

## Association Between Dietary Factors and Cord Serum Zinc Level

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### ABSTRACT

Zinc deficiency in neonates is a common finding in the developing world. The serum zinc level of neonates is affected by the gestational age at delivery, maternal serum zinc level, maternal serum albumin concentration, medications, conditions leading to decreased absorption of ingested zinc as well as increased loss from the body. Zinc deficiency in neonates has been suggested to be related to poor growth, hyperbilirubinemia, seizures, necrotizing enterocolitis, retinopathy of prematurity and bronchopulmonary dysplasia by some investigators. The purpose of this study was to determine association between dietary factors and cord serum zinc levels of neonates delivered at FMC Owerri. Three hundred and thirty mother-neonate pairs who met the inclusion criteria were consecutively recruited; one hundred and eighty (54.5%) of the neonates were males while 150(45.5%) were females. Serum zinc was assayed using Flame Atomic Absorption Spectrophotometer (AAS). The result from this research showed prevalence of low cord serum zinc level was noticed to be least in babies whose mothers took red meat, dairy products and sea food daily when compared with those whose mothers took these items three days or less in a week. However the difference was not statistically significant p-values 0.17, 0.26 and 0.37 respectively.

Keywords: Zinc deficiency, Dietary factors, Cord serum and Zinc level.

### INTRODUCTION

The world Health Organization (WHO) defines neonates as children in their first 28 days of life. Neonates are grouped into preterm, term and post-term into Large for Gestational Age (LGA), Small for Gestational Age (SGA), and Appropriate for Gestational Age (AGA) neonates [1]. Optimal serum zinc level is required by neonates in order to avert postnatal growth failure and other far-reaching consequences of zinc deficiency bearing in mind that the fastest post-natal growth rate in humans is achieved during early infancy [2,3,4]. Zinc is an essential trace element in humans. It is an antioxidant and free radical scavenger which mops up reactive oxygen species and protects the human body from endogenous and exogenous insults [5]. It has a vital role in a wide range of biological activities including the maintenance of cell architecture, protein synthesis, nucleic acid metabolism and immune functions [6]. Zinc is fundamental for growth, development and reproduction while its deficiency has a negative effect on the endocrine system leading to growth failure. It is known to undergo depletion during pregnancy and lactation [7,8]. In pregnancy, maternal zinc depletion is caused by increased uptake of maternal

zinc by foetus and placenta, increased transfer of serum zinc to maternal erythrocytes, expansion of maternal plasma volume and decreased availability of serum albumin which binds zinc in the mother's blood. During lactation, zinc is transferred from the mother into the breast milk to nourish the neonate [9]. Some neonates have been found to suffer zinc deficiency at birth; especially the Low Birth Weight (LBW) neonates which comprises children born preterm and those with Intrauterine Growth Restriction (IUGR) [10]. The reference range for normal serum zinc in the neonate is 9.9-21.4  $\mu\text{mol/l}$  (equivalent to 64.7-139.9  $\mu\text{g/dl}$ ) while that for the mother in the third trimester of pregnancy is 7.6-10.7  $\mu\text{mol/l}$  (equivalent to 49.9-69.9  $\mu\text{g/dl}$ ) [11,12]. Zinc deficiency in neonates has been suggested by some investigators to be implicated in dermatitis, low birth weight, impaired immunity, poor wound healing, necrotizing enterocolitis, seizures, bronchopulmonary dysplasia, hyperbilirubinemia and retinopathy of prematurity [13,14,15]. In the older child, it has been suggested to be associated with acrodermatitis enteropathica, growth failure, poor

wound healing, anaemia, hypogonadism, poor taste and smell sensation [16,17]. There is paucity of data on zinc excess in neonates however a report on zinc excess in total parenteral nutrition of a preterm resulted in death from cardiac failure [18,19]. In older children however zinc excess presents with abdominal pain, diarrhoea, headaches, fatigue and abdominal cramps. It has also been associated with copper deficiency [20]. The bulk of zinc transfer from the mother to foetus takes place in the third trimester and this, therefore, predisposes the premature neonates to zinc deficiency as enough zinc is not transferred from the mother to the foetus before it is born. The IUGR neonates are also predisposed to low serum zinc [21,22]. The risk factors for low serum zinc in neonates are varied and include LBW, genetic defects, maternal malnutrition (from inadequate dietary intake, decreased bioavailability, decreased absorption, excessive losses and increased requirements) as well as from iatrogenic causes like poorly constituted total parenteral nutrition. In addition, male gender, use of thiazide diuretics, dexamethasone, unusually low

alkaline phosphatase, low albumin, large stool or ostomy output and short bowel syndrome may also cause low serum zinc in neonates [22]. The global prevalence of zinc deficiency is estimated at 31% with values ranging from 4-73%. The burden of zinc deficiency is borne most heavily by countries in Africa, the Eastern Mediterranean and South East Asia [23,24], in their study however reported that 25% of the world population is at risk of zinc deficiency. This micronutrient deficiency contributes to over half a million deaths per year in children aged 0-60 months. [21], in Iran, Western Asia reported that the prevalence of low serum zinc in neonates was 11.9%. In Sub-Saharan Africa, zinc deficiency has been found to affect an estimated 68% of the population. The prevalence of low plasma zinc concentration among young children was noted to be 45% and 81% respectively in South Africa and Cameroon respectively. Few studies have been carried out on serum zinc level among neonates in Nigeria. The studies concur that deficiency of zinc in neonates is high across the nation.

#### MATERIALS AND METHODS

##### STUDY AREA

The study was carried out at the delivery room and Obstetrics theatre of FMC Owerri. The population of Imo state is about 3.93 million with about 401,873 people living in Owerri. Most of those living in Owerri are civil servants while traders and artisans constitute a small percentage of the population. The inhabitants of Owerri are predominantly of Igbo tribe. Federal Medical Centre Owerri is the foremost tertiary health institution in Imo state. It however provides primary, secondary and tertiary healthcare services in Paediatrics, Obstetrics and Gynaecology, Internal Medicine, and Surgery. It

provides healthcare for patients from Imo state and parts of Abia, Anambra and Rivers states. The Paediatrics department is made up of the children's emergency, the children's ward, the children's outpatient department and the special care baby unit. The SCBU cares for sick neonates. It has two sections; the inborn and the out born units. The Obstetrics department conducts an average of 1500 deliveries yearly. The delivery room has 8 beds and is opposite the prenatal ward which has 12 beds while the Obstetrics theatre is situated between SCBU and the delivery room.

STUDY POPULATION

This consisted of neonates delivered at FMC Owerri within the study period and their respective mothers.

ETHICAL CONSIDERATION

Ethical approval (Appendix 1) for this proposal was obtained from the research and ethics committee of FMC Owerri.

INCLUSION CRITERIA

- 1 Neonates delivered at FMC Owerri within the study period.
- 2 Mothers who gave consent.

EXCLUSION CRITERIA

- 1 Neonates whose mothers were placed on zinc supplements during pregnancy
- 2 Neonates with gross congenital anomalies.
- 3 Neonates whose mothers had preeclampsia and eclampsia in pregnancy.
- 4 Neonates whose mothers suffered severe heart or lung diseases during pregnancy.

INFORMED CONSENT

A written informed consent was obtained from the mothers once labour was established or as soon as she came in for caesarean section. The informed consent was obtained after providing information to parents regarding the study particularly benefits and risks involved in doing this study.

RECRUITMENT OF STUDY SUBJECTS

Mothers who met the inclusion criteria were recruited as soon as labour was established or as soon as they came for caesarean section and a proforma was administered to her. This included her personal data, parity, socio-economic indices, nutrition while pregnant and medications taken while pregnant. All live neonates delivered in the labour ward and obstetrics theatre of FMC Owerri who met the inclusion criteria were consecutively recruited until the desired sample size was achieved. A quick general examination was carried out on the neonate before blood sample was collected from the umbilical cord. A more detailed examination was carried out on the neonate after sample collection. Warmth was provided using the resuscitator for those that needed warmth.

SAMPLE SIZE ESTIMATION

The sample size for this study was calculated using the formula for calculating sample size when the study population is less than 10,000.

$$nf = \frac{n}{1 + \left(\frac{n}{N}\right)}$$

nf = the desired sample size when population is less than 10,000

n = desired sample size when the population is more than 10,000.  
N = the estimate of the population size

To calculate n, the formula  $n = \frac{z^2 pq}{d^2}$

n = minimum sample size  
z = normal standard deviation set at 1.96 which corresponds to the 95% confidence interval.  
P = prevalence of zinc deficiency in Nigerian neonates. In this study, a prevalence of 39.6%  
q = 1.0 - p

d = degree of accuracy desired (considered significant at the 0.05 level).  
Therefore  $n = \frac{(1.96)^2 (0.39)(0.61)}{(0.05)^2}$   
= 0.9139 / 0.0025  
= 366

$$nf = \frac{366}{1 + \left(\frac{366}{1500}\right)}$$

$$\begin{array}{r} 366 \\ = \\ \hline 1.244 \\ =294 \end{array}$$

Giving room for 10% attrition=29

Calculated sample size= 294+29= 323 neonates.

The respective mothers (323) of these neonates were also recruited and their serum zinc also assayed.

#### SAMPLING METHOD

The neonates and their mothers were recruited consecutively until the desired sample size was attained.

#### STUDY PROCEDURE

The mother was counselled on the procedure and a written informed consent obtained from her. The study proforma was used to record the mother's biodata, parity, origin, address, phone contact. Other information recorded in the proforma included maternal intake of zinc-rich foods during pregnancy, number of antenatal visits and gestational age at delivery. Her height and weight were also measured and her HIV status was also recorded. Then 3 millilitres of venous blood was collected from a prominent vein on the mother's upper limb after cleaning the area with a combination of 2% chlorhexidine and isopropyl alcohol. The sample was put in a pre-labelled sterile anticoagulant free bottle that had been immersed in 10% nitric acid and rinsed in deionized water to make it free from trace elements. Samples were transported in vaccine-rush containers with ice-gel packs (to prevent hemolysis of red cells) to the hematology department of FMC Owerri where samples were centrifuged for 10 minutes by the laboratory scientist and researcher. After centrifugation, the serum was separated from the cells with a bulb pipette and stored in a Thermocool® freezer at a temperature of -20°C until enough samples were pooled for analysis. Upon delivery of the neonate and before delivery of the placenta, the cord was double-clamped and the severed end (also known as the placental end) of the cord was cleaned with a sterile gauze to reduce contamination by Wharton's jelly and maternal blood and was placed into the barrel of a 20 millilitres syringe and the clamp was released to allow the flow of cord blood from the cord to the barrel of the syringe and the blood (3 millilitres) was subsequently transferred to the specimen bottle from the syringe. This was done after ensuring that the

neonate did not have any gross congenital anomaly. The sample was also put into a trace-element decontaminated container, taken to the hematology laboratory for centrifugation and separation of serum from the blood cells, stored in Thermocool® freezer at -20°C same way with the mother's sample. Meanwhile the neonate was dried, provided with warmth on the resuscitaire (for those that needed it) and within this period, the neonate was examined mainly for the weight, length, occipitofrontal circumference; presence or absence of skin changes, palor and jaundice. The New Ballard scoring for preterm neonates was also done and the neonates were classified using the relationship between birth weight and gestational age on a standard growth chart (Colorado). All these measurements and examination findings were recorded in the study proforma. These samples (mothers' and neonates') that had been stored at -20°C were transported by road to the research laboratory at Nnamdi Azikiwe University Awka, Anambra State in vaccine rush containers with ice gel packs. In the research laboratory, the samples were also stored at the same temperature of -20°C before analysis. The researcher and the laboratory scientist analysed the samples using the Flame AAS machine. The serum was diluted five-fold with deionized water and passed through the Atomic Absorption Spectrophotometer; the diluted solution was compared against standards prepared to approximate viscosity in glycerol. The electrons of the atoms in the atomizer (a component of the AAS) were promoted to higher orbitals by absorbing a defined quantity of energy (radiation) in a process called atomization; the wavelength it travels corresponded to only one element giving the technique its elemental selectivity. The radiation

flux with the standard was compared with that of the sample and the ratio between the two also known as the absorbance was converted to the concentration of the analyte(sample). The maternal serum zinc level was low when values below  $49.9\mu\text{g}\text{dl}$  are recorded while the cord serum zinc level was said to be low when values less than  $64.7\mu\text{g}\text{dl}$  are recorded. The cord blood

serum zinc level of the neonate and serum zinc level of the mother were recorded in the proforma. The mothers of the zinc- deficient neonates were contacted to bring their neonates to the neonatology follow-up clinic for treatment;the zinc-deficient mothers were also contacted and referred to the gastroenterology clinic for treatment.

#### QUALITY CONTROL

Samples were collected from the cord immediately the umbilical cord was severed. These samples were centrifuged at the FMC Owerri laboratory, separated with a bulb pipette and then stored in the Thermocool® freezer at -20 degrees Celsius. This was ensured by keeping a dedicated freezer under lock and key at one end of the SCBU call room which had a constant light supply to power the incubators.

These stored samples were transported to Awka in ice pack using a private vehicle in order to shorten the time spent on the road thereby avoiding temperature alterations. At the laboratory the samples were also transferred into a freezer for storage before analysis. Before analyzing the samples, standards were prepared and were run at intervals to ensure similar results were obtained.

#### DATA ANALYSIS

Data was analysed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive analysis such as mean and standard deviation were calculated for continuous variables like cord serum zinc levels; frequency distribution tables and percentages were used for variables like gender and mode of delivery of neonate while bar chart was used to demonstrate the relationship between categories

of gestational age, birth weight and cord serum zinc. Chi-Square was used to determine association between categorical variables like association between cord serum zinc and gender while Pearson's Correlation was used to test for strength and direction of association between cord serum zinc and maternal serum zinc;  $p\text{-value} \leq 0.05$  was regarded significant.

#### RESULTS AND DISCUSSION

##### ASSOCIATION BETWEEN DIETARY FACTORS AND CORD SERUM ZINC LEVEL

Prevalence of low cord serum zinc level was noticed to be least in babies whose mothers took red meat, dairy products and sea food daily when compared with those whose mothers took these items

three days or less in a week. However the difference was not statistically significant  $p\text{-values}$  0.17, 0.26 and 0.37 respectively as shown in table I below.

Table I Association between dietary factors and cord serum zinc level

Dietary factors	Cord Serum Zinc level		Total	$\chi^2$	p-value
	Low	Normal			
<b>Red Meat Intake</b>					
Daily	40 (37.0)	68 (63.0)	108		
4 - 6 days/week	76 (54.3)	64 (45.7)	140		
1 - 3 days/week	38 (51.4)	36 (48.6)	74	5.02	0.17
None	6 (75.0)	2 (25.0)	8		
Total	160 (48.5)	170 (51.5)	330		
<b>Dairy Intake</b>					
Daily	22 (33.3)	44 (66.7)	66		
4 - 6 days/week	84 (53.8)	72 (46.2)	156		
1 - 3 days/day	50 (50.0)	50 (50.0)	100	4.05 <sup>¥</sup>	0.26
None	4 (50.0)	4 (50.0)	8		
Total	160 (48.5)	170 (51.5)	330		
<b>Sea Food</b>					
Daily	40 (39.2)	62 (60.8)	102		
4 - 6 days/week	66 (51.6)	62 (48.4)	128		
1 - 3 days/week	52 (55.3)	42 (44.7)	94	3.15 <sup>¥</sup>	0.37
None	2 (33.3)	4 (66.7)	6		
Total	160 (48.5)	170 (51.5)	330		

Likelihood ratio

## DISCUSSION

The prevalence of zinc deficiency in the index study was lower in neonates whose mothers ingested red meat, dairy products and sea foods daily compared to those whose mothers took these items less than three days in a week, though the relationship was not statistically significant. In contrast, Schulpis et al<sup>9</sup> in Greece showed through a 60-day dietary recall that mothers who were on zinc-rich diets had a higher maternal and cord blood serum zinc

when compared to those who took diets that are poor in zinc content and the relationship was statistically significant. This can be explained by the fact that this study was carried out in a country with geographical indices which are different from that in the index study. In New Zealand it was documented that vegetarian mothers or mothers who stopped ingesting red meat in pregnancy gave birth to neonates with low serum zinc.

## CONCLUSION

Low cord serum zinc level tended to occur more in neonates whose mothers

took red meat, dairy products and seafoods three times or less in a week.

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