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Assessment of Zinc, Selenium and Vitamin C Status in Maternal and Umbilical Cord Blood of Intrapartum Women in Enugu Metropolis, Enugu State, Nigeria

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ABSTRACT

Background: Zinc, selenium, and vitamin C are vital antioxidants that mitigate oxidative stress. Pregnancy-induced metabolic changes may alter their levels, affecting maternal and fetal health. **Aim:** This study evaluated zinc, selenium, and vitamin C concentrations in maternal and umbilical cord blood of women in labor in Enugu Metropolis, Nigeria. **Methods:** A cross-sectional study was conducted among 48 mother-neonate pairs. Maternal and umbilical cord blood samples (5 mL each) were collected postpartum. Zinc and selenium were analyzed using atomic absorption spectrophotometry, while vitamin C was measured colorimetrically. **Results:** Mean maternal and cord serum zinc levels were 41.61 ± 2.45 $\mu\text{g/dL}$ and 42.65 ± 4.7 $\mu\text{g/dL}$, respectively, indicating deficiency. Selenium averaged 168.10 ± 14.47 $\mu\text{g/L}$ in maternal serum and 197.56 ± 16.74 $\mu\text{g/L}$ in cord blood, with neonatal levels exceeding physiological limits. Vitamin C concentrations were 7.53 ± 0.26 mg/L (maternal) and 7.11 ± 0.50 mg/L (cord), both within normal ranges. Correlation analysis showed a weak maternal-cord zinc relationship ($r = 0.11$, $P = 0.46$), a significant positive correlation for selenium ($r = 0.48$, $P = 0.00059$), and a slight negative correlation for vitamin C ($r = -0.022$, $P = 0.88$). **Conclusion:** Zinc deficiency in maternal and cord blood highlights the need for routine monitoring and supplementation. Elevated neonatal selenium suggests potential toxicity risks, requiring further research. Adequate vitamin C levels indicate sufficient nutrition, supporting immune function and oxidative stress reduction. These findings emphasize the importance of maternal micronutrient balance for neonatal health.

KEYWORDS: Maternal blood, micronutrients, selenium, umbilical cord blood, Vitamin C, zinc

INTRODUCTION

Pregnancy is a period of increased metabolic demands with changes in a woman's physiology and the requirements of a growing fetus.^[1] Proper nutrition before and throughout pregnancy is one of the key factors for ensuring a healthy pregnancy.^[2] Pregnant women need to maintain sufficient levels of essential minerals and vitamins to avoid adverse pregnancy outcomes. Inadequate supplies of vital micronutrient minerals and vitamins can harm the mother's and developing embryo's wellbeing.^[3] Nutrition of the fetus begins at conception. This means that the nutritional

status of the mother can affect birth outcomes. It is estimated that 24% of children born underweight are a result of poor nutrition.^[4]

Micronutrients are essential elements that our body requires in small quantities but perform crucial functions. The absence of these micronutrients leads to a condition

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referred to as hidden hunger.^[5] Hidden hunger arises when the food consumed fails to meet our body's nutrient requirements.^[5] In other words, the food lacks the necessary nutrients that our body needs for proper growth and development.^[5] Some micronutrients have antioxidant properties that are beneficial to the body.^[6] These antioxidant substances can stop or slow down damage to cells caused by unstable molecules known as free radicals that the body produces as a result of normal metabolism or by exposure to environmental factors such as smoking, ultraviolet light, and tobacco.^[7] The inability of the body to get rid of these free radicals can lead to oxidative stress.^[7] Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, and respiratory diseases.^[8] Oxidative stress has also been implicated in many reproductive and pregnancy disorders from sub-fertility to miscarriage, maternal vascular disease, and preterm labor.^[9] Antioxidant micronutrients are those micronutrients that can help to eliminate free radicals from the body and they include zinc, selenium, copper, manganese, riboflavin, vitamin A, vitamin C, and vitamin E.^[10]

Zinc, an essential mineral that promotes growth and healing, can be found in various foods such as liver, eggs, nuts, cereals, and seafood. Zinc deficiency can lead to several health challenges, including stunted growth, poor wound healing, impaired cognitive and motor function, anemia, and poor gonadal function. Furthermore, zinc deficiency can worsen pneumonia, diarrhea, and loss of appetite.^[11] Selenium is another crucial nutrient that acts as an antioxidant. Food sources of selenium include oysters, cooked salmon, liver, and Brazil nuts. Selenium deficiency has been implicated in many reproductive complications such as low birth weight, preeclampsia, preterm deliveries, and premature babies.^[12] Vitamin C is a water-soluble lactone essential in the human body for proper functioning. The most common disorder associated with vitamin C deficiency is scurvy. This disorder is distinguished by bleeding gums, pain in the extremities, exhaustion, deformed bone growth in infants, hemorrhagic manifestations, ulcerations, and ultimately death.^[13] Vitamin C is an antioxidant that supports immune function and facilitates the absorption of iron. It plays an important role in collagen, hormone, and amino acid formation. Vitamin C can be found in cabbage, oranges, lemon, spinach, and broccoli.

Lack of specific antioxidant activities linked with the micronutrient zinc and selenium deficiency can lead to some undesirable pregnancy outcomes which include restriction in fetal growth, preeclampsia, and a greater risk of diseases in later life.^[14-16] Premature fetal membrane rupture, miscarriage, and preeclampsia

have also been linked to low levels of plasma vitamin C.^[17,18] To prevent complications for pregnant women, it is essential to assess micronutrient levels among them, particularly in Enugu Metropolis, as these tests are not routinely conducted in antenatal clinics. Therefore, this study aims to examine the concentrations of zinc, selenium, and vitamin C in both maternal and umbilical cord blood of women in labor in Enugu Metropolis. The objectives of the study were to determine the mean concentrations of zinc, selenium, and vitamin C in maternal and umbilical cord blood and to assess the correlation between maternal and umbilical cord blood levels of zinc, selenium, and vitamin C.

MATERIALS AND METHOD

Study area and study design

This study was conducted in Enugu Metropolis, Enugu State, Nigeria. Enugu State, located in the southeastern region of Nigeria, is one of the 36 states in the country. The state is characterized by a landscape dominated by grass and trees, making it conducive for agriculture. Major agricultural products of the state include palm oil, cassava, rice, and maize. The study population consisted of pregnant women attending antenatal clinics in selected health facilities within the study area. A descriptive study design was employed, and participants were recruited using a multi-stage random sampling technique. At stage one, Enugu North LGA was selected out of the 17 Local Government Areas (LGAs) in Enugu State. For stage two, Ogui Township, Asata Township, New Haven, and GRA wards were selected by balloting out of the 13 wards in Enugu North LGA. At stage three, in each of the four selected wards, one hospital was randomly chosen for the study namely, Holy Ghost Maternity and Children's Hospital, St. Mary's Hospital and Maternity, Paulsonic Hospital Limited, and Poly General Hospital, Asata. Finally, at stage four, approximately 24 pregnant women were recruited for the study from each selected hospital.

Sample size

The sample size was calculated using the formula given by (Godden, 2004)^[19]

$$SS = \frac{Z^2 \times p \times (1 - p)}{C^2}$$

where SS denotes sample size, confidence level demonstrated as Z, percentage of population in decimal shown as P and C as confidence interval expressed as decimal.

$$Z = 1.96, P = 0.5, C = 0.1$$

$$SS = \frac{1.96^2 \times (0.5) \times (1 - 0.5)}{(0.1)^2}$$

The calculated sample size (SS) = 96

Informed consent

A detailed explanation of the study objectives and procedures was provided to the pregnant women. Only those who voluntarily provided oral or written consent were included in the study.

Inclusion and exclusion criteria

Healthy pregnant women who gave informed consent and were in their last trimester of pregnancy were included in the study while women who are likely to deliver twins were excluded from the study.

Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching Hospital, Enugu (NHREC/05/03/2008B-FWA00003 452-1RB00003328). Permission was also obtained from the administrative offices of the selected health facilities. Informed consent was secured from all study participants.

Sample collection and processing

Five (5) mL of venous blood was collected from 48 pregnant women before delivery. Immediately after delivery, an additional 5 mL of blood was collected from the umbilical cord using sterile, disposable syringes by the attending nurses. Visibly hemolyzed samples were discarded, and fresh samples were recollected to ensure the integrity of the analysis. The collected blood samples were discharged into sterile plain tubes and allowed to stand at room temperature for 45 minutes to one hour. The serum was separated by centrifugation at 3000 rpm for seven minutes. A micropipette was used to transfer the serum into new sterile plain tubes, which were then stored at -20°C before being transferred to Springboard Research Laboratories for further analysis using standardized laboratory protocols to ensure the accuracy and reliability of the results.

BIOCHEMICAL ANALYSIS

Analysis of zinc, selenium, and vitamin C

Elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA.^[20] The atomic absorption spectrophotometer (AAS) basis of operation is that a sample is atomized by aspiration into flame. The AAS light beam is then directed through the flame into the monochromator and into the detector which measures the amount of light that has been absorbed by

the atomized element in the flame. Different metals have their unique absorption wavelength, so a lamp source composed of that element is used. This helps to eliminate spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element.

Reference solution preparation

A set of standard metal solutions in the requisite concentration scale was prepared. The reference solutions were prepared daily by diluting the single-stock element solutions with water containing 1.5 mL of concentrated nitric acid per liter. A calibration blank was prepared using all the reagents excluding the metal stock solutions. A calibration curve for each metal was prepared by plotting the absorbance of standards against their concentrations.

Sample digestion

Sample digestion was done according to the method of Adrian^[21] Four milliliters (4.0 mL) of distilled water was added to 1.0 mL of the sample. The mixture was thoroughly shaken and boiled at 37°C for 20 minutes. Absorbance was measured using FS240AA Agilent atomic absorption spectrophotometer. The Standard flame emission conditions for zinc and selenium are given in Table 1.

Preparation of stock standard solution

One thousand milligrams per litre (1000 mg/L) of the stock metal solution was dissolved in a minimum volume of (1:1) HNO_3 and diluted to one liter with 1% (v/v) HCL. Appropriate dilutions were carried out to produce 2, 4, and 6 ppm working solutions.

Estimation of vitamin C

Ascorbic acid was analyzed using the spectrophotometric method described by Roe and Kuether.^[22] Ascorbate was treated with activated charcoal which converts it to dehydroascorbate. Dehydroascorbate then reacts with 2,4-dinitrophenyl hydrazine to form an osazone. Osazones give an orange-colored solution when dissolved in sulphuric acid and absorbance is measured spectrophotometrically at 540 nm.

Extracting ascorbic acid

Standard ascorbate varying from 0.2–1.0 mL to 0.5–1.0 mL of the supernatant was measured out and the volume was made up to 2.0 mL with 4% TCA. One-half (0.5) mL of 2,4-dinitrophenylhydrazine reagent was further put in all the tubes. Two (2) drops of 10% thiourea were added to all the tubes. The components of the tube were thoroughly mixed and left to incubate for 180 minutes at 37°C which resulted in the formation of osazone crystals. Two and

a half (2.5) mL of 85% sulphuric acid was used to dissolve the crystals in cold conditions. To the blank alone, 2,4-dinitrophenylhydrazine reagent and thiourea were put after the addition of sulphuric acid. The tubes were cooled on ice and the absorbance of the sample was read at 540 nm using a spectrophotometer (Genesys 10-S, USA).

Concentration of sample (mg / L)

$$= \frac{\text{Absorbance of sample} \times \text{concConc.of standard}}{\text{Absorbance of standard}}$$

Statistical analysis

Test of statistics was done using Statistical Package for Social Sciences (SPSS) Version 23. Data was analyzed and presented as mean \pm standard deviation. Differences in the nutrient concentration of zinc, selenium, and vitamin C in the maternal and cord blood were determined using the Wilcoxon rank test/Spearman's correlation. The result was considered statistically significant at $P < 0.05$.

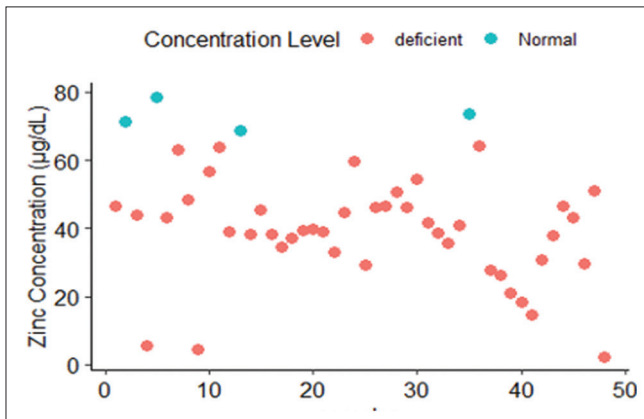


Figure 1: Comparison of zinc concentration in maternal blood with normal range. Normal range: 66–110 µg/dL^[23]

RESULT

The results of maternal blood levels of zinc, selenium, and vitamin C are presented in Table 2. The mean concentrations were 41.61 ± 2.45 µg/dL for zinc, 168.10 ± 14.47 µg/L for selenium, and 7.53 ± 0.26 mg/L for vitamin C. The ranges of these values were 6–78 µg/dL for zinc, 18–396 µg/L for selenium, and 4.2–12.5 mg/L for vitamin C.

The results of cord blood levels of zinc, selenium, and vitamin C are shown in Table 3. The mean concentrations were 42.65 ± 4.76 µg/dL for zinc, 197.56 ± 16.74 µg/L for selenium, and 7.11 ± 0.50 mg/L for vitamin C. The ranges of these values were 9–153 µg/dL for zinc, 22–513 µg/L for selenium, and 1.6–16.8 mg/L for vitamin C.

Comparison of nutrient concentration with normal range

The zinc concentration in maternal blood was compared to a normal range of 66–110 µg/dL and

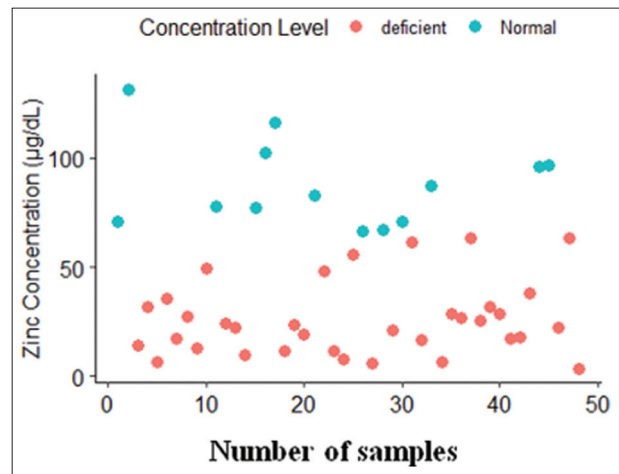


Figure 2: Comparison of zinc concentration in cord blood with normal range. Normal range: >65 µg/dL^[24]

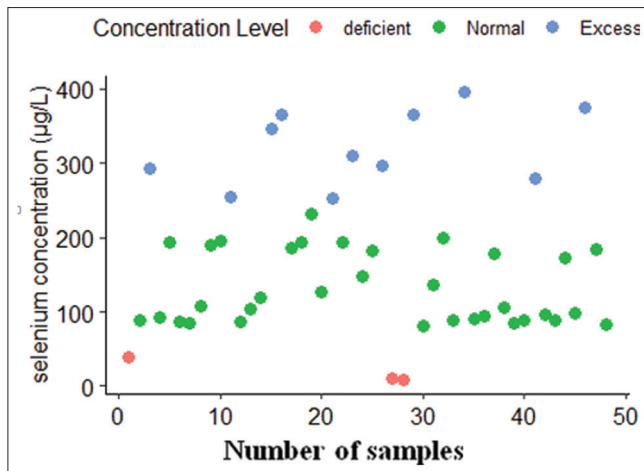


Figure 3: Comparison of selenium concentration in maternal blood with normal range. Normal range: 70–250 µg/L^[23]

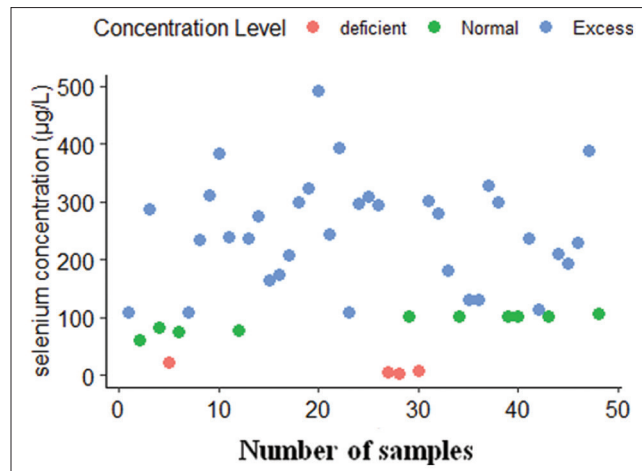


Figure 4: Comparison of selenium concentration in cord blood with normal range. Normal range: 35–107 µg/L^[12]

Table 1: Standard flame emission conditions for zinc and selenium

Metals	Wavelength nm	Slit (nm)	Flame
Zinc	213.9	0.2	Air acetylene
Selenium	196.0	0.2	Air acetylene

Table 2: Maternal blood levels of zinc, selenium, and vitamin C

Variable	Range	Mean±Standard deviation
Zinc (µg/dL)	6–78	41.61±2.45
Selenium (mcg/L)	18–396	168.10±14.47
Vitamin C (mg/L)	4.2–12.5	7.53±0.26

Table 3: Cord blood levels of zinc, selenium, and vitamin C

Variable	Range	Mean±Standard deviation
Zinc (µg/dL)	9–153	42.65±4.76
Selenium (µg/L)	22–513	197.56±16.74
Vitamin C (mg/L)	1.6–16.8	7.11±0.50

Table 4: Shapiro-Wilk Normality Test for distribution of samples

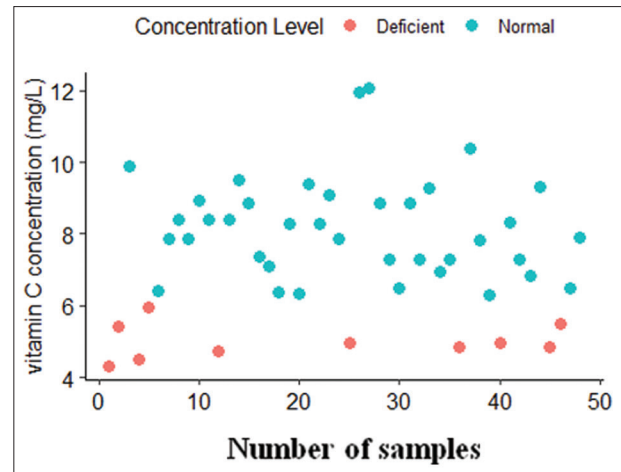
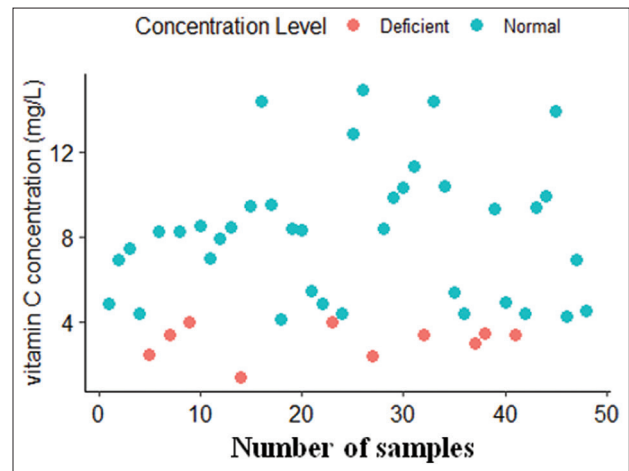
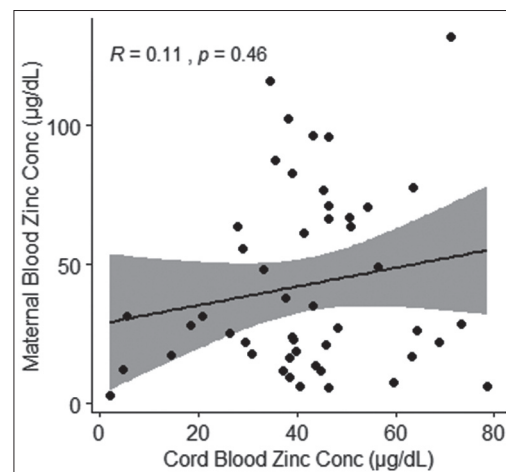
Variable	P Shapiro Test
The zinc level of Mother	0.2571
The zinc level of baby	0.0005*
The Selenium Level of the mother	0.00168*
Selenium Level of baby	0.1276
Vitamin C level of Mother	0.3024
Vitamin C level of Baby	0.01529*

*Statistically significant

it was observed that only 4 samples fell within the normal range. These four samples make up about 8.3% of the total number of samples in this study. The remaining 44 samples fell below the normal range and makeup 91.7% of the total samples used in this study. This shows that a greater percentage of the study population had zinc levels that fell below the normal standard range [Figure 1].

The zinc concentration in cord blood was compared to a normal range of >65 µg/dL and 13 out of 48 samples assessed fell within the normal range. These 13 samples make up 27.1% of the total number of samples studied. The remaining 35 samples fell below the normal range and make up 72.9% of the total samples [Figure 2].

The selenium concentration in maternal blood samples was assessed and compared to a normal range of 70–250 µg/L and 34 out of the 48 samples fell within the normal range. This number makes up 70.83% of the total samples studied. Three (3) out of the 48 samples fell below the normal range and 11 had selenium concentration above the normal range [Figure 3].


Figure 5: Comparison of vitamin C concentration in maternal blood with normal range. Normal range: 6–20 mg/L^[25]

Figure 6: Comparison of vitamin C concentration in cord blood with normal range. Normal range: 4–15 mg/L^[26]

Figure 7: Correlation between maternal and cord serum zinc concentration

The selenium concentration in cord blood was analyzed and compared to a normal range of 35–107 µg/L [Figure 4]. The result of the analysis was

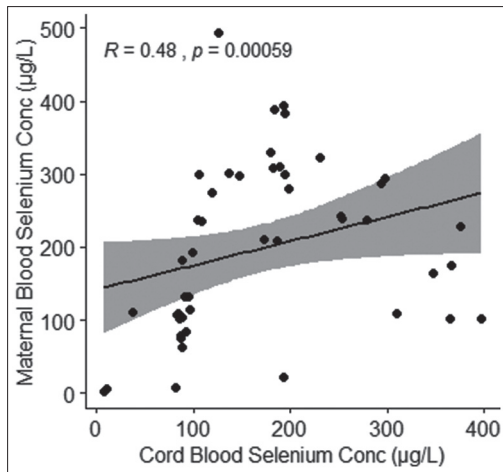


Figure 8: Correlation between maternal and cord serum selenium concentration

categorized as deficient, normal, and excess. 10 samples fell within the normal range and made up 20.83% of the total samples studied. 4 samples fell below the normal range and accounted for 8.33% of the total samples while the remaining 34 samples had selenium concentrations above the given normal range and make up 70.83% of the total samples studied [Figure 4].

The vitamin C concentration in maternal blood was compared to a normal range of 6–20 mg/L and 38 out of the 48 samples fell within the normal range. The remaining 10 samples fell below the normal range. It can be deduced from the result, that the vitamin C concentration of the pregnant women in the studied population is normal because over 79% of the samples studied fell within the normal range [Figure 5].

Below, the vitamin C concentration in cord blood was compared to a normal standard range of 4–15 mg/L and 38 samples fell within the normal range. The remaining 10 samples fell below the normal range and accounted for 20.83% of the total samples studied. The results show that the vitamin C concentration of the babies in the studied population is normal because over 79% of the samples studied fell within the normal range [Figure 6].

Correlation analysis for nutrient pairs

Table 4 shows the Shapiro–Wilk normality test that was done on each of the data columns to test if the columns are normal distributions. Shapiro test value greater than 0.05 shows that distribution can be assumed as a normal distribution. All except zinc level in the baby, selenium level in the mother, and vitamin C level in the baby were normally distributed. From the Shapiro test, none of the 3 pairs of maternal and cord blood fully followed a normal distribution, hence Spearman rank correlation test was used to

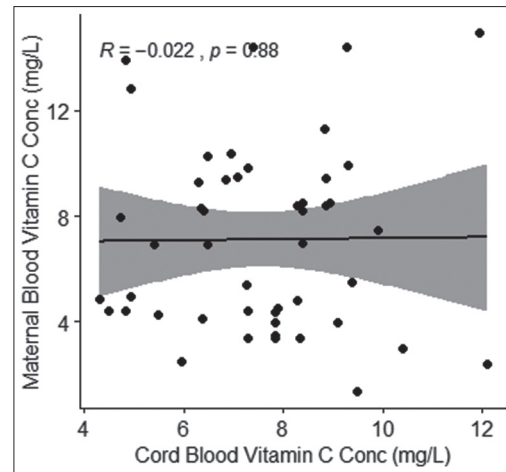


Figure 9: Correlation between maternal and cord serum vitamin C concentration

check the relationship between the maternal and cord blood pair.

Spearman rank correlation

Spearman rank correlation test was carried out on each pair of maternal and cord blood for the three nutrients in the data (zinc, selenium, and vitamin C). The Spearman rank method of correlation was chosen because the data was not normally distributed. In Figure 7, a correlation analysis between the maternal and cord blood zinc concentration shows a slight positive correlation ($r = 0.11$) that is not statistically significant ($P = 0.46$).

Correlation analysis between the maternal and cord blood selenium concentration shows a positive correlation ($r = 0.48$) that is statistically significant at $P = 0.00059$ [Figure 8].

In Figure 9, the result of the correlation analysis shows a slight negative association between the maternal and cord blood vitamin C levels which is not statistically significant ($P = 0.88$; $R = -0.022$).

DISCUSSION

Micronutrients such as zinc, selenium, and vitamin C play critical roles in maternal and fetal health, influencing immune function, antioxidant defense, and enzymatic activities.^[27] These micronutrients in maternal and umbilical cord blood provide insight into their availability for fetal development and the risk of deficiencies or excesses during pregnancy.^[28]

Maternal-fetal nutrient transfer is a complex process governed by maternal dietary intake, placental transport mechanisms, and metabolic demands, all of which contribute to variations in micronutrient concentrations.^[29] The results indicated maternal zinc

levels were mainly below the established normal range. This finding aligns with previous studies that have reported suboptimal zinc levels among pregnant women, often attributed to dietary inadequacies, increased fetal demand, and altered maternal zinc homeostasis during pregnancy.^[30] This study's low maternal zinc concentrations are concerning, given that zinc is essential for fetal growth, immune function, and enzymatic activities.^[30] The umbilical cord blood zinc levels were also predominantly below the normal range, suggesting a limited transfer from the mother to the fetus.^[31] Prior studies have indicated that fetal zinc accumulation relies on maternal stores, and deficiencies in maternal blood often translate to suboptimal fetal levels.^[32]

The selenium concentrations in maternal blood were mainly within the normal range, with a few cases of deficiencies and excesses. This finding supports earlier research indicating that selenium levels in pregnant women tend to be variable due to dietary intake and regional differences in soil selenium content.^[33] However, the umbilical cord blood selenium levels were predominantly above the normal reference range. This could be attributed to the efficient placental transfer of selenium, which has been documented in previous studies.^[34] While selenium is vital for antioxidant defense and thyroid function, excessive concentrations could pose potential risks, including oxidative stress and altered metabolic processes.^[35]

The vitamin C concentrations in maternal blood were mainly within the normal range, indicating an adequate intake among the study population. Studies reported similar findings where maternal vitamin C levels were sufficient, ensuring adequate fetal supply.^[36] The umbilical cord blood vitamin C concentrations also mainly fell within the normal reference range, reinforcing the evidence that maternal vitamin C status significantly influences fetal levels.^[37] Vitamin C is crucial in collagen synthesis, immune function, and oxidative stress mitigation, making its sufficiency critical for maternal and fetal health.^[38]

The correlation analysis between maternal and umbilical cord blood nutrient concentrations revealed a weak and statistically insignificant correlation for zinc. This suggests maternal zinc levels do not directly predict fetal zinc status, consistent with previous findings highlighting the complex regulatory mechanisms governing fetal zinc uptake.^[39] The selenium correlation analysis showed some association, supporting the hypothesis that selenium transfer across the placenta is relatively efficient.^[40] Vitamin C displayed a moderate correlation, reinforcing that maternal vitamin C status directly impacts fetal levels.^[41]

The high prevalence of zinc deficiency observed in maternal and umbilical cord blood samples suggests a need for targeted nutritional interventions. Similar studies have emphasized the importance of dietary supplementation and prenatal nutrition programs to address micronutrient deficiencies in pregnant women.^[42,43] The elevated selenium levels in umbilical cord blood warrant further investigation to determine the potential implications for neonatal health. Previous research has suggested that while selenium is beneficial in appropriate concentrations, excessive levels may contribute to metabolic imbalances and oxidative stress in neonates.^[44] These findings underscore the need to monitor micronutrient status in pregnant women and their neonates continuously. Maternal dietary intake, supplementation practices, and regional dietary patterns should be considered when evaluating micronutrient sufficiency. Addressing deficiencies and excesses through nutritional modifications, supplementation, and public health interventions remains essential for improving maternal and fetal outcomes.^[45,46]

CONCLUSION

This study emphasizes the vital role of zinc, selenium, and vitamin C in maternal and fetal health. The low maternal and umbilical cord blood zinc levels highlight the need for targeted nutritional interventions to prevent deficiencies affecting fetal growth and immune function. While selenium levels in maternal blood were generally expected, the elevated levels in umbilical cord blood suggest efficient placental transfer but raise concerns about the potential risks of excessive selenium. Adequate maternal vitamin C levels were reflected in the umbilical cord, reinforcing the importance of maternal nutrition for fetal development.

The weak correlation between maternal and fetal zinc levels indicates that maternal status alone does not predict fetal zinc availability reliably. In contrast, the moderate correlation between vitamin C and partial association with selenium suggests maternal levels influence fetal nutrient status. These findings underscore the importance of continuously monitoring micronutrient levels during pregnancy and implementing public health strategies to optimize maternal nutrition and prevent imbalances that could affect maternal and neonatal health.

Authors contributions

All authors contributed equally to the manuscript. The manuscript has been read and approved by all the authors.

Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching

Hospital, Enugu (NHREC/05/03/2008B-FWA00003452-1RB00003328). Permission was also obtained from the administrative offices of the selected health facilities. Informed consent was secured from all study participants.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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