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ORIGINAL RESEARCH ARTICLE

Serum Zinc, Copper, and Iron Levels Correlate with Slc39a14 Gene Expression in Pregnant Women Attending a Specialist Hospital in Sokoto, Nigeria

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ABSTRACT

Micronutrient deficiencies, particularly zinc, copper, and iron, have been implicated in poor maternal nutrition and foetal development, yet their interactions with genetic regulators remain underexplored. These studies underscore the importance of both micronutrient status and gene expression in maintaining maternal nutritional balance and support the inclusion of genetic screening in maternal nutrition research. Early detection of micronutrient deficiencies and gene-related absorption inefficiencies may help improve maternal health in resource-limited settings. Evidence from sub-Saharan Africa reveals a high prevalence of micronutrient deficiencies among pregnant women, emphasizing the need for improved nutritional strategies. This study assessed serum concentrations of zinc, copper, and iron and examined SLC39A14 gene expression, a divalent metal ion transporter, among 248 pregnant women aged 17–48 years attending the Specialist Hospital, Sokoto. Serum micronutrient levels were measured using Atomic Absorption Spectrophotometry, while SLC39A14 expression was quantified via Real-Time qPCR. The mean serum concentrations were as follows: zinc ranged from 0.120 ± 0.048 mg/L (15–19 years) to 0.162 ± 0.060 mg/L (30–39 years); iron from 2.100 ± 1.010 mg/L to 3.200 ± 1.250 mg/L; and copper from 0.050 ± 0.060 mg/L to 0.080 ± 0.060 mg/L. Zinc deficiency was highly prevalent across age groups, while copper deficiency was more pronounced in younger women. A statistically significant correlation ($p < 0.05$) was observed between elevated SLC39A14 gene expression (lower Ct values) and increased serum zinc and iron levels (Pearson $r = -0.41$ for zinc, -0.38 for iron), suggesting that SLC39A14 facilitates the uptake and homeostasis of these micronutrients. No significant correlation was found between SLC39A14 expression and copper levels, indicating different regulatory pathways.

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INTRODUCTION

Micronutrient deficiencies remain a major global health challenge, affecting approximately two billion individuals worldwide, with pregnant women and children being the most vulnerable groups (Moloro et al., 2023). During pregnancy, deficiencies in essential micronutrients such as zinc, iron, and copper can lead to a range of metabolic disturbances, including anemia, immune dysfunction, and impaired fetal development (Kanasaki & Kumagai, 2021; Birhanie et al., 2023). Sub-Saharan Africa bears a disproportionate burden of these deficiencies, contributing significantly to global maternal morbidity. In Nigeria, micronutrient malnutrition remains widespread, driven by limited dietary diversity, poverty, and inadequate supplementation programs (Onuigwe et al., 2020; WHO, 2023).

While several studies have documented the prevalence and effects of individual nutrient deficiencies during pregnancy (Afata et al., 2023; Sood et al., 2020), few have explored the interplay among multiple trace elements — particularly zinc, iron, and copper —and the genetic mechanisms that regulate their absorption and homeostasis. One key gene of interest is SLC39A14, a member of the ZIP (Zrt/Irt-like Protein) family that mediates the transport of divalent metal ions, including zinc and iron, into cells (Aydemir et al., 2020; Zhao et al.,

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2015). Although SLC39A14 has been identified as an important regulator of systemic metal ion balance, its expression patterns during pregnancy and relationship with maternal micronutrient status remain poorly characterized in African populations.

Based on the aforesaid, this study was therefore designed to assess serum concentrations of zinc, copper, and iron and to examine the relationship between these micronutrients and SLC39A14 gene expression among pregnant women attending the Specialist Hospital, Sokoto. Understanding these interactions may provide insights into the genetic regulation of micronutrient homeostasis and inform targeted nutritional interventions to improve maternal health. Specifically, this study provides a novel understanding of the interaction between essential micronutrients and the slc39a14 gene in relation to poor maternal nutrition among women of reproductive age. While micronutrient deficiencies have long been recognized as contributors to adverse pregnancy outcomes, this research is among the first to integrate genetic analysis with biochemical assessment of micronutrients in pregnant women, offering new insights into micronutrient metabolism during pregnancy. The contribution includes an innovative integration of genetics and nutrition, as it is among the first to explore the genetic role of SLC39A14 in obstetric outcomes, offering new insights into micronutrient metabolism during pregnancy. By focusing on women in Sokoto metropolis, the study provides region-specific biochemical and genetic data that can inform local public health interventions, dietary policies, and clinical practices tailored to sub-Saharan African populations.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study were of analytical grade to ensure reliable, accurate experimental outcomes. Major reagents included:

Nitric Acid (HNO_3): Used for serum digestion in micronutrient analysis. SYBR Green Master Mix: For quantitative PCR amplification of the SLC39A14 gene. Buffer Solutions: Included lysis buffers for DNA extraction and calibration standards for Atomic Absorption Spectrophotometry (AAS). All reagents were sourced from Sigma-Aldrich (USA) and stored according to the manufacturer's guidelines.

Equipment

The following equipment was utilized, calibrated according to the manufacturer's protocols:

Centrifuge: Eppendorf 5804R (Germany) for serum separation. UV-Vis Spectrophotometer: Shimadzu UV-1800 (Japan) for spectrophotometric assessments. Atomic Absorption Spectrophotometer (AAS): Model AA6300 (Shimadzu, Japan) for quantifying zinc, copper, and iron concentrations. PCR Thermocycler: Bio-Rad CFX96 (USA) for amplification.

Study Area

This study was conducted at the Specialist Hospital, located in Sokoto Metropolis, Northwest Nigeria. The hospital serves as a referral centre and provides comprehensive healthcare services, including antenatal, maternal, and paediatric care to residents of Sokoto State and neighbouring regions. Its central location and accessibility make it a hub for addressing obstetric complications. Ethical clearance was obtained from the Research and Ethics Committee of the Sokoto State Ministry of Health and Specialist Hospital, Sokoto (Ref: SHS/SUB/2024/133).

Study design and population

This study was a cross-sectional study that examined a population at a single point in time. A total of 248 pregnant women aged 17–48 years attending antenatal clinics at the Specialist Hospital, Sokoto, were recruited. This age range was chosen as it represents the typical reproductive age, ensuring relevance to the study objectives. Participation was entirely voluntary, with informed consent obtained prior to enrolment. To encourage participation and express gratitude, light refreshments were provided to all participants, in compliance with ethical standards.

Sample technique and sampling size;

A multistage sampling technique was adopted to ensure a representative sample and minimize selection bias. A systematic sampling technique was employed to select study subjects. The sampling interval was determined by dividing the total eligible population in each cluster by the required sample size.

The sample size was estimated using the formula;

$N=Z^2 \cdot (P) \cdot (1-P)/E^2$

N: is the required sample size.

Z: is the Z-score corresponding to the desired level of confidence=95% (1.96)

P: is the estimated proportion of the population=15%=0.18

E: is the margin of error set at 5% (=0.05)

$N=$ 226

Plus 10% attrition rate (22)

N=248

Data collection

Eligible women attending the Specialist Hospital in Sokoto metropolis who were awaiting delivery were interviewed to assess their dietary intake throughout pregnancy using a semi-quantitative Food Frequency

Questionnaire (FFQ). The FFQ was used to evaluate dietary intake throughout the pregnancy. The collected data included demographic details (age, marital status, income, and education), maternal dietary patterns, nutrient intake across pregnancy, supplement usage, and awareness of micronutrient importance.

Sample Collection

Serum Collection and Preparation

Blood samples were collected from each participant using a standard venipuncture procedure, ensuring aseptic conditions to prevent contamination. A total of 5 mL of blood was drawn using sterile disposable syringes and immediately divided for further processing: approximately 2 mL was transferred into plain (serum) collection tubes to facilitate serum separation, while the remaining 3 mL was placed into EDTA-coated tubes for DNA extraction and micronutrient assessment.

Laboratory Analytical Methods

Assessment of Zinc, Copper, and Iron

Serum levels of zinc, copper, and iron were analysed using Atomic Absorption Spectrophotometry (AAS).

Procedure

For sample preparation, 1 mL of serum was digested with 5 mL of nitric acid (HNO_3) and diluted with 4 mL of distilled water. The mixture was heated for 10 minutes, allowed to cool, and then filtered. The final volume was adjusted to 10 mL with distilled water. For instrument calibration, standard solutions of zinc, copper, and iron with known concentrations were used to generate calibration curves for each element. The concentration of each analyte in the serum was then determined by comparing the sample's absorbance with the calibration curve. Absorbance measurements for zinc, copper, and iron were taken at 213.857 nm, 324.754 nm, and 371.993 nm, respectively, using an Atomic Absorption Spectrophotometer (AAS, Model AA6300).

Detection of SLC39A14 Gene

DNA Extraction.

DNA was extracted from serum samples using a silica membrane-based column extraction method to ensure high-quality DNA for downstream applications. A total of 200 µL of serum was transferred into a sterile 1.5 mL microcentrifuge tube. To lyse the cells, 200 µL of lysis solution was added. The lysate-ethanol mixture was transferred to a silica membrane adsorption column in a 2 mL collection tube and centrifuged at 10,000 × g for 1 minute to bind the DNA to the silica membrane. To remove impurities, the column was washed with 500 µL of Wash Buffer 1, followed by centrifugation at 10,000 × g for 1 minute. This wash step was repeated with 500 µL of Wash Buffer 2. To ensure thorough drying of the silica membrane, the column was centrifuged at 12,000 × g for 3 minutes. For DNA elution, the column was transferred to a sterile, RNase-free 1.5 mL microcentrifuge tube.

Amplification of Slc39a14 Gene Using Real-Time PCR

The amplification was performed using specific primers for Slc39a14 and GAPDH, ensuring accurate and reproducible results. The qPCR amplification was carried out on a Rotor-Gene Q PCR machine using a 96-well optical-grade PCR plate. The cycling conditions were optimized to ensure maximum amplification efficiency: an initial denaturation at 95°C for 5 minutes to denature the DNA and activate Taq polymerase, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50–70°C for 30 seconds (with the specific temperature chosen based on the melting temperature of the primers), and extension at 72°C for 1 minute per kilobase of the target sequence. A final extension step was conducted at 72°C for 5 minutes to complete the synthesis of all amplicons. To ensure the reliability of the data, several controls were included in the experimental setup: a No-Template Control (NTC) to detect any contamination, a positive control containing a validated *Slc39a14* DNA sample to confirm the amplification, and a negative control using DNA from a sample known to lack the *Slc39a14* gene to ensure specificity of the assay.

Genomic DNA extracted from blood serum samples served as the template, and amplification reactions were set up at a final volume of 25 µL (Salisu et al., 2024). To assess the presence and relative expression levels of SLC39A14 in the biological samples, quantitative PCR (qPCR) was performed using SYBR Green chemistry, a widely recognized method for amplification and real-time quantification of specific DNA sequences. This approach enables precise measurement of gene expression levels by selectively amplifying the Slc39a14 gene and normalizing the data to a housekeeping gene, GAPDH, to account for variations in sample input. The PCR mixture included 50–100 ng of template DNA, 12.5 µL of SYBR Green qPCR Master Mix, forward and reverse primers for Slc39a14 (Forward primer: 5'CTACTGAGATGCTGGST-3') SLC39A14 (Reverse primer: 3'GCGTTAACCTGAAAGAG-5') as well as GAPDH (Forward primer: 5'GCTCATTTCCTGGTATG-3') (Reverse primer:3'TCTCTCTTCCTCTTGTG-5'), at a final concentration of 0.5 µM, and nuclease-free water to adjust the volume. Fluorescence data were collected after each cycle to monitor the amplification process in real time. The relative expression of the *Slc39a14* gene was calculated using the ΔCt method, with GAPDH as the internal reference.

Additionally, post-PCR melt curve analysis was performed to assess the specificity of the amplification products and confirm the absence of nonspecific amplification or primer-dimer formation. This meticulous approach ensures accurate quantification and robust data for the analysis of *Slc39a14* gene expression. The expression levels were quantified by calculating the Delta Ct (ΔCt) values, which represent the difference between the cycle threshold (Ct) values of SLC39A14 and the housekeeping

gene GAPDH for each sample. The ΔCt values were determined using the following formula:

$$\Delta Ct = Ct_{SLC39A14} - Ct_{GAPDH}$$

Subsequently, fold change in the expression of SLC39A14 relative to GAPDH was calculated using the following formula: Fold Change = $2^{(-\Delta Ct)}$

Statistical Analysis

The data obtained was analysed using SPSS version 12. The sample size was calculated to compare results between the groups. The result was expressed as a percentage and as Mean ± SD. A one-way Analysis of Variance (ANOVA) was used to compare. Paired comparisons were carried out; a p-value of 0.05 or less was considered significant.

RESULTS

The statistics of serum concentration results for zinc, iron, and copper concentrations in blood samples of the study subjects of different age and trimester categories, ranging from 15-19,20-29- and 30-39, and during the 1st, 2nd, and 3rd trimester are summarised in Table 1. The statistics for the mean and standard deviation for both age groups and trimesters, with significant differences indicated. Zinc levels decrease as pregnancy progresses; iron levels tend to increase in the first two trimesters and then decrease in the last trimester. Copper level, though low, increases in every trimester. Age group 30-39 has the highest level of micronutrient concentration, while the lowest level is observed in age group 15-19.

Table 1. Serum Concentrations of Zinc, Copper, and Iron among Women of Reproductive Age

Category	Zinc (Zn) (mg/L)	Iron (Fe) (mg/L)	Copper (Cu) (mg/L)
Age Group			
15-19 years	0.120a ± 0.048	2.100a ± 1.010	0.050a ± 0.060
20-29 years	0.140a ± 0.055	2.400a ± 1.090	0.070a ± 0.090
30-39 years	0.162b ± 0.060	3.200b ± 1.250	0.080a ± 0.060
Trimester			
1st Trimester	0.150a ± 0.053	2.000a ± 1.050	0.060a ± 0.065
2nd Trimester	0.160a ± 0.058	2.700a ± 1.150	0.080a ± 0.078
3rd Trimester	0.140a ± 0.054	2.400a ± 1.100	0.070a ± 0.075

Keys: Zinc (Zn), Iron (Fe), and Copper (Cu) concentrations are presented as mean ± standard deviation (mg/L)
Superscripts (a, b) within each column indicate statistically significant differences between age groups ($p < 0.05$), as determined by one-way ANOVA
No statistically significant differences were observed across trimesters ($p > 0.05$ for all comparisons).

Table 2: Statistical Associations Between Micronutrients, Gene Expression, and Demographic Variables

Variable	Zinc (mg/L)	Iron (mg/L)	Copper (mg/L)	SLC39A14 Expression (ΔCt)	p-value (ANOVA)
Age Group (15–39 yrs)	↑ with age (p=0.03)	↑ with age (p=0.01)	↑ with age (NS)	↑ in 30–39 yrs (p=0.04)	Significant for Zn, Fe
Trimester (1st–3rd)	Fluctuates (NS)	Fluctuates (NS)	Fluctuates (NS)	No trend (NS)	Not significant
Gene Expression (ΔCt)	r = –0.41 (p=0.02)	r = –0.36 (p=0.03)	r = –0.09 (p=0.62)	—	Significant for Zn, Fe

Keys: Note: ↑ means "increases with", NS = Not Significant; ΔCt inversely proportional to gene expression.
One-way ANOVA was used to compare means across multiple groups (e.g., age groups, trimesters).
Pearson correlation was used to examine associations between *SLC39A14* expression and micronutrient levels.

On the other hand, Table 2 shows the Statistical Associations Between Micronutrients, Gene Expression, and Demographic Variables. From the table, Zinc and Iron increased with age, and these increases were statistically significant ($p<0.05$). Copper also trended upward with age, but this change was not statistically significant (NS).

Across the trimesters, all three micronutrients showed minor fluctuations, none of which were statistically significant.

The Gene Expression measured by ΔCt values from qPCR reflects how active the SLC39A14 gene is. Gene expression increased (ΔCt decreased) with age, with statistical significance at p=0.04, but no significant trend was observed across trimesters.

The Relationship between SLC39A14 Gene Expression and Micronutrient Levels is depicted in Figure 1. The SLC39A14 gene is known to play a role in metal ion transport, potentially influencing the regulation of essential trace elements like Zinc (Zn) and Iron (Fe). The plot displays Zinc (blue points) and Iron (red points) concentrations against SLC39A14 ΔCt values. Ct (cycle threshold) values are inversely related to gene expression, meaning lower Ct values indicate higher expression of the SLC39A14 gene. This visualization helps assess whether increased SLC39A14 expression correlates with elevated micronutrient levels.

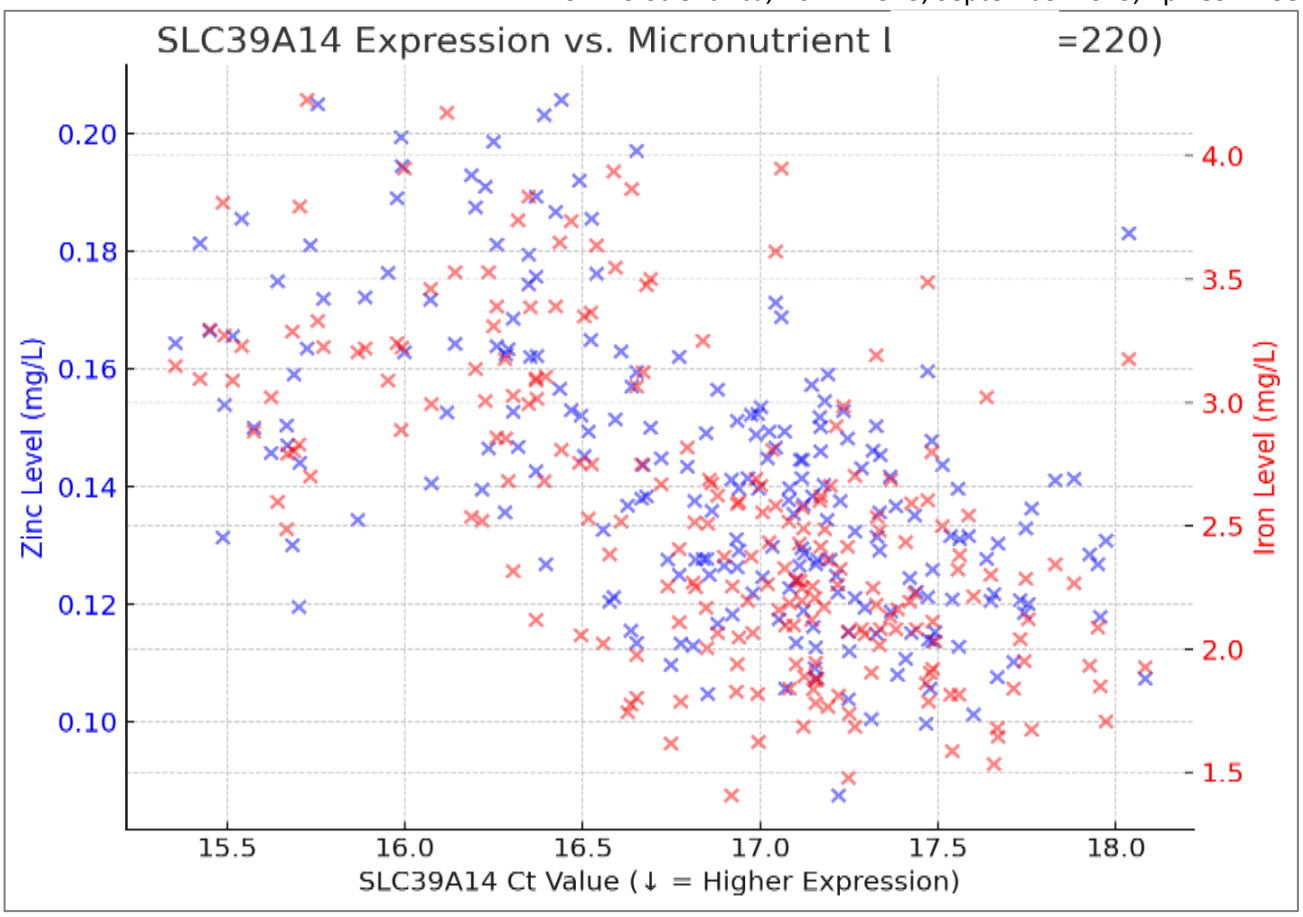

Figure 1: Relationship Between SLC39A14 Gene Expression and Micronutrient Levels
Keys: SLC39A14 Ct Value: Lower ΔCt = Higher gene expression. Zinc and Iron Concentrations: Measured in mg/L.
Data Points: Each point represents an individual study participant.
Color Coding:
Blue = Zinc concentration
Red = Iron concentration

DISCUSSION

Micronutrients such as zinc, iron, and copper are vital for maternal and fetal health, serving as cofactors in numerous enzymatic and physiological processes. The present study examined the relationship between these micronutrients and SLC39A14 gene expression in pregnant women, contributing to the understanding of genetic influences on micronutrient balance during pregnancy. The study found that zinc and iron concentrations increased significantly with age, while copper showed a non-significant upward trend. Older pregnant women (30–39 years) exhibited higher mean serum zinc and iron levels than younger women, possibly reflecting differences in diet quality, supplement adherence, or physiological adaptation. However, the persistently high prevalence of zinc deficiency across all age groups aligns with previous reports of widespread zinc insufficiency among African women of reproductive age (Afata et al., 2023; Gohari et al., 2023). The moderate inverse correlation observed between SLC39A14 ΔCt values and serum concentrations of zinc and iron indicates that higher gene expression corresponded with higher serum levels of these micronutrients. This suggests that SLC39A14 may facilitate the cellular uptake and systemic regulation of zinc and iron, consistent with earlier findings describing its role as a metal ion transporter (Zhou et al., 2015; Aydemir et al., 2020). This finding aligns with a growing body of evidence demonstrating that gene expression levels can be a key determinant of phenotypic traits, as seen in the correlation between DREB1A gene conservation and physiological salt tolerance in rice (Tsamaye *et al.,* 2022). Conversely, no significant association between SLC39A14 expression and copper levels was detected, implying that copper metabolism operates through distinct regulatory mechanisms involving other transport proteins such as ATP7A and CTR1 (Aydemir et al., 2019).

These results highlight the complex relationship between nutritional and genetic factors in determining micronutrient status during pregnancy. Standard antenatal care often emphasizes iron and folate supplementation, yet the high prevalence of zinc and copper deficiencies suggests that broader micronutrient monitoring is warranted. Integrating genetic markers such as SLC39A14 into maternal nutrition assessments could provide a more personalized approach to supplementation and dietary counselling, particularly in regions where deficiencies are common. Further investigation is recommended to evaluate whether SLC39A14 expression changes throughout gestation or in response to dietary interventions, and to determine whether it modulates the absorption of other essential trace elements. Longitudinal

studies could clarify causal pathways linking gene expression with nutrient status and overall maternal well-being.

CONCLUSION

This study demonstrated significant associations between SLC39A14 gene expression and serum zinc and iron concentrations among pregnant women in Sokoto, Nigeria. The findings suggest that SLC39A14 plays a regulatory role in micronutrient metabolism and may influence zinc and iron homeostasis during pregnancy. Although copper showed no significant relationship with gene expression, overall deficiencies, particularly of zinc, remain a major nutritional concern.

These results underscore the importance of combining biochemical and genetic assessments to improve understanding of micronutrient regulation in pregnancy. Further research linking SLC39A14 expression with dietary intake and pregnancy outcomes is recommended to guide the development of evidence-based, personalized nutrition strategies for maternal health in resource-limited settings.

RECOMMENDATIONS

Routine SLC39A14 genetic analysis should be considered in high-risk pregnancies to personalize micronutrient supplementation plans. Community-based awareness initiatives should target adolescent girls and women of reproductive age, emphasizing the importance of zinc, copper, and iron in pregnancy. Maternal supplements should be formulated to minimize competitive inhibition among micronutrients, particularly avoiding excessive iron doses that can impair zinc and copper absorption. Iron, zinc, and copper levels should be routinely assessed at each trimester to guide individualized supplementation and reduce the risk of both deficiencies and toxicities.

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