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Original Article

Correlations of Maternal Serum Zinc Levels with Cord Blood Procollagen Type 1-N Terminal Propeptide Levels and Anthropometric Measurements of Newborns

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ABSTRACT

Background: Zinc is an important micronutrient that is required for optimal fetal growth and development. Zinc deficiency during pregnancy may interfere with intrauterine fetal ossification. Fetal ossification can be measured with specific bone growth markers, such as procollagen type 1-N terminal propeptide (P1NP). This study aimed to investigate the mean maternal serum zinc levels and assess the correlations of maternal serum zinc levels with cord blood P1NP levels and anthropometric measurements of newborns.

Methods: This cross-sectional study was conducted at Universitas Sumatera Utara Hospital and other hospitals in Medan, Indonesia, from April to October 2019. The statistical populations were pregnant mothers and their newborns who met the inclusion criteria. The participants were selected using a consecutive sampling technique. Maternal serum zinc levels and cord blood P1NP levels were measured before delivery; moreover, anthropometric measurements were conducted in this study.

Results: A total of 42 subjects were included in this study with a mean maternal serum zinc level of 52.0±9.0 μg/dL. The proportion of pregnant mothers with low serum zinc levels was high (54.8%). According to the results, there was a significant correlation between maternal serum zinc levels and newborn birth weight (r=0.648) and length (r=0.656), as well as head circumference (r=0.578; P=0.001). There was also a significant and positive linear correlation between maternal serum zinc levels and cord blood P1NP levels (r=0.469; P=0.002).

Conclusion: The mean maternal serum zinc levels during pregnancy were below normal. There were positive significant correlations between maternal serum zinc levels and cord blood P1NP levels with anthropometric measurements of newborns.

Keywords: Anthropometrics of newborns, Cord blood P1NP, Maternal serum zinc

Introduction

Zinc is an important micronutrient that is required for optimal fetal growth and development (1-3). Approximately, 82% of pregnant mothers worldwide are zinc deficient due to increased zinc requirements during pregnancy, consumption of foods with low zinc content, and intake of food that may inhibit zinc absorption (4). Zinc deficiency (serum zinc levels <56 μg/dL) during pregnancy may interfere with intrauterine fetal ossification. It has been shown that maternal serum zinc levels correlate with newborn birth length and head circumference (5).

Other studies have revealed various results regarding the role of zinc in fetal ossification and birth weight (6-8). Specific bone growth marker examinations are required to investigate the correlation between maternal serum zinc levels and fetal ossification. One specific bone growth marker is procollagen type 1 N-terminal propeptide (P1NP). More than 90% of the bone organic matrix is collagen type 1, which will be formed into bones and is derived from procollagen type 1 produced by fibroblasts and osteoblasts. Procollagen type 1 contains (N-)

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amino and (C-) carboxy-terminal propeptides, which will be parsed by the protease enzyme when procollagen is changed into collagen and then the moulded bone matrix. Serum concentrations of P1NP are directly related to the amount of newly formed collagen in the bone. The P1NP is a specific indicator of bone formation, which is released during the formation of type 1 collagen and will enter the bloodstream (9). The examination of N-terminal propeptide can be performed at a clinical laboratory with a cord blood sample. Cord blood P1NP levels describe the rate of the fetal ossification process (10, 11). The P1NP maintains its stability even under the conditions of repeated freezing and thawing. The International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Bone Marker Standards recommended that serum P1NP be used as the reference marker for bone formation (12).

This study aimed to assess the correlation of maternal serum zinc levels with cord blood P1NP levels and anthropometric measurements of newborns (birth weight and length, as well as head circumference). Moreover, it was attempted to discover the role of zinc in the prevention of stunting in infants in later years, which could be beneficial for preventing stunting in Indonesia. This study will help investigators identify the critical role of zinc associated with ossification in the growth and development of newborns. Accordingly, a new theory of stunting may be developed in this regard.

Methods

Study participants

This cross-sectional study was conducted from April to October 2019 at Universitas Sumatera Utara, Malahayati Islamic, and Royal Maternity Hospitals, Medan, North Sumatera, Indonesia. The subjects were pregnant mothers and their newborns who fulfilled the inclusion criteria. The inclusion criteria were healthy pregnant mothers aged 20-35 years old who received regular antenatal care during the third trimester of pregnancy. The patients were selected through a consecutive sampling technique. On the other hand, the pregnant mothers with gestational diabetes, eclampsia, or liver/ kidney diseases, as well as preterm infants (gestational age <37 weeks), and newborns with congenital anomalies were excluded from the study.

Parameters measurement

The parameters assessed in this study were maternal serum zinc levels and anthropometric measurements, including birth weight and length, as well as head circumference. Maternal serum zinc levels were measured with 6 ml from an antecubital vein before delivery. Blood was preserved at room temperature for 30 min and was subsequently centrifuged for 15 min at 3000 rpm. Furthermore, maternal serum zinc was examined as a trace element by inductively coupled plasma-mass spectrometry according to the standard of the Prodia clinical laboratory. Normal serum zinc levels were defined based on a cut-off value of ≥56 µg/dL according to Second National Health and Nutrition Examination Survey data from 1976-1980 (13).

Following that, the P1NP levels were measured using 6 ml of cord blood obtained before delivery. Blood was preserved at room temperature for 30 min, subsequently centrifuged for 15 min at 3000 rpm, and then frozen at -70°C for subsequent analysis of P1NP. Sample collection, transportation, separation, and storage were performed according to standard laboratory procedures. Cord Blood P1NP was examined using an automated Cobas e601 analyzer (Roche Diagnostic, Mannheim, Germany) following Prodia clinical laboratory standards. Anthropometric measurements of birth weight and length were performed on newborns with a SECA 334 digital baby scale for weight and a SECA 232 for length with an accuracy of 0.1 kg and 0.1 cm, respectively. It should be mentioned that the head circumference was measured with a tape with an accuracy of 0.1 cm.

Statistical analysis

The obtained data were analyzed in SPSS software (version 23) (SPSS Inc., Chicago, Illinois, USA) through descriptive statistics, correlation, and linear regression test. A p-value less than 0.05 was considered statistically significant.

Ethical considerations

The study protocol was approved by the Health and Medical Research Ethics Committee of the Faculty of Medicine of Universitas Sumatera Utara/RSUP Haji Adam Malik, Medan, Indonesia (2401/TGL/KEPK FK USU-RSUP HAM/2019).

Results

This study included 50 pregnant mothers with their newborns who met the inclusion criteria from April to October 2019. However, eight subjects were excluded from the study due to lysed blood (n=2) and lack of consent for blood extraction (n=6). Accordingly, 42 pregnant

Table 1. Patients' Characteristics

Characteristics	Mean±SD (Range)	n (%) N=42
Maternal age (years)	27.6±3.4 (21-35)	
Maternal weight (kg)	66.2±10.5 (55-90)	
Maternal weight gain (kg)	12.7±5.5 (9-20)	
Parity status		
Primipara		20 (47.6)
Multipara		22 (52.4)
Newborn gender		
Male		18 (42.9)
Female		24 (57.1)
Gestational age (weeks)	38.5±0.5 (37-40)	
Neonatal birth weight (g)	3323.8±389.3 (2400-4000)	
Neonatal birth length (cm)	49.2±1.6 (44-51)	
Head circumference (cm)	33.9±1.2 (31-37)	
Maternal serum zinc levels*	52.0±9.0 (29-68)	
Value <56 µg/dL		23 (54.8)
Value ≥56 µg/dL		19 (45.2)
Cord blood P1NP levels (µg/dL)	3045.3±1287.1 (1099 -7720)	

*Normal maternal serum zinc levels ≥56 µg/dL (Second National Health and Nutrition Examination Survey)

mothers and their newborns completed the study. Table 1 tabulates the characteristics of the patients in this study. The mean maternal and gestational ages were obtained at 27.6±3.4 years and 38.5±0.5 weeks, respectively. Moreover, the mean maternal weight and weight gain during pregnancy were determined at 66.2±7.5 and 12.7±5.5 kg, respectively. Furthermore, the mean birth weight and length, as well as head circumference were estimated at 3323.8±389.3 kg and 49.2±1.6 cm, as well as 33.9±1.2 cm, respectively. The maternal serum zinc level was 52.0±9.0 µg/dL (range 29-68 µg/dL), and there was a high proportion of serum zinc levels lower than 56 µg/dl (54.8%). The range of the P1NP level in cord blood levels was 1099-7720 (3045.3±1287.1) µg/dL. The correlation and linear regression analyses between maternal serum zinc levels and cord blood P1NP levels with anthropometric measurements of the newborns are described in Table 2 and Figure 1. Maternal

Table 2. Correlation of maternal serum zinc levels with cord blood P1NP levels and anthropometric measurements of the newborns

Variable	Maternal serum zinc levels		
	r	R^2	P
Birth weight	0.648	0.420	0.001
Birth length	0.656	0.430	0.001
Head circumference	0.578	0.334	0.001
Cord blood P1NP levels	0.469	0.220	0.002

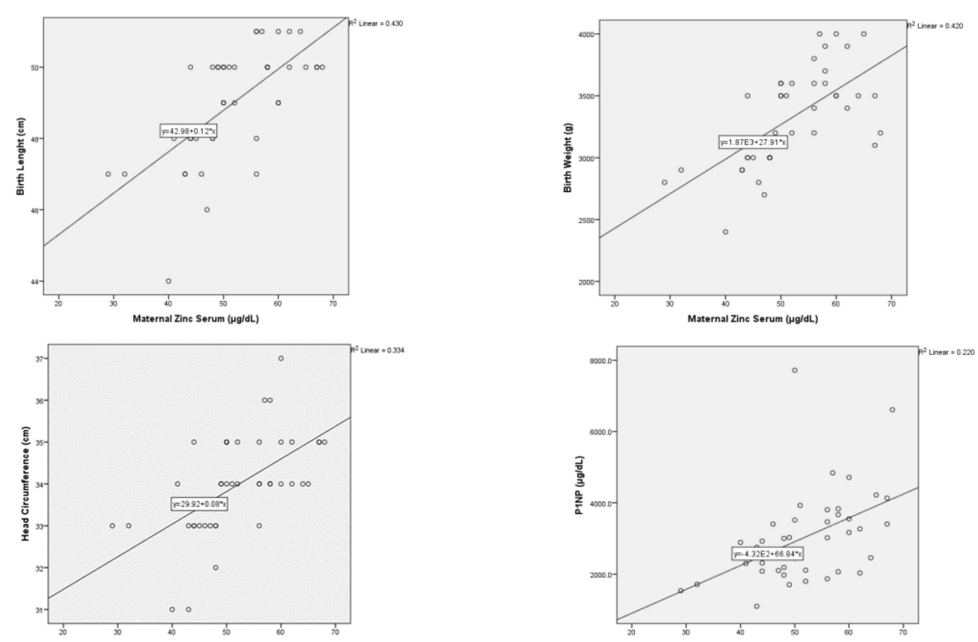

Figure 1. Linear correlation of maternal serum zinc levels with cord blood P1NP levels and anthropometric measurements of newborns

serum zinc levels had a strong positive linear correlation with the birth weight (r=0.688), birth length (r=0.678), and head circumference (r=0.609) of the newborns (P=0.001). In addition, maternal serum zinc levels showed a moderate positive linear correlation with cord blood P1NP levels (r=0.469; R^2=0.220; P=0.002). Figure 1 illustrates the line of positive linear correlation between maternal serum zinc levels and cord blood P1NP levels with anthropometric measurements of newborns (birth weight and length, as well as head circumference).

Discussion

The concentration of serum zinc in pregnant women tends to be lower than that in non-pregnant women and varies among population groups (13, 14) due to an increase in demand, a low-zinc diet, and consumption of foods that inhibit zinc absorption (3). Yasoghara et al. showed that maternal serum zinc levels decreased progressively before pregnancy from 78.1±21.85 μg/dL to 60.5±14.42 μg/dL (15). Maternal serum zinc levels may decrease by 35% due to increased demand during the pregnancy period (16). Normal maternal serum zinc is defined as ≥56 μg/dl (13). Seriana et al. revealed lower mean maternal serum zinc levels of 36.01±18.34 μg/dL at term gestational age (5). A similar study by Wicaksono et al. in Rejang Lebong, Bengkulu, indicated that 39 pregnant mothers in the third trimester had maternal serum zinc levels of 60.35±39.16 μg/dl (17). Similarly, Wang et al. in China demonstrated that 247 cases out of 3187 pregnant mothers had zinc deficiency (<56 μg/dl), and the mean maternal serum zinc level was 91.0 μg/dl (18). The mean maternal serum zinc level was obtained at 52.0±9.0 μg/dl (range 29-68 μg/dl) at a mean gestational age of 38.5±0.5 weeks in the present study. The proportion of pregnant mothers with maternal serum zinc levels <56 μg/dL was as high as 54.8%. This study revealed lower maternal serum zinc levels, compared to those observed in previous studies.

This study showed a strong positive linear correlation between maternal serum zinc levels and newborn infants' birth weight and length, as well as head circumference. In the same line, Awadallah et al. conducted a study in Jordania and reported that the mean maternal serum zinc level in the third trimester was 68±10 μg/dL with a mean birth weight of 3.34±0.44 kg (19). According to a study conducted by Wijaksono et al. in Bengkulu, Indonesia, a strong correlation was found between maternal serum zinc levels and maternal weight gain during pregnancy with the birth weight of newborns (17). The results of a study performed by Seriana et al. indicated a significant correlation of maternal serum zinc levels with the birth length and head circumference of newborns, which was in line with the findings of this study (5). This correlation may be considered an indicator of maternal zinc sufficiency, which affects the growth and development of the fetus. Wang et al. reported lower maternal serum zinc levels in small-for-gestational-age newborns, compared to controls (18).

Low serum zinc levels in pregnant women affect fetal growth and development leading to conditions, such as growth failure and low birth weight (8, 18). Plasma zinc decreases during pregnancy accompanied by a larger decrease observed in women with habitual intakes ≤9 mg/day (20). Zinc supplementation in pregnant women is required, especially in those with low zinc levels, such as those identified in this study. Pregnant women with low zinc levels require approximately 20 mg of zinc per day (21). Several studies suggest that zinc supplementation may improve pregnancy and pregnancy-related outcomes (22). A previous meta-analysis found that the administration of zinc to pregnant mothers could reduce the risk of prematurity and low birth weight (23).

Zinc is the most abundant trace mineral in the human skeleton. Zinc has been shown to stimulate osteoblasts and inhibit osteoclasts to ultimately drive bone formation under *in vitro* conditions (24). A specific study to investigate the association of maternal serum zinc levels with ossification requires feasible markers measured in blood samples. Several biomarkers are known to have specific roles in ossification, such as P1NP, procollagen type 1 carboxy-terminal propeptide (P1CP), osteocalcin, and alkaline phosphatase (ALP). The P1NP is part of the bone matrix. Osteoblasts release the procollagen type-1 precursor. Procollagen then undergoes proteolytic cleavage to produce amino-terminal and carboxy-terminal propeptide from collagen type 1 (P1NP and P1CP). Concentrations of P1NP and P1CP in circulation are presumed to reflect the ossification rate (9, 10, 25).

The present study found a significant and moderate linear correlation between maternal serum zinc levels and cord blood P1NP levels. Several subjects with low maternal serum zinc levels showed high cord blood P1NP levels, while others with normal maternal serum zinc levels also had high cord blood P1NP levels. This condition was due to an increase in zinc demand during fetal ossification; therefore, maternal zinc depletion occurred as reported by Holt et al. (13). Other factors, such as diet and the type of foods consumed, may also play a role in this regard. A previous study exploring the effects of 25 mg zinc supplementation showed clinical improvement in fetal ossification reflected by ultrasonography measurements (6). Choi et al. in Korea reported that the highest P1NP levels were observed during the first year of life; thereafter, the levels decreased until puberty (26). Berger et al. in their study of four-week zinc supplementation among prepubertal girls revealed an increase in P1NP as a bone formation marker (27).

Zinc has been demonstrated to stimulate osteoblastic bone formation and inhibit osteoclastic bone resorption; moreover, mineral supplementation has been shown to support the prevention of bone loss. The role of zinc in the prevention of stunting based on bone mineralization involves the action of alkaline phosphatase, collagen, and deoxyribonucleic acids as indices of bone metabolism. This study showed a significant correlation between maternal serum zinc levels and anthropometric measurements of newborns. This is an important topic to be addressed to understand the role of zinc in preventing stunting in the future.

Regarding the limitation of this study, one can refer to the lack of assessing pregnant mothers' dietary intake or other factors that may affect zinc levels during pregnancy. Therefore, further studies are suggested to assess nutritional intake and zinc levels during pregnancy, as well as the effects of zinc supplementation in pregnant mothers on fetal growth and development at the biomolecular level.

Conclusion

Low levels of maternal serum zinc are commonly found during pregnancy. Moreover, there is a positive correlation between maternal serum zinc levels and cord blood P1NP levels with anthropometric measurements of newborns. This finding is expected to help prevent the occurrence of low birth weight and stunting in newborns.

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Conflicts of interest

The authors declare no conflicts of interest related to financial support or relationships during the proposal and manuscript writing, as well as data collection and analysis.

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