ORIGINAL ARTICLE

A randomized, placebo-controlled trial of zinc supplementation during pregnancy for the prevention of stunting: analysis of maternal serum zinc, cord blood osteocalcin and neonatal birth length

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ABSTRACT

Aim To investigate the influence of zinc supplementation on pregnant women for the prevention of stunting through an analysis of maternal serum zinc, cord blood osteocalcin and neonatal birth length.

Methods This study was conducted with pre-test/post-test control groups and double-blind randomization. Patients were pregnant mothers in second or third trimester and with their newborns who met the inclusion criteria. A total of 71 pregnant mothers and their newborns completed this study. They were divided into two groups of 35 and 36 patients, the supplementation (20 mg/day) and placebo groups, respectively for 12 weeks. The parameters assessed were maternal serum zinc levels, cord blood osteocalcin and birth length measurements.

Results The mean maternal serum zinc level was 54.6±8.7 μg/dL from 71 patients. The mean maternal serum zinc levels after zinc supplementation were significantly higher than those of the placebo group: 55.1±9.9 to 59.1±8.6) μg/dL (p=0.017) and 54.2±7.5 to 50±8.6 μg/dL (p=0.001), respectively. The comparison of mean cord blood osteocalcin levels and median neonatal birth lengths in the supplementation group was higher than in the placebo group: 131.8±35.3 vs 90.6±35.4 ng/ml (p=0.001) and 49.3 (46.5-51.3) vs 48.3 (46-50.8) cm (p=0.004), respectively. Maternal serum zinc levels after zinc supplementation had a positive significant correlation with cord blood osteocalcin and neonatal birth length: r=0.434 (p=0.001) and r=0.597 (p=0.001), respectively.

Conclusion There was a significant correlation of maternal serum zinc with cord blood osteocalcin and neonatal birth length after zinc supplementation.

Key words: bone growth, micronutrients, pregnant, pre-post test design

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Original submission:
31 August 2020;
Revised submission:
27 October 2020;
Accepted:
24 January 2021
doi: 10.17392/1267-21

Med Glas (Zenica) 2021; 18(2):415-420

INTRODUCTION

Stunting is a condition that occurs due to chronic malnutrition, especially in the first 1000 days of life (1,2). Low linear birth length measures generally signal nutritional deficiency due to a lack of energy and protein experienced over time (3). The prevalence of stunting in Indonesia was 30.8%, with 22.7% of all neonatal birth lengths at less than 48 cm (4).

Malnutrition in both macro- and micronutrients in pregnant women causes an inadequate nutritional reserve for the physiological needs of the foetus during pregnancy, which results in growth and developmental disorders, increased morbidity and mortality, and may interfere with long-term intellectual capacity and academic proficiency (1,3,5). Zinc is one of the essential micronutrients, and a lack of zinc intake in small quantities can cause dysfunction in various systems and restriction of physical growth (6). Certain studies have found that low maternal zinc levels are associated with foetal death, congenital malformations, intrauterine growth restriction (IUGR), prematurity, low birth weight infants (LBW) and disorders of childbirth (7,8). Zinc interacts with the development at varying degrees of synthesis, secretions, and hormonal actions that play an important role for linear growth (6), and the formation of foetal bones (9,10).

Zinc deficiency in pregnant women is suspected to be the cause of high birth rates with short birth lengths (9). More specific research into the influence of zinc on bone growth requires a marker that can be measured through the examination of biomarkers in the blood or other tissues (11). Osteocalcin examination can be done in a clinical laboratory with sampling of the cord blood of newborns. Cord blood osteocalcin levels are rated almost equal to the newborn's blood levels (11). This examination can provide an overview of how much bone growth is influenced by zinc or other micronutrients. To date, there have been no studies linking the influence of zinc supplementation in pregnant women on the prevention of newborn stunting through analysis of osteocalcin levels as markers of bone growth in the foetus and the newborn.

The aim of this study was to investigate the influence of zinc supplementation on pregnancy in the prevention of stunting through an analysis of maternal serum zinc, cord blood osteocalcin and neonatal birth length.

PATIENTS AND METHODS

Patients and study design

This was an experimental study with pre-test/post-test control groups, double-blind with randomization block technique. This study was conducted from March to December 2019 in the Obstetrics and Neonatology Unit of Universitas Sumatera Utara Hospital, Malahayati Islamic Hospital and Royal Maternity Hospital in Medan, Indonesia. Patients were pregnant mothers with their newborns who met the inclusion criteria: healthy pregnant mothers in their second or third trimester, 20-35 years old, with a height of >150 cm and no indication of cons following this study, as determined by obstetricians and gynaecology specialists. Births involving twins, congenital abnormalities, prematurity (gestation age <37 weeks) or stillbirth were excluded.

As the pregnant mothers got admitted in the Obstetrics Unit, every consecutive patient was assessed for inclusion and exclusion criteria and was randomized by a blocked randomization method. One hundred and four patients were assessed for this study, 82 were found to be eligible and were randomized, and assigned to two groups, with 41 patients in each group. One group administered zinc supplementation 20 mg/day and the other group administered placebo, in the morning after meals for 12 weeks. Zinc tablets (zinc sulphate) and placebo were each inserted into a capsule of the same shape, colour and taste. All patients were also given iron and folic acid tablets in accordance with the Indonesian Government program.

An informed consent was given by all mothers following the provision of sufficient information prior to the study.

This study was approved by the Health Research Ethical Committee, School of Medicine, Universitas Sumatera Utara (No.179/TGL/FK USU-RSUP HAM/2019).

Trial registration: Clinicaltrials.gov identifier: NCT04559152.

Methods

The parameters assessed in this study were maternal serum zinc levels, cord blood osteocalcin and birth length. Maternal serum zinc levels were measured twice with 6 mL of vein blood each time, both during initial antenatal care and after 12-weeks of supplementation. Samples were subsequently centrifuged for 15 minutes at 3000 rpm. Specimens were processed by the inductively coupled plasma-mass spectrometry (ICP-MS) method using Agilent 7700 analyser (Santa Clara, USA, 2014). Normal serum zinc levels were defined based on a cut-off value of ≥56 μg/dL in accordance with the Second National Health and Nutrition Examination Survey data from 1976-1980 (NHANES II) (12).

Cord blood osteocalcin levels were measured with 6 mL cord blood samples prior to delivery. The serum was separated by centrifugation (15 minutes at 1000 rpm) and was frozen at −70 ^{o}C for subsequent analysis of osteocalcin. Osteocalcin was measured using biotin and ruthenium specific monoclonal antibodies against N-Mid osteocalcin (N-Mid Osteocalcin, Roche Diagnostics, Mannheim, Germany) by the electrochemiluminescence immunoassay (ECLIA) on an automated Cobas e601 analyzer (Roche Diagnostics, Mannheim, Germany). Sample collection, transportation, separation, storage, and analysis were performed according to Prodia clinical laboratory standards, Indonesia (ISO 9001 and ISO 15189).

Neonatal birth length was performed with a SECA 232 digital baby scale (Hamburg, Germany) for length with an accuracy of 0.1 cm, independently duplicate by two trained persons for all births. Maternal zinc intake was calculated in accordance with the NutriSurvey 2007 Indonesian version (13).

Statistical analysis

A Saphiro-Wilk's test was performed to determine the normality of data spread. Descriptive data were expressed as mean±SD and percentage (%); and median (min-max) was for non-normal distribution data. Bivariate analysis was performed with unpaired t-test, paired t-test and $\chi 2$ test; non-normal distribution was analysed with the Mann-Whitney U-test. Correlation was used to measure two variables with Pearson correlation coefficients (r) and Spearman's for nonparametric correlation. Statistical significance was considered at a p-value <0.05 with a 95% confidence interval.

RESULTS

Of the 104 pregnant women assessed during the period between March and December 2019, 22 (21.1%) were excluded. Eighty-two pregnant mothers with their newborns who met the inclusion criteria were enrolled during the study, of which 11 were excluded for the following reasons: seven dropped contact, two stopped taking zinc supplementation, and two cord blood samples were useless. Therefore, 71 pregnant mothers and their newborns completed this study; they were divided into two groups of 35 and 36 patients, the supplementation and placebo groups, respectively (Figure 1).

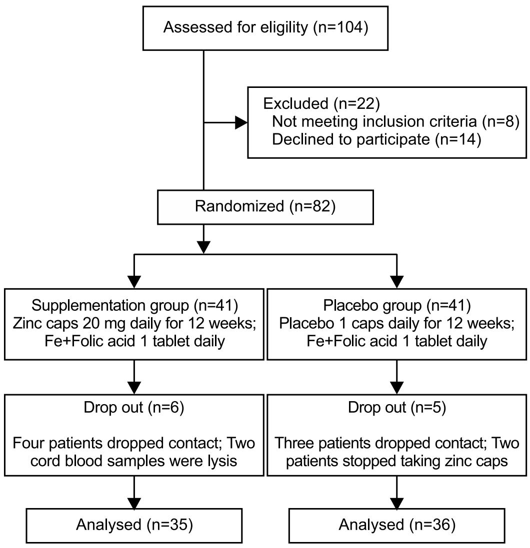

Figure 1. Flow diagram of study patient

Maternal serum zinc levels during pregnancy were of 54.6±8.7 μg/dL (range 40-74 μg/dL), and the prevalence of levels <56 μg/dL was high, 46 (64%) mothers. The mean cord blood osteocalcin levels of the supplementation and placebo groups were 131.8±35.3 ng/dL and 90.6±35.4 ng/dL, respectively (Table 1).

The supplementation group experienced a significant increase of the mean maternal serum zinc level during pregnancy before and after 12 weeks (p=0.017), while the placebo group showed a significant decrease (p=0.001). Using a statistical unpaired t-test, mean maternal serum zinc level between both groups after 12-weeks of zinc supplementation showed significant differences (p=0.001). All values of maternal serum zinc level in both groups were below normal value (<56 μg/dL) (Table 2).

The relationship between maternal serum zinc level and cord blood osteocalcin after 12-weeks of

Table 1. Characteristics of 71 pregnant women involved in the study

Characteristic	Groups			p
	Supplementation (n=35)	Placebo (n=36)	Total (n=71)	
Maternal age (years) (Mean±SD)	28.8±3.6	27.9±3.4		0.265*
Pregnancy period (weeks) (Mean±SD)	22.3±3.3	22.2±2.7		0.930*
Maternal weight (kg) (Mean±SD)	64.2±11.7	66.0±7.2		0.447*
Maternal height (cm) (Mean±SD)	158.4±6.9	157.3±3.7		0.410*
Maternal BMI (kg/m2) (Mean±SD)	25.9±4.8	26.6±3.1		0.442*
Gravida (No, %)				
Primigravida	17 (48.6)	17 (47.2)		1.000†
Multigravida	18 (51.4)	19 (52.8)		
Maternal occupation (No, %)				
Worker	20 (57.1)	21 (58.3)		1.000†
Non-worker	15 (42.9)	15 (41.7)		
Family economic status (No, %)				
Low	1 (2.9)	4 (11.1)		
Average	16 (45.7)	19 (52.8)		0.225†
High	18 (51.4)	13 (36.1)		
Maternal education (No, %)				
High school	5 (14.3)	15 (41.7)		
University, not graduate	2 (5.7)	7 (19.4)		0.002§
University, graduate	28 (80)	14 (38.9)		
Maternal zinc intake (mg/day) (Mean±SD)	6.0±1.3	5.5±0.8		0.061*
Maternal serum zinc (μg/dL) (Mean±SD)	55.1±9.9	54.2±7.5	54.6±8.7	0.082*
Range			40-74	
Value <56 μg/dL No (%)	22 (62.9)	24 (66.7)	46 (64)	0.930†
Value ≥56 μg/dL, No (%)	13 (37.1)	12 (33.3)	25 (35.2)	
Gestational age (weeks) (Mean±SD)	38.8±0.4	38.5±0.8		0.061*
Cord blood osteocalcin (ng/dL) (Mean±SD)	131.8±35.3	90.6±35.4		0.001*
Neonatal birth length (cm) (Median; Min.-max.)	49.3; 46.5-51.3	48.3; 46-50.8		0.004‡

*Unpaired t-test; †Chi Square test; ‡Mann-Whiney U-test; §Significant difference (p<0.05); BMI, Body mass index;

zinc supplementation was positive, moderate and significant (r=0.434; p=0.001). The Spearman rank correlation test showed that the relationship between maternal serum zinc level and the neonatal birth length was positive, strong and significant (r=0.597; p=0.001) (Table 3).

Table 2. Mean maternal serum zinc level before and after zinc supplementation or placebo

Groups	Maternal serum zinc level (Mean±SD) (μg/dL)			p
	Before	After		
Supplementation	55.1±9.9	59.1±8.6	0.017†	0.001*
Placebo	54.2±7.5	50.0±8.6	0.001†	

*Unpaired t-test; †Paired t-test.

Table 3. Correlation between maternal serum zinc level after 12 weeks with cord blood osteocalcin and neonatal birth length

Variable	r	p
Cord blood osteocalcin	0.434*	0.001
Neonatal birth length	0.597†	0.001

*Pearson correlation test; †Spearman's rank correlation test

DISCUSSION

The patients in this study were pregnant mothers because stunting prevention can start at conception (1). The results of the presented study showed mean serum zinc level in pregnant women of 54.6±8.7 μg/dL was under normal value in accordance with the recommendation of NHANES II (12). The proportion of pregnant women with serum zinc level below normal was quite high (64%). Seriana et al. encountered low serum zinc level during pregnancy (36.0±18.3 μg/dL) (14). In a similar study from Ethiopia, Mekonnen et al. reported mean serum zinc level in pregnant women of 58.7 μg/dL, with a proportion of zinc deficiency lower than 55.3% in this study (15).

The average maternal zinc intake of only 5.77±1.09 mg/day was found in our study, which is in accordance with the calculation of the Indonesian version of NutriSurvey 2007 (13). This percentage is low compared to the average needs of pregnant women (13). A study in Bandung, West Java, Indonesia reported an average maternal zinc intake of 5.1 mg/day (16). Gala et al. reported lower levels than those found in this research (4.2±1.2 mg/day) (17). Some of the factors that lead to the prevalence of maternal zinc deficiency are low intake of animal protein sources and plant based diets with high phytate contents, especially in developing countries. Animal proteins are the best dietary source of zinc, as they contain amino acids that increase zinc absorption (3,18,19).

The need of zinc intake increases during pregnancy due to the increasing need for foetus ossification causing a decrease in maternal serum zinc level (12). Choi et al. reported the serum zinc level of pregnant women decreased significantly throughout the first, second and third trimesters of pregnancy (20). This study found that maternal serum zinc level significantly decreased in the placebo group, and increased significantly in the supplementation group. According to WHO, pregnant women need at least 11 mg of zinc intake per day. In pregnant women with a low bioavailability, a zinc intake of 20 mg/day is required (21,22).

Several studies reported the benefits of zinc supplementation in pregnant women and birth outcomes (23-25). This study found that neonatal birth length was higher in supplementation group than placebo. In agreement with this study, Merialdi et al. reported a significant increase in the foetal femur diaphysis length in pregnant mothers who were given 25 mg zinc supplementation concurrently with 60 mg Fe and 250 g folic acid (26). Prawirohartono et al. reported that Zn supplementation during pregnancy improves birth length after adjusting for maternal height and pre-pregnancy weight (27).

Therefore, the examination of osteocalcin is a good parameter for determining the metabolism of bone formation (28,29).This study found that the average cord blood osteocalcin level in the supplementation group was significantly higher than in the placebo group, and they were higher than in previous research studies (30,31). Examination of osteocalcin in other research is taken from the serum, while this study took it from umbilical cord blood. There has been no standard reference value for umbilical cord blood osteocalcin levels. A study in Korea reported that the concentration of osteocalcin in serum was higher at puberty period. This value indicated that osteocalcin greatly increased during growth spurt (31). High levels of umbilical cord blood osteocalcin in this study are thought to be due to the rapid growth rate during the foetal period.

The effect of zinc supplementation of pregnant women on foetal bone growth caused by zinc stimulating osteoblast production and inhibiting osteoclast activity (26). This study showed a positive and significant correlation between maternal serum zinc and cord blood osteocalcin levels as well as neonatal birth length after 12 weeks of zinc supplementation. This relationship indicates that maternal zinc level during pregnancy is one of the factors that can affect foetal growth.

Excess zinc can manifest itself through nausea, vomiting, abdominal cramps, and diarrhoea (19). Nearly none of the patients of this study complained of side effects. Two pregnant mothers reported nausea after taking a zinc tablet, but continued supplementation and reported no complaints afterwards. Nausea could also be attributed to the ongoing state of pregnancy. The limitations of this study include the use of telephone communication to monitor patients taking zinc tablets; it could be possible that the patients forgot to take their zinc tablets, and some of them failed to be contacted again. Also, this study did not control the dietary intake of patients, and did not analyse foods containing zinc inhibitors that could affect the absorption of zinc in digestion.

In conclusion, this study found a high prevalence of zinc deficiency in pregnant women. There was a positive correlation between maternal serum zinc levels with cord blood osteocalcin levels as bone growth markers and neonatal birth length after 12 weeks of zinc supplementation. As a recommendation, pregnant women require regular examination of maternal serum zinc and administration of 20 mg/day zinc prophylaxis dose during pregnancy.

ACKNOWLEDGEMENTS

We thank the Universitas Sumatera Utara Hospital, Malahayati Islamic Hospital, Royal Maternity Hospital and Prodia Clinical Laboratory for technical support during the conduction of this study.

FUNDING

This study was funded by the Ministry of Research and Technology and Higher Education, Republic of Indonesia under the DRPM research grant of the year 2019 (No.9/UN5.2.3.1/PPM/KP-DRPM/2019).

TRANSPARENCY DECLARATION

Competing interests: None to declare.

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