IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

Open Access

Original Article

Effect of Zinc Supplement Provision on Growth and Neurodevelopmental Parameters in Preterm Neonates

Eko Sulistijono[1], Brigitta I.R.V. Corebima[1]*, Atiek Wulandari[2], Stephanie Kusbianto[2]

1. Neonatology Division, Department of Pediatrics, Faculty of Medicine, Universitas Brawijaya, Saiful Anwar Malang General Hospital, Malang, Indonesia
2. Department of Pediatrics, Faculty of Medicine, Universitas Brawijaya, Saiful Anwar Malang General Hospital, Malang, Indonesia

ABSTRACT

Background: Zinc deficiency often occurs in preterm and low-birth-weight neonates. The present study aimed to assess the effect of zinc supplement provision on increased body weight, body length, and head circumference, as well as neurodevelopmental parameters of preterm neonates.

Methods: A true experimental study with a double-blind, randomized clinical trial (RCT) was conducted involving 30 preterm neonates with 30-34 weeks of gestational age who were assigned to two groups, i.e., zinc supplements and placebo, and followed until the three months of corrected age.

Results: The mean score of zinc level in all subjects was 34.47±12.00 μg/dL. There were significant differences in serum zinc level at birth compared to the corrected age of three months, both in the supplement group and placebo ($P< 0.001$, each). Zinc level showed a significant positive correlation with body weight, length, and head circumference at the corrected age of three months ($r=0.469$, $P=0.009$; $r = 0.428$, $P= 0.018$; $r = 0.549$, $P=0.002$). Zinc levels had a significant, positive correlation with BINS at the age of 3-4 months ($r = 0.594$; $P=0.001$), IGF-1 ($r = 0.496$; $P< 0.001$), body weight ($r=0.469$; $P=0.009$), length ($r =0.428$; $P=0.018$), and head circumference ($r =0.549$; $P=0.002$) at corrected age of three months.

Conclusion: The provision of zinc supplements could positively affect the growth of preterm neonates in the form of body weight, body length, head circumference, IGF-1, and neurodevelopmental improvements.

Keywords: Anthropometry, IGF-1, Neurodevelopment, Preterm neonate, Zinc

Introduction

Preterm birth is defined as neonates born alive at less than 37 weeks of gestational age. According to World Health Organization (WHO), preterm neonates are classified into: 1) extremely preterm(< 28 weeks), 2) very preterm(28-32 weeks), and 3) moderate to late preterm (32-37 weeks) (1,2). Preterm neonates are at higher risk of various complications related to the immaturity of organs' growth and development (1). Based on WHO reports, about 15 million neonates are born preterm each year, and more than one million of whom die due to prematurity-related complications (3). On the other hand, Indonesia is the fifth country with the highest preterm births (around 675,700 births per year) (4); accordingly, prematurity is one of the major public health problems in Indonesia.

Zinc (Zn) is an essential micronutrient that has been known to play crucial roles in the body. As a trace element, zinc is mostly found in intracellular compartments, such as cytosol, vesicle, organelle, and nucleus (5). Zinc is involved in many cellular metabolism processes related to growth and development (6). It plays a major role in the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) linked to the cell division process. It also works on a molecular basis to regulate the transcription and synthesis of various enzymes linked to the metabolism of carbohydrates, proteins, and lipids (2). Zinc performs a pivotal role

---

* *Corresponding author*: Brigitta I.R.V. Corebima, Neonatology Division, Department of Pediatrics, Faculty of Medicine, Universitas Brawijaya, Saiful Anwar Malang General Hospital, Malang, Indonesia. Tel: +6282140562689; Email: briggita_vebi@ub.ac.id

Please cite this paper as:
Sulistijono E, I.R.V. Corebima B, Wulandari A, Kusbianto S. Effect of Zinc Supplement Provision on Growth and Neurodevelopmental Parameters in Preterm Neonates. Iranian Journal of Neonatology. 2023 April: 14(2). DOI: 10.22038/ijn.2023.64921.2258

in the neurodevelopmental process through several suggested mechanisms(i.e., Zn-dependent enzymes involved in brain growth, a zinc-finger protein involved in the brain structure development and neurotransmission, and zinc-dependent neurotransmitter related to the brain memory function, as well as its role in the production of neurotransmitter) (7).

Zinc is essential in the regulation of several gene expressions.; moreover, it interacts with some of the hormones critical for development; for instance, somatomedin, osteocalcin, testosterone, thyroid hormone, and insulin (8). Several clinical studies concluded that providing zinc supplements to preterm neonates correlates with an increase in body weight and linear growth (9–11). In addition, zinc stimulates growth by raising the concentration of insulin-like growth factor 1 (IGF-1) hormone produced by the liver as a response to growth hormone. The IGF-1 acts as a growth factor for tissues controlling the process of mitosis, differentiation, chemotaxis, and apoptosis. In growth processes, IGFs are local growth factors functioning as independent growth hormone (GH) through paracrine and autocrine mechanisms for triggering the growth of tissues (12).

Zinc deficiency is defined as having serum zinc concentration at birth < 55 μg/dL (< 8.4 mcmol/L). The concentration of zinc measured at the umbilical cord of preterm neonates is higher than that of term neonates. Clinical manifestation appears in conditions with severe zinc deficiency. Several data suggested significant subclinical symptoms in zinc-deficient conditions of preterm neonates, prompting the need for zinc supplementations for all neonates with low serum zinc concentration (hypozincemia) (13,14). To the best of our knowledge, previous studies were conducted in descriptive or case-control, observational manners (2,15,16). The present study aimed to assess the effect of zinc supplement provision on growth parameters, i.e., anthropometric profiles (body weight, body length, and head circumference) and serum IGF-1 level, as well as the neurodevelopmental condition of preterm neonates, i.e., Bayley Infant Neurodevelopmental Screener (BINS) and Indonesian Developmental Pre-Screening Questionnaire (KPSP).

Methods

Ethics and study area

This present study was approved by the Committee of Research Ethics of the Saiful Anwar General Hospital (SAGH) Malang, Indonesia. The study was conducted at the Neonatology Unit, Department of Pediatrics, SAGH. The serum analysis of the collected samples was conducted at the Analysis and Measurement Units, Laboratory of Chemistry, Faculty of Mathematics and Natural Sciences, Brawijaya University, from March 2019-May 2020. Informed consent to participate in this study was obtained from all respective parents.

Study design and sampling procedure

The study design was a true experimental study, employing a double-blinded randomized clinical trial (RCT) with pre-and post-test control group involving 30 preterm neonates. The subjects, attending neonatologists, and laboratory analysts were blinded to the study, whereas the investigators were blinded to the subjects' randomized grouping. The subjects were allocated into two groups, i.e., Group 1, with zinc supplementation, and Group 2, which was given a placebo. The Zinc and placebo packages were known to the pharmacist in charge.

The inclusion criteria entailed preterm neonates with 30-34 weeks of gestational age, born and treated in the Neonatology Unit, SAGH. On the other hand, the neonates with congenital malformations, asphyxia (APGAR score at 5' < 3), congenital infections or sepsis, and already exposed to Zinc supplements were excluded. Data collection was ceased in case of death, transfer to another hospital, or parents' willingness to withdraw from the study. The sample was determined by means of consecutive sampling, i.e., sample and data collection were carried out sequentially; subsequently, conducting intervention and evaluations were taken at birth (baseline) and third months of corrected age.

The sample size was determined by a two-armed numeric comparative analytical test with $\alpha = 0.05$, $Z\alpha = 1.96$, $\beta = 0.2$, and $Z\beta = 0.84$. The difference in serum Zn level was considered significant if x_1-x_2 = 10 and the standard deviation from the previous study were 9.4 (7,17). The minimum number of subjects was estimated at 14 in each group (28 subjects in total).

Zinc supplementation and data collection

The administered dose was 2 mg/kg/day of elemental zinc per oral. Body weights used for measuring the zinc dose were according to their actual body weight. The subjects received zinc syrup once daily, starting from the first day until the corrected age of three months. The placebo used in this study was an orange-sweetened syrup with orange coloring that contained no active substances (Sirplus). Sirplus syrup was given once per day with a 1-2 ml dose from the first day until the corrected age of three months.

Serum zinc levels were measured using the Atomic Absorption Spectrophotometer (AAS) method in μg/dL unit at the Analysis and Measurement Units, Laboratory of Chemistry, Brawijaya University. The diagnosis of zinc deficiency in preterm neonates was established when their serum zinc level was< 55 μg/dL (8.4 μmol/L) with our normal range between 80-110 μg/dL in accordance with the previous study (9). Growth parameter data were anthropometric profiles comprised of body weight, body length, and head circumference. The laboratory parameter of serum IGF-1 level was measured by enzyme-linked immunosorbent assay (ELISA). Neurodevelopmental parameters were assessed using Bayley Infant Neurodevelopmental Screener (BINS) and Indonesian Developmental Pre-Screening Questionnaire (KPSP).

Statistical analysis

All data were analyzed in SPSS software (version 25). Subjects' demographic data are displayed in descriptive data. The normality test with Shapiro-Wilk and the homogeneity test of variance were carried out as prerequisites for the parametric test. Comparative tests between parameters were using Independent T Test if the data were normally distributed or Mann Whitney non-parametric test. A correlative test between parameters was carried out with the Pearson or Kendall tau correlation test. A p-value of less than < 0.05 was considered statistically significant. Potential confounders of gestational age and clinical presentations were controlled by matching subjects using inclusion and exclusion criteria. Other potential confounders of gender and nutritional type were controlled by stratification.

Results

Clinical characteristics of subjects

This study involved 30 subjects of preterm neonates born in the Neonatology Unit, SAGH Malang, from March 2019- May 2020. The subjects were then randomized (double-blinded) and assigned to the treatment group (zinc-supplemented) and the control group (placebo-administered) (n=15 in each group). The subjects' clinical characteristics are summarized in Table 1.

Table 1. Clinical Characteristics of Subjects

Parameter	Zinc Group(n = 15)	Placebo Group(n = 15)	Total(n = 30)	P-value
Sex [n (%)]*				0.713
Male	7 (46.7)	6 (40.0)	13 (43.3%)	
Female	8 (53.3)	9 (60.0)	17 (56.7)	
Gestational age [weeks (mean ± SD)]*	31.87 ± 1.92	31.33 ± 1.63	31.6 ± 1.77	0.212
Mode of delivery [n (%)]				n/a
Pervaginam	0	0	0	
Caesarian section (SC)	15 (100)	15 (100)	15 (100)	
Nutrition type [n (%)]*				0.519
Breastmilk	5 (33.3)	6 (40.0)	11 (36.7)	
Formula milk	0	1 (6,7)	1 (3.3)	
Breastmilk + formula	10 (66.7)	8 (53.3)	18 (60)	

*Test of normality p > 0.05 is considered homogenous. n/a, not applicable

Effect of Serum Zinc Levels towards growth parameters

Figure 1A depicts an increase in body weight for both groups from the first to the fifth month of observation. There were no significant differences between the two groups in terms of average body weight at birth, 1st up until 4th month (P> 0.05). Nonetheless, the Zn group showed a significantly higher average body weight in the 5th month (equal to a corrected age of 3 months) compared to the placebo group (P= 0.010). Figure 1B presents the increase in average body lengths of both groups from the 1st month to 5th month of observation. It was evident that there was no significant difference in regard to the average body length of both groups at birth, 1st month, and 2nd month (P> 0.05). However, the Zn group exhibited a significantly higher body length in the 3rd to 5th month compared to the placebo group. As illustrated in Figure 1C, it was only by the 3rd to 5th month (corrected age of 3 months) that there were significant differences between the zinc group and the placebo group in terms of average head circumference. The two groups had no significant difference in average head circumference at birth, as well as 1st and 2nd month (P>0.05)).

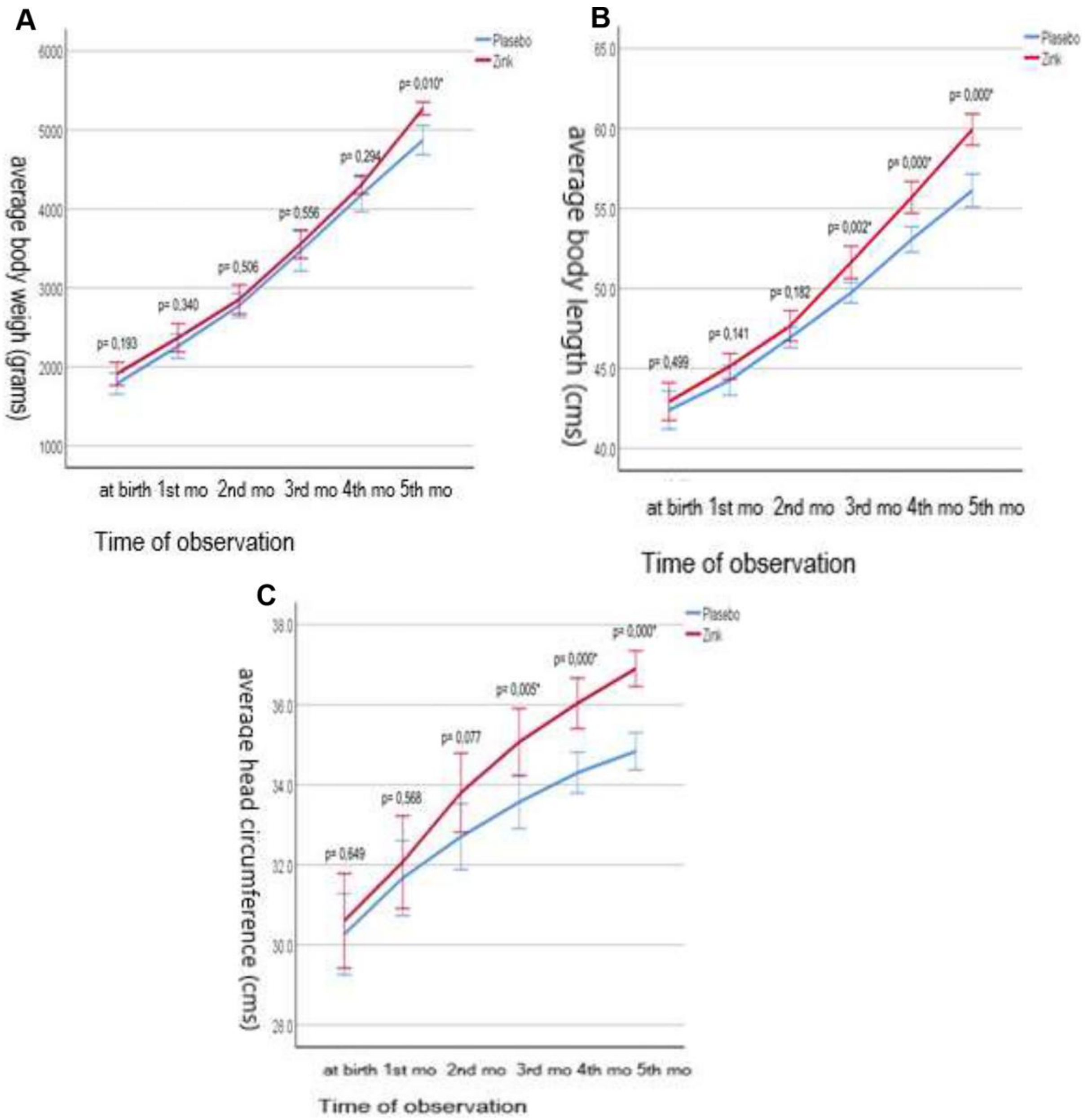

Figure 1. Trends in average growth parameters at each observation time for Zn and Placebo groups
(A) Average body weight; **(B)** average body length; **(C)** average head circumference at each observation time from birth up to 5th months of life, equal to the corrected age of three months old. Comparative analysis using Independent T-test; p < 0.05 indicated a statistically significant difference; error bars: 95% CI

A) Average body weight; (B) average body length; (C) average head circumference at each observation time from birth up to 5th months of life, equal to the corrected age of three months old. Comparative analysis using Independent T-test; p < 0.05 indicated a statistically significant difference; error bars: 95% CI.

The increase in zinc level, body weight, body length, and head circumference, as displayed in Table 2, was defined as the difference between the parameter measurement results of the corrected age of three months and at birth. Based on comparative analysis, the differences in the increase in zinc level, body weight, body length, and head circumference were significantly higher in the zinc group than in the placebo group (P< 0.001; P = 0.001; P< 0.001, and P= 0.001).

Table 2. Comparative Analysis of Serum Zinc Level with the Increase of Body Weight, Length, and Head Circumference

Parameter	Zinc Group(Mean ± SD)	Placebo Group(Mean ± SD)	*p-value*
Δ Zinc Level (μg/dL)	109.07±63.35	30.43±19.66	< 0.001*
Δ Body Weight (gram)	3470.53±204.65	3089.20±352.16	0.001*
Δ Body Length (cm)	18.40±1.67	14.23±1.60	< 0.001*
Δ Head Circumference (cm)	9.76±1.39	7.43±1.84	0.001*

Δ = increase of parameter, measured by the difference between birth and 3-months corrected age. The statistics were analyzed by independent t-test, p-values < 0.05 is considered statistically significant

This study conducted a further analysis of the differences in zinc level, body weight, body length, and head circumference increase between the two groups. The results of comparative tests for these parameters are displayed in Table 3.

Table 3. Correlation Test Results between Zn Level and Growth Parameters in 3-months Corrected Age

Description	Correlation Coefficient (r)	*p-value*
Body Weight	0.469	0.009*
Body Length	0.428	0.018*
Head Circumference	0.549	0.002*

* p < 0.05 means that there is a statistically significant correlation; r = correlation coefficient

Table 3 illustrates the significant, positive correlation of zinc level with body weight, body length, and head circumference at the corrected age of three months. In other words, the higher zinc level resulted in more marked improvements in growth parameters, i.e., body weight, body length, and head circumference. Nevertheless, it should be noted that the correlation strength only showed a medium correlation. It could be inferred that there might be other factors other than serum zinc level correlating with the increase in the body weight, body length, and head circumference of preterm neonates.

Effect of Zinc levels on neurodevelopmental parameters

Table 4 demonstrates the difference in serum zinc levels between the two groups in regards to the Bayley Infant Neurodevelopmental Screener (BINS) score for the age of 3-4 months and Developmental Pre-Screening Questionnaire (KPSP) for the age of three months. The BINS score for the age of 3-4 months showed a median of 11 for the zinc group and 8 for the placebo group. Mann-Whitney test obtained a p-value of < 0.001, indicating a significant difference. N (neurological function) and R (receptive function) parameters displayed significant differences between the two groups (P< 0.05), while the E (expressive function) parameters did not demonstrate any significant difference (P> 0.05). Meanwhile, in the third-month Indonesian Pre-Screening Developmental Questionnaire (KPSP), despite the same median value in both groups, the interquartile range (IQR) was slightly different, whereas the Mann-Whitney test illustrated a significant difference (P = 0.016).

Table 4. Difference between Zinc and Placebo Groups in Neurodevelopmental Parameters

Parameter	Zinc Group(n = 15)	Placebo Group(n = 15)	p
Serum Zinc (μg/dL)			
At birth (mean±SD)	33.7 ± 11.6	35.3 ± 12.7	0.720
At the age of 6 months (median, IQR)#	110 (90 – 210)	38 (24.7 – 46)	< 0.001*
BINS (median, IQR)	11 (10 – 11)	8 (8-10)	< 0.001*
N (Neurological function) (median, IQR)	5 (5 – 5)	4 (3 - 4)	< 0.001*
E (Expressive function) (median, IQR)	2 (2 – 2)	2 (2 - 2)	0.550
R (Receptive function) (median, IQR)	4 (4 – 4)	3 (2 - 4)	0.002*
Third-month KPSP	10 (10 – 10)	10 (9 -10)	0.016*

* p-*value* < 0.05 was considered statistically significant. #actual age of 6 months which was equal to the corrected age of three months in our subjects. BINS: Bayley Infant Neurodevelopment Screener; KPSP: Indonesian Developmental Pre-Screening Questionnaire. IQR: Inter-quartile range; SD: standard deviation

Table 5. Correlation of Serum Zinc Level at Corrected Age of 3 Months with Neurodevelopmental & Growth Parameters BINS and Third-Month KPSP

Parameter		*p-value*	*r*
	Neurodevelopmental Parameters		
BINS	Zinc at the corrected age of three months	0.001	0.594**
N		0.002	0.535**
E		0.613	-0.096
R		0.001	0.582**
Third-Month KPSP		0.028	0.400*
	Growth Parameters		
IGF-1	Zinc at the corrected age of three months	< 0.001*	0.496
Body weight		0.009*	0.469
Body length		0.018*	0.428
Head circumference		0.002*	0.549

BINS: Bayley Infant Neurodevelopment Screener; KPSP: Developmental Pre-Screening Questionnaire; r = correlation coefficient. IGF-1: Insulin-Like Growth Factor 1
*p<0.05;**p<0.01

According to Table 5, there was a significant, positive correlation between zinc level and the BINS score for the age of 3-4 months (P= .001; r=0.594). Moreover, the N and R parameter also showed significant correlations (P=0.002; r = 0.535; and P= 0.001; r = 0.582). A positive correlation value indicated that the higher the zinc level, the more the BINS score achieved at the age of 3-4 months would be. The correlation strength was considered to be relatively strong. Meanwhile, the third-month KPSP parameter with zinc level yielded a value of P= 0.028 and r = 0.400. The positive correlation coefficient suggested that the higher the zinc level, the higher the KPSP score would be. The correlation strength was considered to be quite strong.

Table 5 also illustrates the positive and significant correlation of zinc level with IGF-1, body weight, body length, and head circumference at the corrected age of three months (P< 0.001, r = 0.496; P=0.009, r = 0.469; P= 0.018, r =0.428; P=0.002, r = 0.549). To put it in another way, the higher the zinc level, the higher the average IGF-1, body weight, body length, and head circumference would be. Nonetheless, the correlation strength in this case only showed a medium correlation.

Discussion

The present study was designed in a prospective manner to evaluate the effects of zinc supplementation on the improvement of growth parameters, represented by body weight, length, and head circumference, and neurodevelopmental parameters, comprised of BINS and KPSP, in a temporal view in a group of preterm neonates. The subjects were randomized (double-blinded) and assigned to two treatment groups: zinc provision and placebo. No significant differences were found between the two groups in terms of gender, gestational age, and nutrient intake types. Due to the homogenous nature of both groups, clinical factors such as gender, gestational age, and nutrient intake types could be controlled as potential confounders for this study.

Regarding gender, the majority of subjects (56.7%) were female. A previous study by Terrin et al. (13) mentioned no zinc level differences between male and female preterm neonates. Despite this, a study reported that male gender could be one of the risk factors for hypozincemia in preterm neonates (15). On average, the mean gestational age of all the preterm neonates included in this study was 31.6±1.77 weeks (range: 30-34 weeks). The selection of these gestational ages was based on literature findings suggesting that the mortality rate was higher in neonates born under the gestational age of 28 weeks accounting for 32% of total births while this rate was only 1.3% for those born after the 32-week gestational age (18). In addition, the age selection was justified by a literature finding arguing that preterm neonates are extremely vulnerable to zinc deficiency due to less-than-optimal zinc reserve during the third trimester of pregnancy (16). A previous study mentioned that zinc transport via the placenta occurs in the third trimester of pregnancy, especially at the end of the third trimester (19).

A study by Kaban et al. (15) conducted at several hospitals in Jakarta, Indonesia, pointed out that low zinc levels were found in neonates born preterm (gestational age< 34 weeks) and low birth weight (LBW, < 2000 grams) neonates, in whom growth problems were most commonly identified symptoms. Low levels of zinc in preterm neonates might be caused by the lack of Zn reserves in the body, high Zn requirements after birth to catch up growth, limited capacity to absorb and retain nutrients, and inadequate intakes (16). The clinical features of zinc deficiency were growth problems, dermatitis, irritability, oral candidiasis, diarrhea, poor bone mineralization, motor and cognitive function disorders, increased risk of infections, and growth retardation (13).

Nutrient intake during the early phases of life would influence serum zinc concentration in preterm newborns; therefore, nutrition fulfillment during these periods is pivotal to preventing hypozincemia. A case report discussed transient neonatal zinc deficiency in preterm neonates receiving exclusive breast milk (13). Another study described that zinc content in breast milk varied at a range of 0.7–1.6 mg/L and decreased as time goes. This might lead to inadequate zinc concentration for preterm neonates' needs (2). Preterm neonates who received 180 mL/kg of breast milk would take 0.5–1 mg/kg of zinc per day, whereas those who consumed formula milk would get 1.5–3 mg/L of elemental zinc per day (20). A study by Mahmood et al. (16) reported that zinc level was significantly lower in the preterm neonate group receiving exclusive breast milk than those with formula milk. Other literature also mentioned that the provision of breast milk without zinc supplementation did not adequately meet the zinc needs of preterm neonates (13).

This study found that the average zinc level for all subjects at birth was 34.47±12.00 µg/dL with a minimum value of 14.6 µg/dL and a maximum value of 59 µg/dL. Moreover, 29 (96.7%) subjects have already suffered from zinc deficiency at birth. The diagnosis of zinc deficiency in preterm neonates was established when their serum zinc level is < 55 µg/dL (8.4 µmol/L) since the normal concentration ranges around 80–110 µg/dL, according to a previous study (9). Zinc deposit in the fetus occurs during the third trimester, especially at the end of the third trimester. Some other factors predisposed preterm neonates to zinc deficiency originated from the combination of i) low reserve in the body due to decreased duration of zinc delivery through the placenta, ii) inadequate intake, and iii) elevated endogen loss (21). This condition might explain why zinc reserve in preterm neonates was very low, i.e., the main cause of zinc deficiency in them. Furthermore, preterm neonates are a population with a plethora of needs, trauma, infections, and physiological intestinal insufficiency, with the addition of high antibiotics use, making them very vulnerable to zinc deficiency (9).

A number of clinical tests have reported that there had been an increase in serum zinc levels in preterm neonate groups receiving zinc supplementation throughout the chronological age of three months (12,22). Another study by Díaz-Gómez et al. (23) that provided zinc supplementation up to the corrected age of six months showed significant sALP (skeletal alkaline phosphatase) and serum zinc level at the corrected age of three months while not exhibiting a significant increase in serum zinc level at the corrected age of six months. Several other research reported comparable results but with a duration of zinc supplementation up to the chronological age of four months (7). Zinc supplementation with a longer duration of 6-month chronological age was also revealed to be effective in the elevation of serum zinc levels (9). A similar result was also found in this study. There had been significant increases in serum zinc levels after the provision of zinc supplements up to the corrected age of three months compared to the level at birth.

Based on this study, it was evident that the average body weight was significantly higher in the zinc group than in the placebo at the 5th month of measurement, i.e., corrected age of three months. Moreover, a significantly higher increase in average body length and head circumference was observed in the 3rd month, 4th month, and 5th month of measurement for the zinc group compared to the placebo. Analogous to the result of this study, Díaz-Gómez et al. (23) reported that there was a significant increase in sALP and serum zinc level at the corrected age of three months while not exhibiting the same significant increase in serum zinc level at the corrected age of six months. A comparable result was found in a study by El Farghali et al. (9), who asserted that there were higher increases in body weight, body length, head circumference, and other anthropometric parameters in preterm neonates who received zinc compared to the placebo group during the 3rd and the 6th month of visit.

Other similar results also revealed a significantly higher increase in the average body weight, length, and head circumference in preterm neonates receiving zinc supplementation than in those who received a placebo during measurements on the 2nd and 4th month of chronological age (7). An observational study conducted in the Karyadi Semarang Hospital, Indonesia, in 2012 reported increased body weight, body length, and head circumference in neonates born preterm and small-for-gestational-age who were given zinc supplementation for three months (22). The present study also conducted analyses of the increase or *delta* zinc level, body weight, body length, and head circumference between measurement results at birth and at the corrected age of three months. The delta zinc level, body weight, body length, and head circumference of the zinc-supplemented group were found to be higher than those in the placebo group. In conclusion, the provision of zinc for preterm neonates since their early stages of life positively contributed to their linear growth (23).

Here, zinc supplementation was given from birth to the corrected age of three months. This was based on references from several clinical studies suggesting zinc deficiency in preterm neonates (24). Literature findings also mentioned that zinc concentration in preterm neonates decreased drastically in their first month of life without adequate nutrition or supplementation. As a result, zinc supplementation was essential for preterm neonates (13). Similar to the present findings, a previous study by El Farghali et al. (9) suggested that growth catch-up for preterm neonates who received zinc supplementation started to occur from the 3rd to the 6th month (or equal to the corrected age of three months). Other research results also displayed similar findings, though the zinc supplementation only spanned 2 and 4 months of chronological age (7). Research findings by Ram Kumar and Ramji (11) suggested that zinc supplementation over 60 days for low-birth-weight neonates could significantly increase their linear growth.

This study used a zinc dose of 2 mg/kg/day for preterm neonates throughout the corrected age of three months, as stated in a previous study (7). Several clinical tests using various zinc doses have been conducted and found that a minimum of 1.4-2 mg/kgBW/day of zinc intake was required to achieve optimal growth for preterm neonates (11). The adverse effects of excess zinc dose are rare at a dose of < 25 mg/day. Some reported adverse effects were nausea, vomiting, epigastric pain, lethargy, or symptoms due to disturbances in mineral absorption (25). No subjects in this study experienced any adverse effects related to zinc supplementation. El Sadek et al. (7) also conducted a zinc supplementation study with the same dose at 2 mg/kg/day for four months with no report of adverse effects.

This study pointed to the significant, positive correlation of zinc level with body weight, body length, and head circumference at the corrected age of three months. In other words, the more zinc level, the higher the average body weight, body length, and head circumference would be. Zinc plays a role in growth by elevating the concentration of IGF-1 produced by the liver as a response to growth hormones. Insulin-Like Growth Factor 1 (IGF-1) hormone acts as a growth factor in tissues controlling mitotic, differentiating, chemotactic, and apoptotic processes. In the growth process, IGFs are local growth factors functioning as independent growth hormone (GH) through paracrine and autocrine mechanisms for triggering the growth of tissues. Research shows that zinc supplementation could increase the level of IGF-1 and accelerate the linear growth of pediatric patients (12). This study revealed a positive correlation between IGF-1 level and growth parameters at the corrected age of three months. The IGF-1 level in preterm neonates was found to be correlated with growth parameters in a previous study by de Jong et al. (26), who also observed body weight, body length, and head circumference.

There was a significant difference in the BINS score at the age of 3-4 months between the zinc and the placebo group. Moreover, the third-month KPSP score demonstrated a significant difference between the two groups. A positive correlation value means that the higher the zinc level, the higher the BINS score would be. This finding also applied to the third-month KPSP score, which showed a similar positive correlation. El Sadek et al. (7) performed a randomized controlled study at the Benha University Hospital involving 80 preterm neonates with a gestational age of less than 37 weeks. They were given zinc supplementation at 2 mg/kg/day and observed for four months. Zinc supplementation in preterm neonates improved their growth (body weight and body length), increased their hemoglobin level, and matured their central nervous system. Zinc plays a pivotal role in the neurodevelopmental process since zinc-dependent enzymes are involved in brain growth, zinc-finger protein participates in the brain structure development and neurotransmission, the zinc-dependent neurotransmitter is related to the brain memory function, and zinc also plays a role in the production of neurotransmitter.

Zinc is an essential component of DNA and RNA polymerase required for the synthesis of DNA and RNA and protein structure stabilizer. It also works molecularly by regulating the transcription and synthesis of various enzymes linked to the metabolism of carbohydrates, proteins, and lipids (2). In addition, Zn was also closely associated with important hormones involved in the growth process, such as growth hormone, somatomedin-c, osteocalcin, testosterone, thyroid hormone, insulin, and vitamin D (10). Zinc also takes part in the regulation of the sense of taste and smell, all of which are linked to hunger and food intake (27). These are some of the explanations regarding how zinc correlates with linear growth.

Limitations of the study

This study was a randomized, double-blinded clinical trial (RCT) with pre and post-test control group. Nonetheless, this study had some limitations due to its lack of serum Zn level stratification. This means that the provision of zinc supplementation was not differentiated among subjects with normal, insufficient, or deficient serum zinc levels; therefore, further investigation is encouraged. Factors that might affect zinc level changes, such as nutrition intake, were not included as parameters in this study since the nutrition intake proportions be it breast milk or formula milk were difficult to supervise when the subjects were at home. Moreover, the trust in their compliance with the consumption of supplementation greatly depended on patients' honesty, though controls were implemented in the form of a medication card. Another limitation of this study was the difficulty in obtaining subjects who were able to participate until the end of the study due to the COVID-19 pandemic. Therefore, their full commitment to visiting appointments throughout this study was relatively hard to be achieved.

Conclusion

This present study concluded that there was a significant difference between preterm neonates receiving zinc supplementation and the control group in the average serum zinc level. There were also differences in terms of growth and neurodevelopmental parameters, represented by average body weight, body length, head circumference, serum IGF-1 level, BINS score for the age of 3-4 months, and the third-month KPSP score. The preterm neonates receiving zinc supplementation had significantly higher scores compared to the control group.

Acknowledgments

None.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. IDAI. Consensus on nutritional care for premature infants. Jakarta: Ikatan Dokter Anak Indonesia; 2016.
2. Manish P, Gupta B, Suman B, Poonam P, Pramod S, Manisha G, et al. Role of zinc supplementation in growth and neuro-developmental of premature and small for gestational age (SGA) babies. Natl J Community Med. 2012; 3:736–9.
3. Soon BT. The global action report on preterm birth. Geneva: World Health Organization; 2012.
4. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012; 379(9832):2162-72.
5. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. J Res Med Sci. 2013; 18:144–57.
6. Classen HG, Gröber U, Löw D, Schmidt J, Stracke H. Zinc deficiency. Symptoms, causes, diagnosis and therapy. Med Monatsschr Pharm. 2011; 34(3):87–95.
7. El Sadek AE, El Faiky OA, Behiry EG, El Said WN. The effect of zinc supplementation on growth and development in preterm neonates. Int j Adv Res Pub. 2016; 4:2713–20.
8. Salgueiro MJ, Zubillaga M, Lysionek A, Sarabia MI, Caro R, De Paoli T, et al. Zinc as an essential micronutrient: A review. Nutrition Research. 2000; 20:737–55.
9. El-Farghali O, El-Wahed MA, Hassan NE, Imam S, Alian K. Early zinc supplementation and enhanced growth of the low-birth weight neonate. Open Access Maced J Med Sci. 2015; 3(1):63–8.
10. Imdad A, Bhutta ZA. Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. BMC Public Health. 2011; 11(3):1-4.
11. Ram Kumar TV, Ramji S. Effect of zinc supplementation on growth in very low birth weight infants. J Trop Pediatr. 2012; 58(1):50–4.
12. Hamza RT, Hamed AI, Sallam MT. Effect of zinc supplementation on growth hormone-insulin growth factor axis in short Egyptian children with zinc deficiency. Ital J Pediatr. 2012; 38:1-7.
13. Terrin G, Berni Canani R, Di Chiara M, Pietravalle A, Aleandri V, Conte F, et al. Zinc in early life: a key element in the fetus and preterm neonate. Nutrients. 2015; 7:10427–46.
14. Thureen PJ. Neonatal nutrition and metabolism. Cambridge University Press; 2012.
15. Kaban RK, Dharmasetiawani N, Siswanto JE. Prevalence and risk factors for hypozincemia in low birth weight infants at a correction age of near-term or term. Sari Pediatri. 2016; 13:207.

16. Mahmood T, Saeed T, Hussain S, Zulfiqar R. Zinc levels among preterm infants. J Rawalpindi Med Coll. 2015; 18:65–7.
17. Dahlan MS. Sample Size in medical health and research. 4th ed. Jakarta: Epidemiologi Indonesia; 2016.
18. Yeoh PL, Hornetz K, Dahlui M. Antenatal care utilisation and content between low-risk and high-risk pregnant women. PLoS One. 2016; 11(3):e0152167.
19. Wang H, Hu YF, Hao JH, Chen YH, Su PY, Wang Y, et al. Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. Sci Rep. 2015; 5(1):11262.
20. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin. 2013; 60(1):49–74.
21. Fukada T, Yamasaki S, Nishida K, Murakami M, Hirano T. Zinc homeostasis and signaling in health and diseases: Zinc signaling. J Biol Inorg Chem. 2011; 16:1123–34.
22. Setiawan CN, Sarosa GI, Setiawati M. Linear growth patterns in small for gestational age and preterm infants after zinc supplementation. Paed Indonesiana. 2015; 55:23.
23. Díaz-Gómez NM, Doménech E, Barroso F, Castells S, Cortabarria C, Jiménez A. The effect of zinc supplementation on linear growth, body composition, and growth factors in preterm infants. Pediatrics. 2003; 111:1002–9.
24. Vázquez-Gomis R, Bosch-Gimenez V, Juste-Ruiz M, Vázquez-Gomis C, Izquierdo-Fos I, Pastor-Rosado J. Zinc concentration in preterm newborns at term age, a prospective observational study. BMJ Paediatr Open. 2019; 3:e000527.
25. Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. Int J Environ Res Public Health. 2010; 7(4):1342–65.
26. de Jong M, Cranendonk A, Twisk JWR, van Weissenbruch MM. IGF-I and relation to growth in infancy and early childhood in very-low-birth-weight infants and term born infants. PLoS One. 2017; 12:e0171650.
27. Ratnasari W. Role of Zinc against the taste function and changes in body weight (a study in less nutrition toddlers with low levels of Albumin in Bojonegoro. Sain Med. 2012; 4:63–7.