Association between cord serum zinc level and birth weight, gender, socioeconomic class and maternal 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. 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 normal range for serum zinc is 9.9-21.4µmol/l (equivalent to 64.7-139.9µg/dl) for cord blood and 7.6-10.7µmol/l (equivalent to 49.9-69.9µg/dl) for maternal blood. For the purpose of this study, cord serum zinc less than 64.7µg/dl and maternal serum zinc less than 49.9µg/dl were considered low. The cord serum zinc levels of neonates was normal in 51.5% of cases but low in 48.5% of cases. There was a significant positive association between cord blood serum zinc and maternal serum zinc however cord serum zinc level was not significantly associated with birth weight, length, gender and socioeconomic status. In conclusion from this study included that the prevalence of hypozincemia was high in neonates delivered at FMC Owerri, maternal serum zinc is a strong factor influencing cord serum zinc and that low cord serum zinc is commoner among Low Birth Weight (LBW) neonates, female neonates, neonates from low socioeconomic class families and in neonates whose mothers took little of zinc-rich foods in pregnancy. This study therefore recommends improved maternal nutrition in pregnancy especially with zinc-rich foods and to give zinc supplements to pregnant mothers during antenatal care visits.

Keywords: Cord serum, zinc level, birth weight, gender and socioeconomic class

INTRODUCTION

The world Health Organization (WHO) defines neonates as children in their first 28 days of life [1]. Neonates are grouped into preterm, term and postterm into Large for Gestational Age (LGA), Small for Gestational Age (SGA), and Appropriate for Gestational Age (AGA) neonates [2,3]. 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 [3,4]. Zinc is an essential trace element in humans [5]. It is an antioxidant and free radical scavenger which mops up reactive oxygen species and protects the human body from endogenous and exogenous insults. 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 [6,7]. 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 [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 [8,9]. 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 9.9-21.4µmol/l(equivalent to 64.7-139.9µg/dl) [11] while that for the mother in the third trimester of pregnancy is 7.6-10.7µmol/l(equivalent to 49.9-69.9µg/dl) [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 [16,17,18]. 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 [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 [21]. The IUGR neonates are also predisposed to low serum zinc [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.16In 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.

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 DESIGN
STUDY POPULATION
This consisted of neonates delivered at FMC Owerri within the study period and their respective mothers.
ETHICAL CONSIDERATION

Ethical approval for this proposal was obtained from the research 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 consecutively recruited until the desired sample size was attained. 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 resuscitare 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.

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

\[ nf = \text{the desired sample size when population is less than 10,000} \]
\[ n = \text{desired sample size when the population is more than 10,000} \]
\[ N = \text{the estimate of the population size} \]

To calculate \( n \), the formula \( n = \frac{z^2pq}{d^2} \) is used.
\( n \) = 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%.
\( q = 1 - p \)
\( d \) = degree of accuracy desired (considered significant at the 0.05 level).

Therefore \( n = (1.96)^2 (0.39) (0.61) / (0.05)^2 \)
\[ = 0.9139 / 0.0025 \]
\[ = 366 \]

\[ \frac{366}{1 + \left( \frac{366}{1500} \right)} \]
\[ = 366 \frac{1.244}{1.244} \]
\[ = 294 \]

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 resuscitator (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 49.9µg/dl are recorded while the cord serum zinc level was said to be low when values less than 64.7µg/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.

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.

**QUALITY CONTROL**

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-value ≤ 0.05 was regarded significant.

**FACTORS AFFECTING ZINC STATUS OF NEONATES**

Low cord serum zinc level was found to be more common among neonates whose mothers had low serum zinc level 102 (58.6%) when compared with mothers with high serum zinc level 14 (24.1%) in a statistically significant relationship. Low cord serum level was also found to be commoner among low birth weight neonates, female gender, and low socioeconomic status but this did not reach statistically significant level p values were 0.247, 0.078 and 0.077 respectively as shown in table IV below.

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**Table I Association between Cord Serum Zinc Level and Birth Weight, Gender, Socioeconomic class and Maternal Serum Zinc Level.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cord Serum Zinc Status</th>
<th>Total</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Birth Weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>16 (53.3)</td>
<td>14 (46.7)</td>
<td>30</td>
<td>2.80*</td>
</tr>
<tr>
<td>Normal</td>
<td>144 (48.6)</td>
<td>152 (51.4)</td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>4 (100)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>160 (48.5)</td>
<td>170 (51.5)</td>
<td>330</td>
<td>2.80*</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>76 (42.2)</td>
<td>104 (57.8)</td>
<td>180</td>
<td>3.11</td>
</tr>
<tr>
<td>Female</td>
<td>84 (56.0)</td>
<td>66 (44.0)</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>160 (48.5)</td>
<td>170 (51.5)</td>
<td>330</td>
<td>3.11</td>
</tr>
<tr>
<td><strong>Social economic Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>54 (39.1)</td>
<td>84 (60.9)</td>
<td>138</td>
<td>5.125</td>
</tr>
<tr>
<td>Middle</td>
<td>80 (52.6)</td>
<td>72 (47.4)</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>26 (65.0)</td>
<td>14 (35.0)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>160 (48.5)</td>
<td>170 (51.5)</td>
<td>330</td>
<td>5.125</td>
</tr>
<tr>
<td><strong>Maternal serum Zinc</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>102 (58.6)</td>
<td>72 (41.4)</td>
<td>174</td>
<td>10.713</td>
</tr>
<tr>
<td>Normal</td>
<td>44 (44.9)</td>
<td>54 (55.1)</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>14 (24.1)</td>
<td>44 (75.9)</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>160 (48.5)</td>
<td>170 (51.5)</td>
<td>330</td>
<td>10.713</td>
</tr>
</tbody>
</table>

*Likelihood ratio

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THE DISTRIBUTION OF CORD SERUM ZINC ACCORDING TO BIRTH WEIGHT

The distribution of cord serum zinc according to birth weight is shown in Figure1 below. Neonates with normal birth weight had normal cord serum zinc (89.4%) followed by those with low birth weight (8.2%) and macrosomic neonates (2.4%).
Figure 1: Bar chart showing the distribution of cord serum zinc by birth weight.
THE DISTRIBUTION OF CORD SERUM ZINC ACCORDING TO GESTATIONAL AGE

The distribution of cord serum zinc according to gestational age is represented in figure 2 below. Neonates delivered between 37 and 42 weeks had the highest normal cord serum zinc (95.3%) followed by those delivered below 37 weeks (4.7%) while non of those delivered after 42 weeks had normal serum zinc (0%) .

![Figure 2 Bar chart showing the distribution of serum zinc status by gestational age.](image)

DISCUSSION

The index study found that there was no significant association between cord blood serum zinc and gender indicating that gender is not a significant risk factor for low cord serum zinc [25]. This finding is in tandem with other studies like that by [26] However,[27] in North Central Nigeria reported that more females were zinc deficient and this might have arisen because they also studied older children (under-fives); however in New Zealand, male gender was found to predispose to zinc deficiency in neonates [28]. The finding of the index study was that cord serum zinc was lower among neonates whose parents were from low socioeconomic class although this relationship was not statistically significant. The findings of the index study are corroborated by [29] in Turkey. In contrast, a group of Nigerian investigators found a statistically significant relationship between cord blood serum zinc and socioeconomic class [30]. This finding may have arisen from the fact that these investigators recruited their subjects from two different venues (a tertiary hospital and a rural hospital) while the index study recruited subjects only from a tertiary centre. [31] in Egypt also found a statistically significant relationship between socioeconomic class and cord serum zinc but this may have arisen because these investigators excluded neonates who had needs for admission as against the present study.
CONCLUSION

In conclusion, results from this study suggested that there was no significant association between cord blood serum zinc and gender. The results also indicated that cord serum zinc was lower among neonates whose parents were from low socioeconomic class although this relationship was not statistically significant.

REFERENCES


12. Vargas ZCL, Melo MRR, Donangelo CM. Maternal placental and cord zinc components in healthy women with different levels of serum zinc.*Biol Neonate* 1997; 72:84-93


