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Original Article

Effect of Zinc Supplementation in Prevention of Necrotizing Enterocolitis in Preterm Neonates

Khola Binet Mansoor[1*], Mehdi Abbas[1], Aneela Anjum[1], Naveed Haider[2], Shadab Fatima[3], Sameen Anwar Rao[4] and Ferheen Shahbaz[5]

[1]Pediatric Medicine Unit II Mayo Hospital, King Edward Medical University, Lahore, Pakistan
[2]Department of Pediatric Surgery, District Head Quarter Hospital, DG Khan, Pakistan
[3]CMH Medical College, Lahore, Pakistan
[4]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[5]Department of Public Health, University of The Punjab, Lahore, Pakistan

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***Corresponding Author:**
Khola Binet Mansoor
Pediatric Medicine Unit II Mayo Hospital, King Edward Medical University, Lahore, Pakistan
dr.khola6@gmail.com

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ABSTRACT

Necrotizing enterocolitis is a serious condition involving the gastrointestinal tract and is one of the most common causes of death in neonates. It occurs due to various factors including the altered immune response of the premature intestinal tract against gut microbiota, resulting in inflammation and damage to the gut. **Objective:** To find out the effect of Zinc in the prevention of necrotizing enterocolitis in very preterm infants in the first 2 weeks of life. **Methods:** It was a double-blind randomized control trial held in the Neonatology section of Pediatric Medicine unit-II, Mayo hospital Lahore. The duration of the study was 11 months after the approval of the synopsis from August 2019 to July 2020. A total of 94 patients were included in the studies (95% confidence level, 7% absolute precision). Probability sampling, a simple random sampling technique was used. The study included 94 patients, 28 weeks to 32 weeks of gestational age. **Results:** In this study, the mean age of neonates was 54.85±11.60 hours, 51(54.26%) patients were male whereas 43(45.74%) patients were females. A birth weight having <1 kg was observed in 20(21.3%) patients and a birth weight between 1-2 kg was observed in 49(52.1%) patients. Necrotizing enterocolitis was developed among 6(6.38%) patients. Among the zinc supplementation group, the NEC developed in 2(4.3%) patients whereas, among the placebo group, the NEC developed in 4(8.5%) patients (p-value=0.677).**Conclusion**: This study concluded that zinc supplementation did not prove any preventive effect against necrotizing enterocolitis in very preterm infants.

INTRODUCTION

Necrotizing enterocolitis (NEC) is the major cause of neonatal death and a leading cause of gastrointestinal tract-related emergencies in newborns [1]. Up to 10% of premature neonates with less than 29 weeks of gestational age, develop necrotizing enterocolitis [2]. It is an inflammatory and ischemic response of the gut mainly occurring in premature neonates [3]. The exact mechanisms of pathogenesis of necrotizing enterocolitis are not completely known. Immaturity of the intestine, hypoxic-ischemic encephalopathy, impaired/abnormal inflammatory response, and infections are the possible underlying mechanisms of developing it [4]. Enteral feeding, prematurity of the gut, and normal gut flora play important roles in gut damage and ultimately the development of necrotizing enterocolitis [5]. The imbalance between pro- and anti-inflammatory factors in premature neonates results in abnormal activation and inactivation of inflammatory mediators and mediators like

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platelet-activating factors are thought to be involved in the pathogenesis of necrotizing enterocolitis. Abnormal toll-like receptor 4 signaling in the premature intestine and increased activation of nuclear factor-κB may play a role in the pathogenesis of necrotizing enterocolitis [4]. Zinc is essential for the synthesis of many enzymes involved in the metabolism of nucleic acid, protein synthesis, immune functions, and organ formation [6]. It has a role in controlling of epithelial barrier mechanism and developing proper immune responses [7]. It also acts on toll-like receptor 4 and modulates the immune mechanism. Zinc is essential for the brain and gut development during organogenesis [8]. Zinc deficiency in premature neonates occurs due to reduced transfer of zinc from the placenta resulting in low stores and excessive endogenous losses and less intake [9]. Zinc supplementation can play in the etiology and therapy of a wide range of gastrointestinal diseases [10]. Those Subjects who had low zinc levels expressed a poor immune response and some antioxidant activities [11]. There is extensive research going on the supplementation of Zinc in preterm infants for the reduction of morbidity and mortality. There are multiple studies supporting the evidence that zinc when given at different doses plays a positive effect on mortality, morbidity, and growth in infants and children [12]. Some studies demonstrate that zinc is equally effective as probiotics in the prevention of necrotizing enterocolitis in preterm infants [13]. A randomized controlled trial conducted in low birth-weight very preterm infants showed that the occurrence of necrotizing enterocolitis was significantly lower with high doses of zinc in those infants [14]. However, no systematic study has been carried out so far on the possible effect of zinc in the development of necrotizing enterocolitis [15-16]. The rationale of this study was to identify the effect of Zinc therapy on preventing necrotizing enterocolitis in very preterm infants. So if the effect of zinc is established in the prevention of this complication of neonates, it will be a major step in reducing neonatal morbidity/mortality. Objective of this study was to evaluate that Zinc supplementation prevents the development of Necrotizing enterocolitis in very preterm infants.

METHODS

Randomized control trial, double-blind. Neonatology unit of Pediatric Medicine unit-II, Mayo Hospital Lahore. The duration of the study was 11 months after the approval of the synopsis from November 2019 to April 2020. The sample size of 94 patients (47 patients in each group) was estimated by using a 95% confidence level, and 7% absolute precision with expected Zinc% as 0% and the control group as 6.3% [17].

$$n = \frac{Z^2_{1-\alpha/2}[P_1(1-P_1)+P_2(1-P_2)]}{}$$

Probability sampling, a simple random sampling technique was used. Neonates admitted in the ward with age meeting inclusion criteria were examined for Ballard's score before they were enrolled for the study. Ballard's scoring was done by a panel of 3 members (2 residents and the senior registrar of the section) in order to reduce any chance of poor scoring an average of 3 scores was taken. All the residents were properly guided in calculating the APGAR score beforehand. After taking informed written consent, patients admitted to the neonatology section and meeting the inclusion criteria were included in the study. Randomization was performed by using computer-generated numbers. Group A was given zinc while group b received a placebo. It was a double-blind randomized control study because the staff administering the drug was not informed about the contents of the bottle and doctors measuring the outcome were also informed about the drug or placebo given. Before that, enteral feeding was initiated on the 2nd or 3rd day of life at 5-10ml/kg/day in all stable infants. Feeds were divided into 8 equal parts. Expressed breast milk was the first preference but if it was not available appropriate preterm formula feed will be used. Once the patient has tolerated enteral feed (no gastric residual volume or vomitus) for one day, the trial drug was added on the next day. It was given in two divided doses. Feeding was stopped if there are any signs of feed intolerance. Inclusion criteria: Very Preterm infants (gestational age 28 to < 32 completed weeks), Age 0-72 hrs, both sexes, Mode of delivery NVD/ C-Section, Born elsewhere. Exclusion criteria: Congenital malformation (major on gross physical examination). Obvious dysmorphic/ Syndromic baby. Asphyxia –stage II, III. Diagnosis of necrotizing enterocolitis was recorded according to Modified Bells staging, once infants completed two weeks study period. Whether the patient developed NEC or not was measured according to Modified Bell's staging criteria and was recorded by the resident. X-ray was reported by a consultant radiologist. Data were tabulated and analyzed by SPSS version 26. Quantitative variables like age were presented as Mean± Standard deviation. Qualitative variables like gender were presented as frequency and percentages. Difference of two groups (Zinc supplemented VS placebo) between gender, gestational age, placebo or drug was given pt. developed NEC or not, pt. developed vomiting or diarrhea or not was done by applying the chi-square test. p-value ≤ 0.05 was taken as significant.

RESULTS

In our study total of 94 neonates were enrolled. The data were normally distributed as the p-value of the KS test was

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0.100. According to this study among the zinc supplementation group a birth weight <1 kg was found in 11(23.4%) patients, a birth weight 1-2 kg was found in 22(46.8%) patients and a birth weight >2 kg was found in 14(29.8%) patients. Similarly among the placebo group a birth weight <1 kg was found in 9(19.1%) patients, a birth weight of 1-2 kg was found in 27(57.4%) patients and a birth weight >2 kg was found in 11(23.4%) patients. This difference was statistically insignificant. i.e. p-value=0.586 (Table 1).

Table 1: Comparison of the weight of neonates between study groups

Birth Weight (kg)	Study Group		Total	p-value
	Zinc Supplementation	Placebo		
<1	11(23.4%)	9(19.1%)	20 (21.3%)	0.586
1-2	22(46.8%)	27(57.4%)	49(52.1%)	
>2	14(29.8%)	11(23.4%)	25(26.6%)	
Total	47(50.0%)	47(50.0%)	94(100.0%)	

NEC developed in 4(8.5%) patients. This difference was statistically insignificant. i.e., p-value=0.677(Table 2).

Table 2: Comparison of NEC development between study groups

Develop NEC	Study Group		Total	p-value
	Zinc Supplementation	Placebo		
Yes	2(4.3%)	4(8.5%)	6(6.4%)	0.677
No	45(95.7%)	43(91.5%)	88(93.6%)	
Total	47(100.0%)	47(100.0%)	94(100.0%)	

In our study among the zinc supplementation group, vomiting was observed in 3(6.4%) patients while among the placebo group, the vomiting was observed in 2(4.3%) patients. Similarly, among the zinc supplementation group diarrhea was observed in 4(8.5%) patients while among the placebo group diarrhea was observed in 4(8.5%) patients. This difference was statistically insignificant. i.e. p-value=0.688(Table 3).

Table 3: Comparison of side effects between study groups

Side Effects	Study Group		Total	p-value
	Zinc Supplementation	Placebo		
Vomiting	3(6.4%)	2(4.3%)	5(5.3%)	0.688
Diarrhea	4(8.5%)	4(8.5%)	8(8.5%)	
Nil	40(85.1%)	41(87.2%)	81(86.2%)	
Total	47(100.0%)	47(100.0%)	94(100.0%)	

DISCUSSION

Our study aimed to evaluate the effect of zinc supplementation on the incidence of necrotizing enterocolitis (NEC) in very preterm infants. We followed a double-blind randomized controlled trial design with a sample size of 94 patients, divided equally into a zinc supplementation group and a placebo group. The results showed that there was no statistically significant difference between the two groups regarding birth weight, the incidence of NEC, vomiting, or diarrhea. While in literature mostly observational study and reviews are available. The study by Kreb and colleagues highlighted the importance of zinc in growth, cell differentiation, gene transcription, metabolism, hormone and immune function, as well as wound healing [18]. In preterm neonates, zinc stores are low due to inadequate absorption and storage mechanisms. Terrin and Berni's study found that zinc can prevent NEC through its effects on the intestinal mucosa and modulation of intestinal permeability. However, their study did not use a high dose of zinc. Their study had significant output and our study had non-significant results due to limited sample size [12]. Two meta-analysis by Livingstone and Mashad, *et al.*, provide us a comparison of effects of zinc on neonates. Zinc is digested and absorbed in the upper gastrointestinal tract into enterocytes and is entered into a small plasma pool. Humans have no dedicated zinc stored in any specific organ. This analysis highlights the efficacy of zinc like our study [19, 20]. Our study of double-blinded randomized control trial at the Neonatology unit of pediatric Medicine unit-II, Mayo hospital Lahore aimed to investigate the effect of high-dose zinc supplementation on the prevention of NEC in very preterm infants. The study found no preventive effect against NEC with the use of high-dose zinc supplements in the diet of very low birth weight neonates. While previous studies available were focused on the dosage of zinc, occurrence of necrotizing enterocolitis was significantly higher in the placebo group as compared to the zinc supplementation group. The dose of zinc used in this study was 4mg/kg, which is within the Tolerable Upper Intake Level for zinc intake set by the Institute of Medicine for infants in the first 6 months of age. A hospital based study done by Terrin and Berni, which was a randomized control trial had similar methodology as our study. Zinc can prevent NEC through its trophic effects on the intestinal mucosa and modulation of intestinal permeability so it affects the morbidity and mortality of neonates. While our study had no follow-up indicators involved and highlighted the efficacy of zinc supplementation [21, 22]. Canani *et al.*, compared zinc with probiotics in the prevention of NEC and found no statistically significant difference between the two groups. However, there was a slightly better outcome in the probiotic group as compared to the zinc group [23]. By comparing all these studies with our study we can say that, zinc supplementation can benefit the development and growth of neonates, reduce illness, and mortality. Zinc dose is crucial in the efficacy of treatment, as very high doses may lead to toxicity and reduced absorption of copper and iron. The supplement dose used in the studies mentioned in this article was within safe limits [24]. There were some limitations in our study. Because our study

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included only very preterm neonates, we cannot generalize it to other preterm neonates. There was also some difference in formula feed and human milk usage in the two groups of study. Our study also didn't include any measurements of serum zinc and hemoglobin levels. It was because of avoiding additional procedures in the preterm neonates due to minimal handling. Also, blood sampling in very preterm neonates can cause iatrogenic anemia. So separate studies can be done showing levels of zinc and then its association with replacement of it in preterm infants for prevention of NEC.

CONCLUSIONS

This study concluded that the use of zinc supplementation has no preventive effect for necrotizing enterocolitis in very preterm infants within the neonatal period. So in light of this study, we did not find any convincing evidence regarding the prevention of NEC by zinc supplementation for breastfed LBW neonates. However, further studies can be carried out to find out the positive effects of Zinc.

Conflicts of Interest

The authors declare no conflict of interest.

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