (zinc group) or not receive (control group) oral zinc supplementation. Total prescribed zinc intake ranged from 9.7 to 10.7 mg/d in the zinc group and from 1.3 to 1.4 mg/d in the placebo control group. The main endpoint was the rate of neonates with ≥ 1 of the following morbidities: late-onset sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, periventricular leukomalacia, and retinopathy of prematurity. Secondary outcomes were mortality and body growth. Their results showed that morbidities were significantly lower in the zinc group (26.8% compared with 41.7%; P = 0.030). The occurrence of necrotizing enterocolitis was significantly higher in the control group (6.3% compared with 0%; P = 0.014). Mortality risk was higher in the placebo control group (RR: 2.37; 95% CI: 1.08, 5.18; P = 0.006). Daily weight gain was similar in the zinc (18.2 ± 5.6 g · kg^{-1} · d^{-1}) and control (17.0 ± 8.7 g · kg^{-1} · d^{-1}) groups (P = 0.478). They concluded that oral zinc supplementation given at high doses reduces morbidities and mortality in preterm neonates.

Abrams SA, et al[36] in their review reported the dosing provided in a recently published study. The authors provided ~10 mg supplemental Zn/d to a group of very-low-birth-weight (<1.5 kg) infants beginning at 1 week of age and continued supplementation for their hospitalization or until 42 weeks postmenstrual age. Mean weight during the entire intervention period was not provided, but assuming a weight of 1.2–1.5 kg, this dose provided ~6–8 mg Zn/kg during most of the study. A beneficial outcome was shown for morbidity and mortality in the supplemented group compared with the unsupplemented group, primarily driven by a rate of necrotizing enterocolitis (NEC) of zero in the supplemented group.

Terrin G, et al[37] in their narrative review, focused on the role of dietary zinc in early life (including embryo, fetus and preterm neonate), analyzing consequences of zinc deficiency and adequacy of current recommendations on dietary zinc. They selected and analyzed 81 studies. Results of their analysis showed that preservation of zinc balance is of critical importance for the avoidance of possible consequences of low zinc levels on pre and post-natal life including NEC.

From the above studies, there is evidence of efficacy of preventive strategies with regards to the occurrence of NEC in premature neonates. Head to head comparative studies are required to assess the most effective strategy. Our study was aimed at such an endeavor and the results showed that probiotics (specifically lactobacillus acidophilus) fared better in the prevention of NEC as compared to Zinc with 2.4% of the probiotic group developing NEC compared to 4.9% of the Zinc group. However, our study was limited by its small sample size. Hence we recommend further studies to determine the most effective preventive preparations, timing and length of therapy to be utilized. The distinction between a physiologic dose needed for growth and a much higher dose is critical when using supplement doses that are several times the physiologic requirements for growth. Concerns about toxicity and other complications need to be evaluated in adequately powered controlled trials.

Conclusion

In this study, we conclude that NEC was observed with lesser frequency in preterm infants receiving probiotics as compared to those receiving Zinc. However, our study was limited by its small sample size, therefore, we recommend further better-powered studies.

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