RESEARCH PAPER

Oral Zinc Supplementation for Reducing Mortality in Probable Neonatal Sepsis: *A Double Blind Randomized Placebo Controlled Trial*

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Objective: To study the role of Zinc in the treatment of neonatal sepsis.

Design: Double blind, randomized, placebo controlled trial.

Setting: Tertiary Care Hospital.

Participants: 614 neonates with probable neonatal sepsis.

Intervention: The drug group (*n*=307) received 1mg/kg/day of elemental zinc, and placebo group (*n*=307) received the placebo, in addition to antibiotic therapy and supportive care, till the final outcome (discharge/death).

Outcome Measures: Decrease in mortality rates (primary outcome), duration of hospital stay and need of higher lines of antibiotic therapy (secondary outcomes) were tested.

Results: Baseline characteristics of the two groups were similar. No statistically significant differences between drug and placebo group were found in mortality rate (9.77% *vs 7.81 %; P*=0.393), mean duration of hospital stay (142.85±69.41 hrs, *vs*. 147.99±73.13 hrs; *P*=0.841), and requirement of higher lines of antibiotic therapy (13.35% *vs* 12.05%, *P*=0.628) after supplementation.

Conclusions: This study does not report decrease in mortality rates, duration of hospital stay and requirement of higher lines of antibiotic therapy following zinc supplementation in neonatal sepsis.

Key words: Mortality, Newborn, Outcome, Sepsis, Zinc.

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Despite advances in neonatal care, the mortality and morbidity from neonatal sepsis still remains high. The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia [1,2]. In Nepal, out of the total infant mortality rate of 46/1000 live births, more than two-third, i.e. 33/1000 live births is contributed by neonatal mortality [3]. A similar situation exists in India and other developing countries in South-East Asia [4].

Multiple factors contribute to the increased susceptibility of neonates to infection. These include developmental, quantitative and qualitative neutrophil defects, decreased bone marrow neutrophil pool, and quantitative and qualitative deficiencies in immunoglobulins [5]. Zinc is known to play a central role in the immune system.It is crucial for normal development and function of cells mediating innate immunity, neutrophils, macrophages and natural killer cells. Phagocytosis, intracellular killing, cytokine production, and T and B cell function are all affected by zinc deficiency [6,7]. Decreased rates of infection have been observed following zinc supplementation in several population-based studies of different diseases, notably diarrhea, pneumonia and malaria [8].

Earlier studies of zinc supplementation in neonates have shown significantly reduced mortality in small for gestational age (SGA) infants [9], increased growth among low birth weight (LBW) [10,11] and very low birth weight (VLBW) infants [12]. However, there have been no published studies of zinc supplementation in neonates with sepsis. This study was done to evaluate whether therapy with zinc in neonates with sepsis would decrease mortality, lead to earlier discharge from hospital, and decrease the requirement of higher lines of antibiotic therapy.

METHODS

This study was conducted in the Pediatric wards of BP Koirala Institute of Health Sciences (BPKIHS), a level III tertiary care hospital in the Eastern region of Nepal, between May 2010 and January 2011. A sample size of 614 was calculated to be sufficient to detect 50% difference between study and control groups with 80% power and alpha of 0.05.

Ethical clearance was obtained from the Institutional Ethical Review Board, BPKIHS. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2008. Written informed consent was taken from the parents of all neonates enrolled in the study.

Intramural and extramural neonates of >32 weeks presenting to BPKIHS with diagnosis of 'Probable Neonatal Sepsis', during the study period, whose parents consented to be a part of the study were included. Probable neonatal sepsis was defined as per the guidelines [1] formulated by the National Neonatology Forum of India based on the duly approved tenth revision of the International Classification of Diseases (ICD) [13] by the WHO. In an infant having a clinical picture suggestive of sepsis, the presence of any one of the following criteria was considered enough to suspect the diagnosis of bacterial infection [1,13]:

(*a*) Existence of any of the predisposing factors like premature rupture of membranes (PROM), foul smelling liquor, amnionitis/funisitis, gastric polymorphs >5/HPF.

(*b*) Positive 'sepsis screen' i.e. presence of at least two of the following five parameters, namely, total leucocyte count <5000/mm^3, low absolute neutrophil count (as per standard charts), bands to total neutrophil ratio (IT ratio) of >0.2, C-reactive protein>1mg/dL, micro ESR >15mm in first hour on any day of life/age in days+3.

(*c*) Radiological evidence of pneumonia

In case of parental refusal to consent, neonates <32 weeks, neonates with severe birth asphyxia (5 min Apgar score <5), congenital malformations and necrotizing enterocolitis, the neonates were excluded from the study. The primary outcome measure was mortality and the secondary outcomes were duration of hospital stay, and requirement of higher lines of antibiotic therapy.

Randomization and blinding: The neonates who enrolled into the study were randomized in two groups, the drug and placebo group. The sequence used to enroll the neonates in either group was generated by using restricted randomization by using the permuted block design of 1:1 to ensure an equal sample size in either group. The person who generated the sequence of drugs was not involved in monitoring the study. Patients were allocated a specific numbered strip of either zinc or placebo tablets, without revealing its identity. The tablets were identical in appearance, consistency and taste. The sequence of code numbers were kept in a sealed envelope which was opened by a nursing officer, not a part of the study, who identified the groups after the completion of the study. Stratification of the study population was done on the basis of onset of neonatal sepsis. All study participants and personnel including care providers, evaluators and monitors were blinded to treatment assignment for the entire duration of the study to avoid any kind of bias. Neither the study participants nor the person distributing the medication were able to identify the drug/placebo during the course of the study. Patient blinding was evaluated by asking questions to the parents to indicate which type of treatment they believed their baby had received, but no one was able to identify the drug/placebo. Similarly, study personnel were asked questions as to which formulation they thought they were providing to the participants, but none could identify the drug/placebo correctly.

Intervention: A detailed history reviewing the antenatal, natal and postnatal factors was taken and tabulated. Gestational age of the neonates was estimated by using the Modified Ballard scoring system. Weight was measured to 5 g with an electronic scale (SECA Corporation, Columbia, MD). Baseline investigations like sepsis screen, appropriate cultures, chest radiographs and lumbar punctures were done as needed. In addition to antibiotics (Cefotaxime and Gentamicin, first line, as per hospital protocol) and standard supportive care, the neonates in the drug group received zinc at 1mg/kg/day dissolved in expressed breastmilk (formulation: Zinc Sulphate Dispersible tablets of 10 mg) either orally or via a nasogastric tube in neonates kept NPO, while the neonates in the placebo group received a safe placebo. In both groups, the formulation was given till the final outcome (discharge/death). During the course of therapy, the neonates were evaluated daily and in case the need to revert to higher lines of antibiotic therapy (2nd line, Ceftazidime and Amikacin; 3rd line, Vancomycin and Meropenem; as per hospital protocol), the decision to do so was taken by the treating pediatricians.

Statistical analysis: The data obtained was entered into Microsoft Excel. All analysis was carried out using the statistical software SPSS (Version 16; SPSS Inc., Chicago, IL). Pearson Chi-square test and Fisher exact test were used to test for statistical significance between the parameters and clinical criteria. Odds ratio was used for comparison of case and control. Two sided significance tests were used throughout and *P* value <0.05 was considered to be statistically significant.

RESULTS

A total of 1540 neonates with probable neonatal sepsis were admitted during the study period, of which 840 were

excluded. Of the remaining 700 neonates, 86 were lost to follow-up and a total of 614 neonates with probable neonatal sepsis were analyzed in this trial. Of these 614 neonates, half received zinc and the other half received a placebo along with standard antibiotic therapy and supportive care (***Fig* 1**). No side-effects were noted in either group.

The baseline characteristics of both the groups were similar (***Table* I**). The neonates enrolled in the two groups had similar risk factors for neonatal sepsis. Culture/ sensitivity and sepsis screen outcomes were also similar in the two groups.

When the final outcome in the two groups was compared (***Table* II**), we found that 30 neonates in the drug group and 24 in the placebo group expired (P=0.393). Further, 41 neonates in the drug group as compared to 37 in the placebo group required higher lines of antibiotic therapy (P=0.628). The mean duration of hospital stay, 142.85 ± 69.41 hours in the drug group as compared to 147.99 ± 73.13 hours in the placebo group was also not statistically significant (P = 0.841).

TABLE I BASELINE CHARACTERISTICS IN THE TWO GROUPS

Characteristics	*Drug Group (n=307)*	*Placebo Group (n=307)*
Male	193	200
Gestational age (wk)*	38.05 ± 2.11	38.11 ± 2.11
Apgar@1 min*	6.56 ± 1.46	6.76 ± 1.32
Apgar@5 min*	7.80 ± 0.98	7.93 ± 1.01
Apgar@10 min*	8.73 ± 0.85	8.83 ± 0.81
Early onset sepsis	256	244
Late onset sepsis	51	63
Intramural patients	166	141
Cesarean delivery	65	68
Vaginal delivery	232	230
Vehicle delivery	10	9
Birth weight (g)*	2461.7 ± 640.16	2652.9 ± 652.72

** Values in mean (SD)*

TABLE II COMPARISON BETWEEN DRUG AND PLACEBO GROUPS BASED ON OUTCOME MEASURES

Measure	*Drug Group (n=307) No. (%)*	*Placebo Group (n=307) No. (%)*
Mortality	30 (9.77)	24 (7.81)
Use of 2/3 line antibiotics	41 (13.35)	37 (12.05)
Hospital stay (hrs)*	142.85 (69.41)	147.99 (73.13)

** Values in mean (SD); P >0.05 for all three measures in column 1.*

DISCUSSION

We aimed to study the role of zinc in the treatment of neonatal sepsis. After the analysis of 614 neonates with probable neonatal sepsis, we did not find any significant difference in terms of decrease in mortality rate, duration of hospital stay and requirement of higher lines of antibiotic therapy between the zinc supplemented and placebo groups.

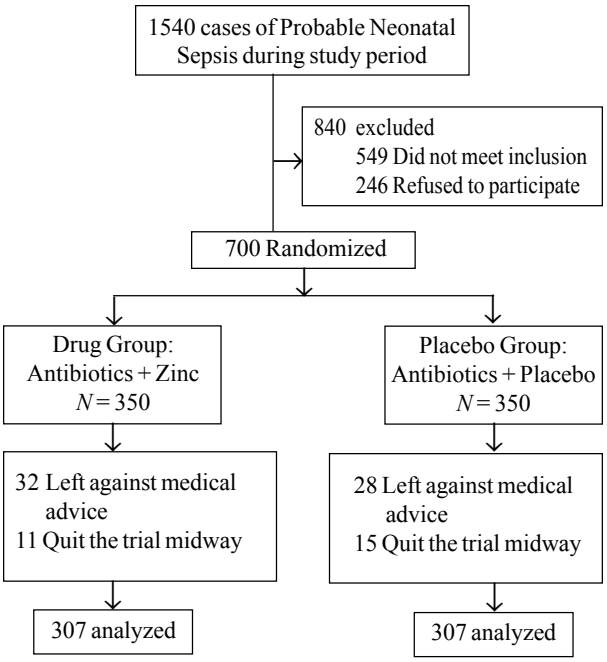

FIG.1 *Study flow-chart.*

We would like to emphasize on the fact that till date, there are no published studies to evaluate the role of zinc in neonatal sepsis. The strengths of this study included its robust randomization, allocation concealment, double-blind design, large sample size with adequate power and homogeneity between the two groups, all of which add to the internal validity of the study. The larger interpretation of this study would need to consider some potential limitations which include this being a single center study and inability to estimate serum zinc levels to rule out any underlying zinc deficiency prior to the onset of therapy. Also, there is paucity of literature regarding the exact role of zinc on the neonatal immune system, a clear understanding of which would aid in designing such studies.

WHAT IS ALREADY KNOWN?

- Zinc supplementation reduces rates of diarrhea and pneumonia in young infants.

WHAT THIS STUDY ADDS?

- This study did not find any benefit of oral zinc supplementation for the treatment of probable neonatal sepsis.

As this study is the first of its kind reported in literature, we were unable to compare the results with those obtained in other similar studies. The lack of significant differences between the zinc supplemented and placebo groups in this study could be explained by the fact that the duration of therapy in the neonates in both arms of the trial was not standardized (the formulation was given till the subject was discharged/expired, and not for a standardized unit of time to both arms). The duration of treatment (mean, 145 hours) may not have been sufficient enough for zinc to significantly augment the immune system. Lack of a significant role of zinc in augmenting the neonatal immune system in particular, and a possible absence of zinc deficiency in the neonates enrolled in our trial could also be other reasons for these findings.

Based on the findings of the present study, it can be concluded that there was no difference in terms of decrease in mortality rate, duration of hospital stay and requirement of higher lines of antibiotic therapy between the zinc supplemented and placebo groups of neonates. Further studies conducted after a thorough understanding of the role of zinc on the neonatal immune system, incorporating paired sample observations of serum zinc levels (before and after supplementation) and subsequently meta-analyses are required to further clarify the role of zinc in the treatment of neonatal sepsis.

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