Hormozgan Medical Journal

doi 10.34172/hmj.2022.37

Hormozgan Med J. 2022; 26(4): 213-217

Research Article

The Effect of Zinc Supplementation on the Treatment of Neonatal Sepsis: A Randomized Controlled Trial

Seyed Hossein Saadat[1], Rakhshaneh Goodarzi[1], Simin Yazdi[1*], Shahram Zare[2]

[1]Clinical Research Development Center of Children's Hospital, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[2]Department of Biostatistics, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Abstract

Background: Zinc plays an important role in some metabolic and signaling pathways of the immune system and may improve the signs and symptoms of neonatal sepsis. This study aimed to evaluate the efficacy of zinc supplementation in neonatal sepsis.

Methods: This randomized controlled trial included 50 neonates with sepsis admitted to Bandar Abbas Children's hospital, Iran, from 2018 to 2019. Patients were randomly allocated into two groups: the zinc group received standard antibiotics plus 1 mg/kg zinc gluconate twice a day for 7 days starting within the first 24 hours after admission, while the control group only received antibiotics. Complete blood count (CBC) with differential, C-reactive protein (CRP), and platelets were measured on the first day of admission. Blood sampling was done again after 48 hours based on the patients' condition. Patients' information such as age, sex, gestational age, birth weight, time to the improvement of clinical and laboratory findings, hospital length of stay, mortality, change in the antibiotic regimen, and signs of sepsis were noted.

Results: The two study groups were similar concerning age and sex. Birth weight, gestational age, duration of hospital stay, time to the improvement of clinical findings, baseline CRP, and change in the antibiotic regimen were comparable in both groups ($P>0.05$). The time to the improvement of laboratory findings was significantly lower with zinc supplementation compared with controls (6.56 ± 2.95 vs. 8.36 ± 3.34 days, $P=0.022$). Further, final CRP significantly decreased compared to baseline CRP in both groups ($P<0.001$); however, this reduction was greater in the zinc group (final CRP: 3.60 ± 1.87 vs. 5.12 ± 2.11 mg/L, $P=0.015$). Moreover, no mortality was reported in either of the groups.

Conclusion: Zinc supplementation had no effect on hospital length of stay in neonatal sepsis; however, it reduced the time to the improvement of laboratory findings, especially CRP.

Keywords: Neonatal sepsis, Zinc supplementation, C-reactive protein

***Correspondence to**
Simin Yazdi,
Tel: +989173605726,
Email: siminyazdi.hums@gmail.com

Received January 7, 2022, **Accepted**: February 14, 2022, **Published Online**: October 10, 2022

Background

Many advances have been made in neonatal care; however, its mortality rate is still high with an incidence of 2022 in every 100 000 live births and a mortality rate ranging from 11% to 19% (1). Different inflammatory cytokines are believed to be involved in neonatal sepsis; nevertheless, the mechanism and pathophysiology of this condition remain unknown (2).

Zinc is necessary for the development of the brain and respiratory and intestinal systems in both prenatal and postnatal periods (3). In addition, zinc is found in hormones, proteins, and nucleotides and is essential for various enzymes in metabolic pathways, including immune function, protein synthesis, nucleic acid metabolism, and organogenesis (3-5). More importantly, Prasad et al found that zinc deficiency can suppress immune function leading to infections (6).

Some trials have proposed high doses of zinc early in life as co-treatment against infections (7, 8). It has been reported that zinc has a beneficial role in children and low birth weight infants with bacterial infections (9, 10). Mortality has been reported to be significantly reduced in infants with zinc supplementation in a previous study (11). In some studies, substantial reduction occurred in inflammatory cytokines and serum calprotectin with zinc supplementation in neonates with sepsis (12-15). On the contrary, some randomized clinical trials have reported no marked impact of zinc supplementation on serum zinc levels, mortality rate, and hospital length of stay in neonatal sepsis (16, 17).

Data regarding the efficacy of zinc supplementation on reducing mortality and improving outcomes in neonatal sepsis are insufficient. Furthermore, previous studies have demonstrated conflicting results in this regard. Therefore, the current study aimed to investigate the effect of zinc supplementation on the treatment of neonatal sepsis.

Methods

Participants

In this randomized controlled clinical trial, 50 neonates

with sepsis admitted to Bandar Abbas Children's Hospital, Iran, from 2018 to 2019 were evaluated. The sample size contained 25 patients in each group with $\alpha = 0.05$ at 95% confidence level with 5% mortality rate in the control group versus 15% in the zinc group and a maximum of 15% difference in mortality rate in the studies by Banupriya et al (12, 17). The inclusion criteria were neonatal sepsis (based on the definition demonstrated in detail in the study design below), age under 28 days, gestational age over 37 weeks, and parents' informed consent to participate in the study. The exclusion criteria were any hypersensitivity to or contraindication of treatment with zinc, hypoxic-ischemic encephalopathy, major congenital anomalies, co-existing surgical abnormalities, intracranial hemorrhage, inborn errors of metabolism, and candida infection.

Study Design

Sepsis was diagnosed according to the following criteria (18):

- The presence of at least two clinical signs, including generalized jaundice or pallor; cardiovascular manifestations (bradycardia or tachycardia, poor perfusion, or shock); hypo- or hyperthermia; respiratory manifestations (intercostal retraction, apnea, grunting, or cyanosis); neurological manifestations (lethargy, irritability, hypotonia, or seizure); gastrointestinal manifestations (hepatosplenomegaly or abdominal distension).
- C-reactive protein (CRP) > 5 mg/dL
- At least one more positive laboratory finding, including white blood cell count < 5000/μL or > 25 000/μL at birth, or > 30 000/μL 12-24 postnatal, or > 21 000 on the second day or thereafter; absolute neutrophil count < 1800/μL; and immature/total neutrophil ratio < 0.16 on the first day or < 0.13 on the second day or thereafter.

Age, sex, birth weight, and gestational age were recorded for each participant. Patients were consecutively randomized into two groups using a randomization table provided by the Random Allocation Software. Parents' written informed consent was obtained after providing them with information about the study objectives and design. Venous blood samples were taken from all patients on the first day of admission and complete blood count (CBC) with differential, CRP, and platelets were measured. Blood culture was also performed on the samples. Blood sampling was done again after 48 hours based on the patients' condition. In addition to antibiotics, neonates in the zinc group received 1 mg/kg zinc gluconate (ALHAVI pharmaceuticals, Iran, every milliliter of the syrup contains 1 mg zinc) twice a day for 7 days, starting within 24 hours of admission, orally or via the nasogastric or orogastric tube (based on the patient's condition), while the control group only received antibiotics. Then, all patients were visited and examined daily, and their symptoms were recorded. Any change in the antibiotic regimen was also recorded.

Data Analysis

Mean and standard deviation were used to describe continuous variables, and frequency and percentage were utilized to describe categorical variables. To compare categorical data between groups, the chi-squared and the Fisher's exact tests were employed, while the independent *t* test or the Mann-Whitney test was run for continuous variables. To compare CRP values at the beginning and the end of the treatment in each group, the paired t-test or the Wilcoxon test were employed. *P* values less than 0.05 were regarded as statistically significant. The Statistical Package for the Social Sciences (SPSS) software was used to analyze the data (version 26.0, Armonk, NY: IBM Corp., USA).

Results

The mean age of the participants was 14.15 ± 8.79 days (range: 0.5-27), of which 39 (78%) were male and 11 (22%) were female. Age, birth weight, and gestational age were comparable between groups (Table 1). Further, 72% (18.25) of patients in the zinc group and 84% (21.25) in the control group were males ($P = 0.306$).

A comparison of baseline clinical findings in Table 2 revealed no significant difference between groups with respect to fever, pallor, jaundice, respiratory and gastrointestinal manifestations, neutrophil count, and platelet count ($P > 0.05$). However, the frequency of cardiovascular manifestations was significantly higher in the zinc group ($P = 0.007$). As observed in Table 2, among the neurological manifestations, lethargy and poor feeding were significantly higher in the control group, while hypotonia and seizure were higher in the zinc group ($P = 0.008$).

According to Table 3, the time to improvement of laboratory findings was significantly lower in the zing

Table 1. Comparison of Baseline Demographic and Anthropometric Variables

Variable	Zinc (n = 25) Mean ± SD	Control (n = 25) Mean ± SD	Total (N = 50) Mean ± SD	*P* Value[a]
Age (days)	13.02 ± 9.66	15.28 ± 7.87	14.15 ± 8.79	0.346[b]
Gestational age (weeks)	38.24 ± 0.97	38.20 ± 1.04	38.22 ± 0.99	0.815
Birth weight (g)	3360 ± 368.78	3280 ± 378.42	3320 ± 371.98	0.455

Note. N: Number; SD: Standard deviation.
[a] Analyzed by Mann Whitney test. [b] Analyzed by independent *t* test.

Table 2. Comparison of Baseline Clinical Findings Between Cases and Controls

Variable		Zinc (n = 25) No. (%)	Control (n = 25) No. (%)	Total (N = 50) No. (%)	*P* Value[a]
Fever		2 (8)	7 (28)	9 (18)	0.138
Pallor		2 (8)	2 (8)	4 (8)	1.000
Jaundice		6 (24)	1 (4)	7 (14)	0.098
Respiratory manifestations	Distress	16 (64)	19 (76)	35 (70)	0.562
	Tachypnea	6 (24)	3 (12)	9 (18)	
	Cyanosis	9 (36)	2 (8)	11 (22)	
Cardiovascular manifestations	Bradycardia	2 (8)	0 (0)	2 (4)	0.007
	Tachycardia	1 (4)	0 (0)	1 (2)	
Neurological manifestations	Lethargy	5 (20)	10 (40)	15 (30)	0.008
	Poor feeding	0 (0)	3 (12)	3 (6)	
	Hypotonia	3 (12)	0 (0)	3 (6)	
	Seizure	4 (16)	0 (0)	4 (8)	
Gastrointestinal manifestations	Vomiting	3 (12)	0 (0)	3 (6)	0.235
Neutrophil count	Neutropenia	0 (0)	2 (8)	2 (4)	1.000
	Normal	25 (100)	24 (96)	49 (98)	
Platelet count	Thrombocytopenia	0 (0)	2 (8)	2 (4)	0.235
	Normal	25 (100)	22 (88)	47 (94)	
	Thrombocytosis	0 (0)	1 (4)	1 (2)	

Note. N: number.
[a] Analyzed by Fisher's exact test.

Table 3. Comparison of Hospital Length of Stay and Time to Improvement of Clinical and Laboratory Findings

Variable	Zinc (N = 25) Mean ± SD	Control (N = 25) Mean ± SD	Total Mean ± SD	*P* Value[a]
Time to improvement of clinical findings (days)	4 ± 2.14	3.92 ± 2.84	3.96 ± 2.49	0.792
Time to improvement of laboratory findings (days)	6.56 ± 2.95	8.36 ± 3.34	7.46 ± 3.25	0.022
Hospital length of stay (days)	10.76 ± 4.07	12.40 ± 4.64	11.58 ± 4.39	0.237

Note. N: Number; SD: Standard deviation.
[a] Analyzed by Mann-Whitney test.

group ($P = 0.022$). Conversely, time to improvement of clinical findings was slightly higher in the zinc group ($P = 0.792$), and hospital length of stay was higher in the control group ($P = 0.237$); nevertheless, the difference between groups was not statistically significant.

The mean baseline CRP was 25 ± 16.61 mg/L, and the mean final CRP was 4.36 ± 2.12 mg/L. Moreover, baseline CRP did not differ between groups ($P = 0.236$), while final CRP values reduced significantly in both groups compared to baseline values ($P < 0.001$). However, final CRP values were significantly lower in the zinc group ($P = 0.015$) compared to the control group as illustrated in Table 4.

Change in antibiotic regimen was observed in eight patients (32%) in the zinc group and 12 (48%) in the control group; nonetheless, no statistically significant difference was found ($P = 0.248$). No mortality was reported in either of the groups.

Table 4. Comparison of Baseline and Final CRP

Variable	Zinc (N = 25) Mean ± SD	Control (N = 25) Mean ± SD	Total Mean ± SD	*P* Value[a]
Baseline CRP (mg/L)	21 ± 12.33	29 ± 19.45	25 ± 16.61	0.236
Final CRP (mg/L)	3.60 ± 1.87	5.12 ± 2.11	4.36 ± 2.12	0.015
P Value[b]	< 0.001	< 0.001		

Note. N: Number; SD: Standard deviation; CRP: C-reactive protein.
[a] Analyzed by Mann-Whitney test. [b] Analyzed by Wilcoxon test.

Discussion

Sepsis is considered one of the leading causes of mortality and morbidity in neonates (19). Zinc deficiency can lead to immune dysregulation, cognitive and motor impairment, vulnerability to infections, and growth retardation (20). The role of zinc in improving survival rate and reducing inflammation and organ damage has been confirmed in animal models (21, 22). In addition, previous studies have demonstrated that zinc supplementation is beneficial in preterm neonates with extremely low birth weight, and it can reduce mortality in this group of patients (9, 11).

In a double-blinded randomized placebo-controlled trial in Nepal, zinc (1 mg/kg/d) was dissolved in breast

milk. No significant reduction was observed in the hospital length of stay, requirement of higher lines of antibiotic therapy, and mortality rates (23). Although the present study administered a higher daily dose of zinc (2 mg/kg/d), the results were consistent with the findings of this study. The present study found that zinc has no significant effect on hospital length of stay and the need to change antibiotic regimens. Further, no deaths were reported in either group of the current study; thus, the mortality rate could not be compared between groups. Banupriya et al used even higher doses of zinc (6 mg/kg/d) but still found no significant difference in mortality between cases and controls (12).

El Frargy and Soliman found no significant difference in CRP between the zinc and control groups before starting zinc, while CRP was significantly lower in the zinc group after 5, 10, and 15 days. CRP decreased significantly in both groups by the end of the study with a higher decrease in the zinc group compared to the control group (24). Rashidi et al also showed that the decrease in CRP towards the normal range occurs more rapidly in the septic group compared to those who only received antibiotics (25). High-sensitivity CRP was measured in both studies, which was different from what was done in the current study; however, the present study's findings were similar. In this study, baseline CRP did not differ between groups, final CRP values reduced significantly in both groups compared to baseline values, and final CRP values were significantly lower in the zinc group compared to the control group.

The present study was not without limitations. Firstly, this study was single-centered, and the sample size was relatively small. The probability of overestimating the treatment effects is higher in smaller trials. On the other hand, there were many exclusion criteria which led to even fewer eligible participants. Finally, treatment with zinc was difficult in critically ill neonates or those in septic shock.

Conclusion

According to the findings of the present study, zinc supplementation does not affect the duration of hospital stay in neonatal sepsis; however, it can reduce the time to the improvement of laboratory findings, especially CRP in these patients. The findings need to be confirmed by future studies. Accordingly, further studies with larger sample sizes are required to evaluate the efficacy of different doses and treatment durations of zinc supplementation on neonatal sepsis.

Acknowledgments

We are sincerely thankful to our counselors in the Clinical Research Development Center of Children's Hospital, Bandar Abbas, Iran.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's Contribution

SY designed the study and was a major contributor in writing the manuscript. RG was consulted for the design of the study and the expected outcomes. SZ determined the sample size, analyzed the acquired data, and interpreted the results. SHS wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that they have no competing interests.

Ethics Approval

The study received ethics approval from the Ethics Committee of Hormozgan University of Medical Sciences under the ethics code: HUMS.REC.1397.301 and it complies with the statements of the Declaration of Helsinki. It has also been retrospectively registered at the Iranian Registry of Clinical Trials (IRCT) under IRCT20200810048347N1 available at http://irct.ir/trial/50191. Written informed consent was obtained from the parents/guardians of each patient.

Funding/Support

Hormozgan University of Medical Sciences funded the current study.

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