RESEARCH PAPER

Zinc Supplementation in Preterm Neonates and Neurological Development: *A Randomized Controlled Trial*

NB MATHUR AND DEVENDRA K AGARWAL

From Department of Neonatology, Maulana Azad Medical College, New Delhi, India.
Correspondence to: Dr NB Mathur, Director Professor and Head, Department of Neonatology, Maulana Azad Medical College, New Delhi 110 002, India. drnbmathur@gmail.com
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Objective: To assess the effect of zinc supplementation on neuro-development and growth of preterm neonates.

Setting: Referral neonatal unit of a teaching hospital**.**

Design: Open-labeled Randomized controlled trial.

Participants: 100 preterm neonates.

Intervention: Participants randomized to receive oral zinc (study group) or not (controls).

Main Outcome Measures: *Primary*: Neuro-development status at 40 weeks post conceptional age and at 3 month corrected age using Amiel-Tison neurologic assessment. *Secondary*: anthropometry and serum alkaline phosphatase at 3 months corrected age.

Results: At 40 weeks post-conceptional age, greater number of zinc supplemented infants demonstrated alertness and attention pattern normal for their age *(P*=0.02). Higher number of controls showed signs of hyper-excitability at 40 week post-conceptional age *(P*=0.001) and 3 months corrected age *(P*=0.003). At 3 month corrected age, mean serum alkaline phosphatase level was significantly higher in the study group compared to controls.

Conclusion: Zinc supplementation till 3 month corrected age in preterm breastfed infants improves alertness and attention pattern; and decreases signs of hyperexcitability, and proportion with abnormal reflexes.

Keywords*: Micronutrient, Neurodevelopment, Outcome, Prematurity.*

Zinc may be essential for brain function as well as for growth in the fetus and child [1]. Zinc deficiency may be particularly relevant to early development, growth and function of many organ systems, including the neurologic system [2,3]. Studies have shown reduced levels of zinc in low birth weight infants [4,5], that may account for increased morbidity and growth failure in such neonates. With a small liver, very limited hepatic stores of zinc [6] and increased requirements for catch-up growth, they are at risk for zinc deficiency. There is a paucity of studies evaluating effect of zinc on neuro-development in preterm neonates. The present study evaluated the effect of zinc supplementation on neurological development, anthropometry and serum alkaline phosphatase at 3 month corrected age in preterm infants.

METHODS

This randomized controlled trial was conducted in the referral neonatal unit of a teaching hospital providing care to neonates born in community hospitals or at home. The participants were 100 consecutively admitted preterm neonates <7 days old at admission. The predefined sample size was based on convenience. Neonates with major congenital malformations and those not receiving anything orally for 7 days were excluded. Eligible neonates admitted during the study period (March 2011 through December 2011) were randomized either to receive zinc gluconate (2 mg/kg/day of elemental zinc) [7] or no zinc using website generated random allocation sequence. Randomization sequence was concealed using sealed opaque envelopes. Written informed consent was obtained from the parents. The study was approved by the institutional ethical committee.

Zinc was given once daily till 3 months of corrected age in the study group. Syrup zinc was procured from hospital supply and administered by nursing staff till discharge. After discharge from hospital, the mother administered the supplements. Before discharge, the mother was trained to administer the supplements and advised to give a repeat dose if the baby vomited the supplement within 30 minutes of administration. Infants in both groups were given 4 mL/kg/day of an oral calcium and vitamin D preparation (elemental Calcium 125 mg, Phosphorus 55 mg, Vitamin D3 125 IU per 5 mL), 1 mL/day of multivitamin drops (containing: Vitamin B1 2 mg, Niacinamide 10 mg, D-panthenol 3 mg, Tocopherol acetate 1.5 mg, Vitamin B6 1 mg, Biotin 20 mcg, Vitamin C 40 mg, Vit A 1000 IU, Vit D2 400 IU), and vitamin E

drops containing Tocopherol acetate 5 mg/kg body weight daily. Iron drops 2 mg/kg body weight daily was started at 4 weeks of postnatal age [7]. Weight, length and head circumference were measured daily till discharge, after which infants were followed up weekly till 40 weeks post-conceptional age, and then monthly till 3 months of corrected age. Nude weight was recorded using an electronic weighing scale with an accuracy of 5 g. Length was measured from crown to heel in supine position, using an infantometer, to the nearest of 1 mm. The occipito-frontal circumference was recorded using non-stretchable measuring tape with an accuracy of 1 mm using cross-tape technique.

At each visit, history related to pattern of feeding, diarrhea, respiratory illness, fever, lethargy, vomiting or any other illness in the intervening period was recorded. Adverse effects of oral zinc supplement (vomiting following administration) were enquired at each visit. All mothers were counseled at each visit about care of infants particularly maintaining temperature, breastfeeding, nutritional supplements, prevention of infections, immunization and date of next follow up. All infants were exclusively breastfed during the entire study period. Compliance to zinc administration was monitored using a compliance sheet given to mother at discharge. Compliance was checked at each visit by entries in the sheet, and also by measuring residual volume of drug in the bottle.

Neuro-development status was evaluated at 40 weeks post conceptional age and at 3 months corrected age using Amiel-Tison method [8,9] by a single observer. The examiner circled a score of 0,1 or 2, according to the information given in the technical description of this method for each maneuver. The examiner was not blinded. A score of 0 indicates a typical result for that age, within the normal range. A score of 1 indicated a moderately abnormal result for that age. A score of 2 indicated a definitely abnormal result. Serum alkaline phosphatase was estimated by Olympus auto analyzer using spectrophotometric method at enrolment and at 3 months corrected age.

Cranial ultrasound was done in all neonates <35 weeks at enrolment and on follow-up. Brainstem evoked auditory response (BERA) was performed in all infants at 40 weeks post-conceptional age and at 3 months corrected age.

The data were analyzed using SPSS version 12. Continuous variables were tested using student t-test and for categorical variables, Pearson Chi-square test was applied. For variables with non- Gaussian distribution, Mann-Whitney test was applied. Wilcoxan signed rank test was used to compare pre- and post- intervention variables within the same group. A *per protocol* analysis was done.

RESULTS

During the study period, 50 neonates were randomized in each of zinc and control arm. The study was completed by 37 neonates in zinc group and 35 neonates in control group. Overall loss to follow up at 3 month corrected age was comparable in both zinc (13%) and control group (15%) (***Fig.* 1**). Baseline demographic characteristics were comparable in both groups (***Table* I**).

***Table* II** shows findings of Amiel-Tison neurologic assessment in study population. The scores for all parameters were similar in both the groups at enrolment. At 40 weeks post conceptional age, none of the infants in Zinc group and 13% in control group showed moderate deficit in attention span ($P = 0.02$). At 3 month corrected age, most infants had normal alertness and attention in both groups, with moderate deficit in 3% in zinc group and 6% in controls.

Higher number of neonates in control group had signs of hyperexcitability compatible with normal life and brisk

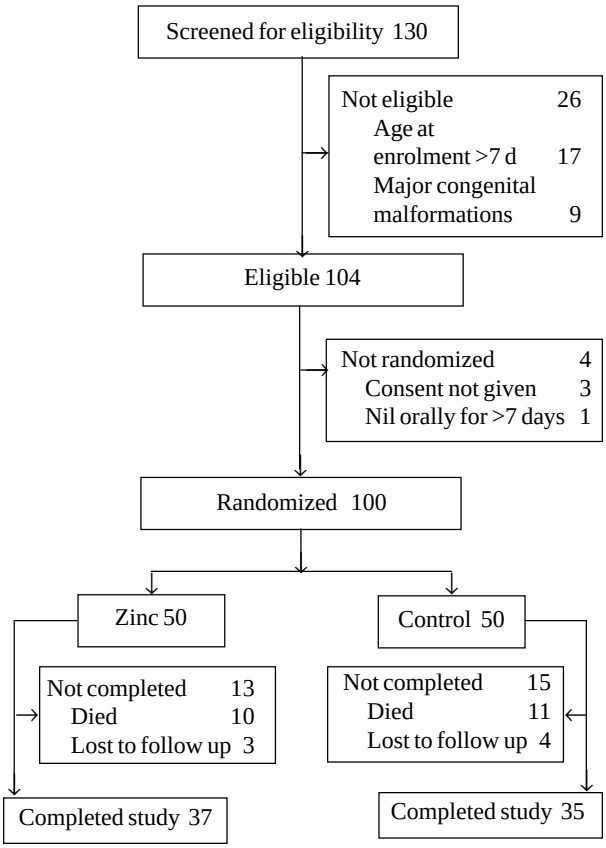

FIG. 1 *Flow of the Participants in the Study.*

TABLE I BASELINE CHARACTERISTICS AT ADMISSION IN THE STUDY POPULATION

Variable	*Zinc Group N=50*	*Control Group N=50*
Gestational age (wks)[#]	33.4 (2.2)	33.4 (2.3)
Maternal age (y)[#]	24 (3)	24 (8)
Age at enrollment (d)[#]	2.6 (1.5)	2.9 (1.7)
Admission weight (g)[#]	1603.7 (452)	1630.8 (479)
Male gender	33 (66)	32 (64)
Small for gestation (SGA)	33 (66)	35 (70)
Intra ventricular hemorrhage	3	6
Hypoglycemia	2 (4)	1(2)
Apnea	12 (24)	14 (28)
Shock	6 (12)	5 (10)
Pneumonia	14 (28)	17 (34)
Hyperbilirubinemia[$]	12 (24)	15 (30)

[#]Values in mean (SD); all other in no.(%); [$]requiring phototherapy.

bicipital reflex response at 40 weeks post conceptional age and at 3 month corrected age. Brisk patellar reflex at 40 weeks and 3 months was also more frequently seen in control group.

There was no difference in both groups at 40 weeks post conceptional age and 3 month corrected age with respect to visual and ocular signs, hearing abnormality, muscle tone, motor activity, involuntary movements, dystonia, cutaneous reflex, primitive reflex and asymmetric tonic neck reflex.

Body weight, length and head circumference were comparable in both the groups 3 month corrected age. (***Web Table* I**). The mean (SD) serum alkaline phosphatase levels were significantly elevated at 3 month corrected age in zinc group [298 (54) *vs.* 272 (55) IU/L, *P*=0.046] (***Fig.* 2**).

During the post-discharge follow up till 3 month corrected age, infants in zinc group had fewer re hospitalization episodes (8 *vs.* 19); episodes of respiratory infections were 2 *vs.* 6, diarrhea 3 *vs* 8 and sepsis 3 *vs.* 5 (OR 0.23; CI 0.08-0.64; *P*=0.05). Post-discharge mortality was 4 in control group (9.3%) compared to 1 in zinc group (2.4%) (OR 0.21). The four deaths in control group were due to respiratory infection, diarrhea, septicemia and unidentified cause in one neonate each. The death in zinc group was due to sudden infant death syndrome.

TABLE II AMIEL-TISON NEUROLOGIC ASSESSMENT IN ZINC-SUPPLEMENTED AND CONTROL INFANTS

Timing	*Score*						*P value*
	Zinc group			*Control group*			
Score	0	1	2	0	1	2	
Alertness and attention							
At enrolment (*n*=100)	37	12	1	31	17	2	0.38
At 40 weeks (*n*=77)	38	0	0	34	5	0	0.02
At 3 months (*n*=72)	36	1	0	33	2	0	0.54
Hyper-excitability							
At enrolment (*n*=100)	46	4	0	47	3	0	0.33
At 40 weeks (*n*=77)	37	1	0	30	9	0	0.001
At 3 months (*n*=72)	36	1	0	25	10	0	0.003
Bicipital reflex							
At enrolment (*n*=100)	4	11	35	12	9	29	0.08
At 40 weeks (*n*=77)	27	10	1	14	21	4	0.01
At 3 months (*n*=72)	34	3	0	22	10	3	0.01
Patellar reflex (knee jerk)							
At enrolment (*n*=100)	4	12	34	9	11	30	0.31
At 40 weeks (*n*=77)	27	11	0	14	21	4	0.002
At 3 months (*n*=72)	33	4	0	20	12	3	0.006

Alertness and attention: Moderate deficit (1), Severe deficit (2); Hyper-excitability: No signs (0), Signs compatible with normal life (1), Uncontrollable(2); Bicipital reflex: Normal (0), Very brisk (1), Clonus (2), Absent (2); Patellar reflex (knee jerk): Normal (0), Very brisk (1), Clonus (2), Absent (2).

WHAT IS ALREADY KNOWN?

- Zinc has a role in cellular growth and production of enzymes necessary for the synthesis of RNA and DNA.

WHAT THIS STUDY ADDS?

- Zinc supplementation till 3 month corrected age in preterm breastfed infants improves alertness and attention pattern and decreases signs of hyperexcitability and abnormal reflex.

No adverse event of zinc supplementation was observed.

DISCUSSION

In the present study, higher number of zinc supplemented infants demonstrated alertness and attention pattern normal for age compared to non-supplemented infants at 40 weeks post conceptional age, but not at 3 month corrected age. Infants in non-supplemented group were more likely to show signs of hyper-excitability, including insufficient sleep, excessive crying and frequent startling when assessed at 40 weeks and 3 month corrected age. We found no significant difference in gain in weight, length and head circumference between groups. Serum alkaline phosphatase in the zinc group was significantly higher.

The strength of our study was a robust tool for early evaluation of neurodevelopment status. For assessing the efficacy of neuroprotection, it is important to evaluate occurrence of early transient signs before they disappear due to neuroplasticity. Limitations of the study are the small sample size without *a priori* calculation. We did not use any placebo in control group. The study was not blinded and serum zinc and copper levels were not estimated. The long-term consequences of these findings are also largely unknown.

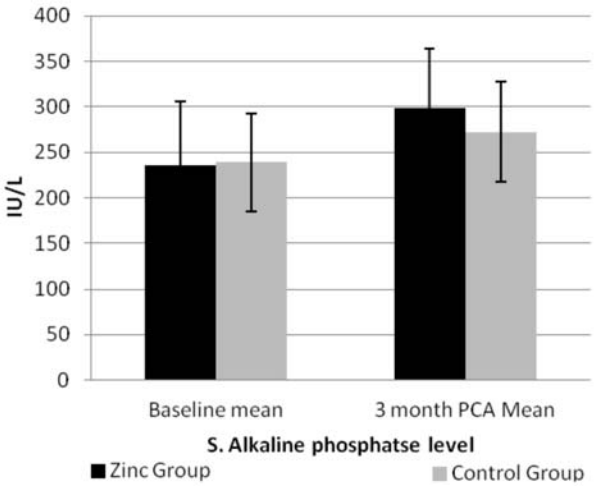

FIG. 2 *Change in serum Alkaline phosphatase through study period.*

Friel, *et al*. [18] assessed 52 preterm infants for neurological development using Griffith's Mental Development Scale, and showed that maximum motor developmental score at 12 months were higher in the zinc-supplemented than the placebo group. However, a trial in low birth weight term infants using Bayley Scale of Infant Development found that zinc supplementation for eight weeks from birth did not improve the mental and psychomotor scores at 6 and 12-months [11]. Preterm infants are likely to have higher zinc deficit and dietary requirements as nearly 60% fetal zinc is acquired during third trimester of pregnancy [12]. Therefore, the response to zinc supplementation may be variable amongst preterm and term SGA low birth weight neonates. However, most of the zinc supplementation trials have been done in heterogeneous population of low birth weight neonates including term AGA, and have not segregated the data for preterm babies [13-18].

To conclude, zinc supplementation till 3 month corrected age in preterm breastfed infants improves alertness and attention pattern, and decreases signs of hyper-excitability and abnormal patellar and bicipital reflex.

Contributors: NB: conceived and designed the study, interpreted the results, finalized the manuscript and shall be guarantor for the paper; DK: collected the data and drafted the manuscript. Both authors approved the final version of manuscript.
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