Journal of Medical Laboratory Science, 2021; 31 (4): 47-57
http://jomls.org; info@jomls.org
http://doi.org/10.5281/zenodo.5802237

Cord Serum Copper-to-Zinc Ratio Correlates with Birth Weight among Neonates in Benin City, Nigeria

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ABSTRACT

Background: Low Birth weight of babies is a risk factor for infant morbidity, survival, and health status. **Objective:** To determine cord serum levels of zinc, copper and copper-to- zinc ratio in neonates and their association with neonatal birth weight in order to provide health policy makers and care-providers with data necessary for appropriate intervention. **Materials and Methods:** Two hundred newborn, 41 small for gestational age (SGA) and 159 appropriate for gestational age (AGA) were evaluated. Cord blood sample was collected immediately after birth and zinc and copper levels were determined using atomic absorption spectrophotometry method. Copper-to-zinc ratio was calculated from the copper and zinc values. **Results:** Cord serum zinc was significantly lower ($p<0.001$) while copper-to-zinc ratio was significantly higher ($p<0.001$) in small for gestational age than appropriate for gestational age babies. There was no significant difference ($p>0.05$) in the cord serum copper level between appropriate for gestational age and small for gestational age neonates. The cord serum zinc correlated positively ($R=0.33;p<0.001$) while copper-to-zinc ratio correlated negatively ($R=-0.23;p<0.001$) with birth weight of neonates. However, there was insignificant inverse relationship between cord serum copper with birth weight of neonates. Cord serum zinc increased with increasing ponderal index while copper-to-zinc ratio decreased with increasing ponderal index among neonates. The changes in cord blood copper levels with increasing ponderal index were insignificant. **Conclusion:** Intensive implementation of zinc supplementation in pregnancy at all health care levels is suggested to reverse the observed relationship between zinc, Cu/Zn ratio with low birth weight among newborn.

Keywords: Small for gestational age, cord serum zinc, copper.

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Author's contributions: This work was carried out and approved in collaboration between all the authors who take responsibility for its intellectual contents, accuracy and integrity. MAE designed the study; OJA sourced for funding; EMA, AOJ wrote the protocol; EMA, AOJ, OOP contributed in literature search; AOJ, OOP did Lab experiments; EMA, AOJ, OOP did the statistical data analysis; EMA, OOP contributed in the discussions; AOJ, OOP drafted the manuscript; EMA supervised the study; MAE, AOJ, OOP Proof-read the galley proof for final publication.

Received: September/19, 2021; **Accepted**: November/25, 2021; **Published**: December/26, 2021.

Citation: Agbonlahor OJ, Emokpae MA, Osuntade OP. Cord Serum Copper-to-Zinc Ratio Correlates with Birth Weight among Neonates in Benin City, Nigeria. **J Med Lab Sci, 2021; 31 (4): 47-57**

INTRODUCTION

Maternal micronutrient deficiency is a major risk factor responsible for higher incidence of small for gestational age (SGA) and intrauterine growth retardation (IUGR) babies. Inadequate zinc intake and metabolism are common among pregnant women in developing countries (1,2). Some authors have reported that up to 73.5% pregnant women suffer from zinc deficiency in some parts of India (3). Low birth weight (LBW) is a major health challenge in developing countries, especially in sub-Saharan Africa, including Nigeria. Birth weight of babies is risk factor for infant morbidity, survival, and health status (4). Neonatal low birth weight (<2500 g) has several adverse outcomes such as higher incidence of diarrhea, respiratory infections, septicemia and malnutrition and increased susceptibility to adulthood diseases (e.g., coronary heart disease, diabetes mellitus, hypertension, and obstructive lung disease) (4,5).

Zinc and copper are important trace elements that are needed in the body for optimum human growth and development. Zinc and copper perform important functions in the body; they act as cofactors in many antioxidants enzymes that protect against oxidative stress (6). Zinc is essential for the normal metabolic and physiological processes that control cell growth. Zinc requirements are higher during pregnancy and low levels of zinc and copper might correlate independently with a risk of LBW neonates (2,4). One important determinant of the future growth and development of a neonate is intra-partum period of growth (7). It is during pregnancy that fast growth and cell differentiation takes place in both mother and foetus. During this period, both the mother and foetus are susceptible to dietary deficiencies particularly to those of the essential micronutrients.

Copper as an important micro-nutrient is essential for maintaining the strength of the skin, blood vessel, epithelial and connective tissues. It is also involved in the production of haemoglobin, myelin, melanin and proper functioning of thyroid gland(8). Copper is important for normal foetal development (9). Physiological changes during pregnancy increase serum copper concentration due to increase of ceruloplasmin as a result of elevated levels of estrogen and move across the placenta by passive transfer (10).

Deficiency of copper may occur during pregnancy due to low estrogen level, low dietary intake and metabolic defect (11). Low serum copper during pregnancy strongly affects fetal growth as well as length of gestation. Again, low serum copper status in pregnant mother may occur due to severe protein calorie malnutrition, malabsorption states and prolonged diarrhea or gastrointestinal (GIT) disturbance which contributes to the depletion of the hepatic stores for copper (12). It is therefore imperative to know the levels of these important trace elements in neonates and their association with neonatal birth weight in our setting to provide health policy makers and care-providers with data necessary for appropriate intervention. .

The association between zinc to copper ratio and neonatal birth weight has not been reported in our setting to the best our knowledge. Evidence indicates that maternal zinc and copper levels are affected not only by intakes but by environmental factors (13) and these could have adverse effects on both the mother and intrauterine growth and development of the fetus (14). Many studies elsewhere have evaluated the association between copper-to-zinc ratio and birth weight of neonates and the findings have not been consistent. Whereas some authors have reported positive associations between neonatal serum zinc and copper concentrations and birth weight (15,16), others observed negative associations while some did not find any significant relationships (17). This study seeks to determine the relationship between copper-to-zinc ratio and birth weight of neonates in Benin City.

MATERIALS AND METHODS:

This is a cross-sectional study of 200 neonates delivered at the Departments of Obstetrics and Gynecology, Stella Obasanjo Hospital, Benin City. They were consecutively enrolled for the study. The gestational age was calculated by counting in weeks from the first day of the last menstrual period.

Inclusion Criteria: All apparently healthy neonates delivered by women of 18years and above expecting singleton, who attended antenatal clinic throughout the pregnancy and reported for delivery were included. Pregnant women who carried their pregnancy to full term and delivered either by vaginal and cesarean were also included.

Exclusion Criteria: Babies delivered by pregnant women with complications such as diabetes mellitus, cardiovascular diseases, and those who had parity more than four (4) were excluded. Obstetric conditions that could cause small for gestational age babies like preterm deliveries, bad obstetric history, abruption placenta previa, and congenital anomalies of the baby, pregnancy-induced hypertension, polyhydramnios, endocrine disorders, or other severe maternal illnesses, clinical signs of infection, benign tumors and malignancies were excluded. Demographic and clinical information were obtained using structured questionnaires.

Ethical Consideration: Ethical approval and clearance for this study was sought and obtained from the Ethics and Research Committee of the Edo State Hospitals Management Board, Benin City, Nigeria (Reference: A.926/438 dated 5/1/2018). Individual informed consent was obtained before the commencement of study.

Sample size determination: The sample size for this study was determined using the sample size determination for health studies formula:
$N=Z^2pq/d^2$ (18), and 14% estimate of small for gestational age in Nigeria (19).

Sample Preparation: The pregnant women were recruited at the third trimester of pregnancy and samples were collected immediately after delivery, the cord was clamped at both ends and cut. Five milliliters (5mL) of blood was collected from the umbilical vein into pyrogen-free plain tube containers and labeled appropriately. The cord blood was spun at 3000 rpm for 15minutes to obtain serum. The Plasma and serum was stored at -20^0 C until analysis for zinc and copper using Atomic Absorption spectrophotometric method.

Anthropometric Measurements
Birth weight of the neonates, head circumference, and recumbent length were measured by digital infant scale, flexible metal tape measure, and Seca 416 portable Infantometer respectively. The ponderal index (PI) was calculated as Birth weight (gr)/ Body length $(cm)^3$ x 100, to assess the fetal growth pattern. Gestational age, measured in weeks completed, was based primarily on the last menstrual period.

Estimation of Copper and zinc levels

The cord serum copper and zinc concentrations were measured by atomic absorption spectrometry (Buck Scientific Model VGP-210, Germany). Samples were digested by adding nitric acid diluted with deionized water (1:4). This was aspirated into the instrument after adequate calibration with series of copper and zinc standards at appropriate wavelengths (20).

Principle of the Test

Serum trace metals (Zinc and Copper) will be determined with flame Atomic Absorption Spectrophotometer (AAS) using direct method. The atoms of the elements, when aspirated into the AAS, vapourized, and absorb light of the same wavelenght, as that emitted by the metal when in the excited state i.e. in the vapourized ground state (unexcited), atom of a trace metal in the excited state. The amount of light absorbed is proportional to the trace metal in the solution.

Quality Control: The calibration standards were first prepared by making serial dilutions of the Perkin Elmer Cu standard 1000mg/L using 1% HNO_3 and the working range tested according to the manufacturer's recommended conditions.

Statistical Analysis

The data obtained were analyzed using statistical package for Social Science Program (SPSS) Version 21.0 (Chicago, IL, USA). The values obtained in this study are represented as Mean ± Standard Deviation. Student's t- test, Chi-Square and Analysis of Variance (ANOVA), were used to compare means while Pearson correlation coefficient was used to associate the measured parameters and birth weight. The $P<0.05$ was considered as statistically significant.

RESULTS:

The findings from this study are presented in table 1-4. Table 1 shows the comparison of anthropometric measurement of babies based on neonatal birth weight. The results obtained showed that birth weight ($p<0.01$), head circumference ($p<0.04$), Recumbent height ($p<0.04$) and Ponderal index ($p<0.01$) were significantly lower in Small for Gestational Age (SGA) than Appropriate for Gestational Age (AGA) neonates. Table 2 shows the comparison of the levels of zinc, copper and copper-to-zinc ratio in Cord blood of neonates based on birth weight. The results indicate that cord blood zinc was significantly lower ($p<0.001$) while copper-to-zinc ratio was significantly higher ($p<0.001$) in small for gestational age compared with appropriate for gestational age babies. There was no significant difference ($p>0.05$) in the cord blood copper level between appropriate for gestational age and small for gestational age neonates.

Table 3 shows the correlation of zinc, copper and copper-to-zinc ratio in cord blood with birth weight of neonates. The results indicate that cord blood zinc correlated positively ($R=0.33;p<0.001$) while copper-to-zinc ratio correlated negatively ($R=-0.23;p<0.001$) with birth weight of neonates. However, there was insignificant inverse relationship between cord blood copper with birth weight of neonates.

Table 4 shows the comparison of the levels of zinc, copper and copper- to-zinc ratio in cord blood based on Ponderal Index of SGA and AGA babies. The results indicate that cord blood zinc increased with increasing ponderal index while copper-to-zinc ratio decreased with increasing ponderal index among neonates. The zn levels in babies with ponderal index <22 was significantly lower than babies with ponderal index 22-30 among SGA and AGA babies. There was no significant difference in the mean Cu/Zn ratio among SGA babies based on ponderal index, but Cu/Zn ratio was significantly lower in babies with ponderal >30 among AGA babies.

Table 1: Comparison of Anthropometric measurements of Babies based on Neonatal Birth Weight

Anthropometric Parameters	SGA n=41	AGA n=159	P-value
Birth weight (Kg)	2.34±0.3	3.47±0.4	0.01
Head Circumference (cm)	32.3±1.3	34.4±2.8	0.04
Recumbent length (cm)	50.2±0.5	54.6±0.3	0.04
Ponderal Index (g/cm^3)	2.14±0.5	2.45±0.2	0.02

SGA=small for gestational age; AGA= Appropriate for gestational age

Table 2: Comparison of the levels of zinc, copper and copper-to-zinc ratio in Cord Blood of Neonates based on birth weight (Mean ± SD)

Parameters	Small for gestational age (<2.5 kg) n=41	Appropriate for gestational age (>2.5kg) n=159	t value	P value
Zinc (ug/dL)	61.8±2.44	75.6±1.31	4.83	0.001
Copper (ug/dL)	32.6±14.0	30.3±10.3	1.17	0.245
Cu/Zn ratio	0.59±0.061	0.43±0.06	3.78	0.001

Cu=copper; Zn=zinc

Table 3: Correlation of zinc, copper and copper-to-zinc ratio in Cord Blood with Birth weight of neonates

Correlation	R-Value	P-Value
Zinc (ug/dL)	0.33	0.001
Copper (ug/dL)	-0.067	0.357
Cu/Zn ratio	-0.23	0.001

Cu=copper; Zn=zinc

Table 4: Comparison of the Levels of zinc, copper and copper- to-zinc ratio in Cord Blood Based on Ponderal Index of SGA and AGA Babies(Mean ± SD)

Ponderal index	SGA <22.0(n=32)	(<2.5kg) 22-30(n=09)	AGA 22-30(n=101)	(>2.5Kg) >30(n=58
Zinc(µg/dL)	66.0±1.02[a]	68.0±1.0[b]	72.8±0.91[b]	78.5±1.52[b]
Copper(µg/dL)	30.5±1.08[a]	31.0±1.21[a]	31.6±1.02[a]	31.8±1.03[a]
Cu/Zn ratio	0.46±0.01[a]	0.46±0.02[a]	0.43±0.01[bc]	0.41±0.02[b]

a=p>0.05; b=p<0.001; c=p<0.05

DISCUSSION

Zinc and copper are important trace elements that are needed in the body for optimum human growth and development. Zinc and copper perform important functions; they act as cofactors in many antioxidants enzymes that protect against oxidative stress (21). We hypothesized that copper-to-zinc ratio may be associated with birth weight of neonates, since the ratio was previously reported to be associated with immune defense, growth stimuli and stress (22), inflammation and early onset of infection among neonates (23) and sickle cell anemia (24). Cord serum zinc was significantly higher and copper-to-zinc ratio significantly lower in AGA neonates than SGA neonates, while copper level in cord serum was not significantly higher in AGA than SGA babies. Copper-to-zinc ratio correlated inversely with birth weight among neonates and may be a better marker to assess health status than individual parameter. Also, it may be an important early prognostic biomarker of nutritional and health status in LBW neonates. The finding of association Cu/Zn ratio with LBW is consistent with previous study. It was reported that Cu/Zn ratio correlates independently with risk of LBW neonates (2). An inverse regulation of plasma Cu and Zn is a well-established and the Cu/Zn ratio has been observed to be a diagnostic marker of several disorders in humans such as pediatric infectious diseases (25), tuberculosis (26,27), autism, attention-deficit hyperactivity disorder, hypertension, inflammatory and malignant diseases (28-31). The ratio has also been used as a potential biomarker of inflammation, nutritional status and for mortality predictor in geriatric medicine (32). Others have reported that it can be helpful in the assessment of inflammation, immune system, and oxidative stress in peritoneal dialysis (33). Conversely, this finding is inconsistent with that of Maamouri et al.(34). The authors did not observe any significant alteration between cord serum zinc of LBW neonates and neonates weighing more than 2500 g (P=0.49).

In this study, there was an association between cord serum zinc with birth weight of neonates. This is consistent with previous study by Rwebembera (35), who reported a significant association between LBW and neonatal zinc. A positive association between gestational age and cord serum zinc has been reported (36). Conversely, Saleh et al. (37) did not observe significant correlation between neonatal cord blood zinc and gestational age in their study. Maamouri et al.(34) reported that 25% of their study population with LBW neonates had SGA and there were no significant changes in maternal zinc serum levels. They therefore suggested that the alteration between zinc level of LBW and normal neonates were mainly attributed to gestational age rather than the difference of neonatal birth weight. The limitation in their study was the small population size and they suggested the need for further study with higher population size in order to prove the hypothesis of the association between cord serum zinc and Cu/Zn ratio with birth weight of neonates. Interestingly, only term babies were evaluated in this present study as against both term and pre-term babies in the earlier studies. Therefore, pooling the mean cord serum zinc concentration for term and preterm babies as was done in other studies may have contributed to the variations observed in the mean cord serum zinc concentration and Cu/Zn ratio.

The result of this study showed that cord serum zinc was significant higher ($p<0.001$) in appropriate for gestational age neonates than small for gestational age neonates. The mean cord serum zinc concentration is consistent with the studies done by several authors (38). This is an indication of deficit in zinc supply from the mothers to their foetus thus causing intrauterine growth restriction reflected in cord serum zinc concentration. Similar lower cord serum zinc levels were reported by other authors elsewhere (2). Significantly lower zinc levels and association with LBW was reported by other authors (39,40). Term babies have been reported to have higher cord serum zinc concentration than preterm babies because of a longer period of zinc accretion in the term babies than the preterm babies (41, 42).

On the other hand, no significant difference ($p>0.05$) was observed in the cord serum copper level between appropriate for gestational age neonates compared with small for gestational age neonates. The finding partially aligns with previous study by Bermúdez *et al.* (43) who reported that copper was the only trace element in umbilical cord blood observed to be inversely and independently related to birth weight the highest copper concentrations were reported in the SGA group. Interestingly, copper is a trace element that is involved in the function of several cuproenzymes that are essential for life, and their requirement increases during pregnancy (43). Conversely, this result is inconsistent with that of Maamouri et al.,(34) who reported that neonatal serum copper level was lower in LBW neonates than neonates with normal birth weight.

Finally, the results of this study showed significant difference ($p<0.05$) in the levels of zinc and Cu/Zn ratio in cord blood based on Ponderal Index, while copper levels did not show any significant difference ($p>0.05$). This finding is in tandem with previous studies (44). Low birth weight is a public health challenge in developing countries and a risk factor for infant development, and increased mortality and morbidity. Majority of the LBW neonates are SGA and intrauterine growth restriction (IUGR). The readily identifiable contributing factor to LBW is maternal micronutrient deficiencies including zinc. Several cost effective ways have been promoted by international organizations to minimize the delivery of LBW neonates in developing countries. These include promotion of antenatal use of multiple micronutrients supplementation and the ways to minimizing zinc deficiency as part of the Millennium Development Goal 1(45).

In conclusion, cord serum zinc was higher in neonates with SGA and AGA babies while Cu/Zn ratio was lower in neonates with AGA than SGA neonates. Cord serum zinc and Cu/Zn correlated negatively with SGA neonates and ponderal index. Intensive implementation of zinc supplementation in pregnancy at all health care levels is suggested to reverse the observed relationship between zinc, Cu/Zn ratio with low birth weight.

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