

Research Article

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Maternal and Umbilical Cord Serum Zinc, Iron, and Copper Levels in Preterm and Term Neonates: A Prospective case control Study

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Abstract: Background: Zinc, iron, and copper contribute to fetal growth, oxygen transport, enzymatic activity, and antioxidant defence. Their relationship with preterm birth remains uncertain, particularly in resource-limited settings. **Objective:** To compare maternal and umbilical cord serum zinc, iron, and copper concentrations between preterm and term neonates. **Methods:** This prospective case-control study was conducted during 2018–2019 at a rural tertiary-care hospital in Nellore, Andhra Pradesh, India. Fifty neonates born at 30–36 weeks of gestation and 50 term appropriate-for-gestational-age neonates were enrolled. Maternal venous blood and cord blood were collected at delivery. Serum zinc, iron, and copper were measured by atomic absorption spectrophotometry. Group comparisons used the unpaired t test or chi-square test, with statistical significance set at $p < 0.05$. **Results:** Mean maternal zinc was lower in the preterm group than in the term group (77.12 ± 23.22 vs 89.28 ± 29.90 $\mu\text{g/dL}$; $p = 0.02$), as was maternal iron (0.74 ± 0.143 vs 0.87 ± 0.197 ; $p = 0.05$). Maternal copper did not differ significantly (98.74 ± 22.37 vs 105.78 ± 28.34 $\mu\text{g/dL}$; $p = 0.08$). Cord zinc was lower among preterm neonates (89.40 ± 18.13 vs 119.00 ± 33.36 $\mu\text{g/dL}$; $p = 0.004$), as was cord iron (1.53 ± 0.478 vs 1.93 ± 0.59 ; $p = 0.05$); cord copper did not differ significantly (69.00 ± 16.53 vs 73.00 ± 13.50 $\mu\text{g/dL}$; $p = 0.13$). **Conclusion:** Lower maternal and cord zinc and iron concentrations were associated with preterm birth in this study population. Copper concentrations did not show a significant case-control difference. Larger studies with antenatal sampling and multivariable adjustment are needed. **Keywords:** preterm birth; zinc; iron; copper; umbilical cord blood; maternal serum; atomic absorption spectrophotometry.

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INTRODUCTION

Trace elements are micronutrients that constitute less than 0.01% of body weight. They are incorporated into metalloproteins and perform diverse structural and enzymatic functions, including roles in the skeletal system, teeth, skin, and hair. Zinc, iron, copper, and selenium are components of enzyme systems involved in antioxidant protection [1,2] This function may be particularly important during pregnancy, which is accompanied by increased oxidative activity. Bakacak *et al.* reported an association between the plasma copper-to-zinc ratio and pre-eclampsia [3]. Pre-eclampsia is associated with increased lipid peroxidation, which may be related to elevated copper concentrations and an increased copper-to-zinc ratio in maternal serum.⁴ Preterm and low-birth-weight infants are particularly vulnerable to free-radical injury because their antioxidant defence systems are immature. The role of

trace elements in pregnancy progression and fetal development, however, remains incompletely understood. Accordingly, this study compared maternal and umbilical cord serum zinc, iron, and copper concentrations between preterm and term neonates and assessed maternal–cord differences within each group.

MATERIALS AND METHODS

Study design and setting: This prospective case-control study was conducted over one year during 2018–2019 in the neonatal intensive-care unit and Department of Paediatrics at Narayana Medical College and Hospital, Nellore, Andhra Pradesh, India. The hospital was described in the source dissertation as a tertiary-care centre in a rural setting.

Study subjects: The preterm group comprised 50 neonates born at 30–36 weeks of gestation. The control

group comprised 50 term appropriate-for-gestational-age neonates. Maternal age was restricted to 21–30 years. Gestational age was assessed using maternal dates and Dubowitz scoring; postnatal assessment also included New Ballard scoring. Preterm births were described as spontaneous. Mothers with BMI <18 kg/m², chronic liver disease, or refusal of consent were excluded.

Data Collection: Maternal age, socioeconomic status according to the modified Kuppaswamy scale, gravidity, ferrous-sulphate tablet intake, medical and obstetric illness, and identifiable causes of prematurity were recorded. Neonatal variables included gestational age, sex, weight, length, head circumference, congenital malformations, morbidity, and outcome until discharge or death.

Specimen collection and laboratory analysis: After informed consent, approximately 3 mL of maternal venous blood and 3 mL of cord blood were collected after cord clamping. Samples were centrifuged, serum was separated, and specimens were stored at approximately –20°C until analysis. Zinc, iron, and copper were measured by atomic absorption spectrophotometry after dilution and calibration with standards. The reported wavelengths were 213.9 nm for zinc, 248.3 nm for iron, and 324.8 nm for copper.

Ethics: Approval was obtained from the Institutional Ethics Committee. Written informed consent was obtained from the parent or guardian according to the dissertation consent form.

Statistical analysis: Continuous variables were summarised as mean±standard deviation and categorical variables as frequencies and percentages. The study used unpaired t tests for continuous variables and chi-square tests for categorical variables, with $p < 0.05$ considered statistically significant. Maternal–cord comparisons were reported separately for preterm and term groups. Because adjusted effect estimates were not presented, the analyses reported here are unadjusted.

RESULTS

Participant characteristics

Fifty preterm and 50 term neonates were included. The groups were reported as comparable with respect to socioeconomic status, gravidity, ferrous-sulphate tablet intake, neonatal sex, maternal age, BMI, and maternal haemoglobin. Mean maternal age was 23.64±3.498 years in the preterm group and 22.90±2.808 years in the term group. Corresponding BMI values were 23.02±3.61 and 24.04±3.32 kg/m², while haemoglobin values were 9.72±0.93 and 9.81±0.71 g/dL.

Table-1: Participant characteristics

Variable	Preterm (n=50)	Term (n=50)	p value
Maternal age, years	23.64±3.498	22.90±2.808	0.30
BMI, kg/m ²	23.02±3.61	24.04±3.32	0.21
Maternal haemoglobin, g/dL	9.72±0.93	9.81±0.71	0.63
Male sex, n (%)	35 (70)	32 (64)	0.28

Maternal trace elements

Maternal serum zinc was significantly lower in mothers of preterm neonates than in mothers of term neonates. Maternal serum iron was also lower in the preterm group at the reported significance threshold. Maternal copper did not differ significantly between groups.

Table-2: Mean Trace elements levels in Preterm & Term neonates.

Element	Preterm mean±SD	Term mean±SD	p value
Zinc, µg/dL	77.12±23.22	89.28±29.90	0.02
Copper, µg/dL	98.74±22.37	105.78±28.34	0.08
Iron, reported units*	0.74±0.143	0.87±0.197	0.05

Cord trace elements

Cord serum zinc and iron were lower among preterm neonates than term neonates, whereas cord copper did not differ significantly.



Table-3: Mean Cord Trace elements levels in Preterm & Term neonates.

Element	Preterm mean±SD	Term mean±SD	p value
Zinc, µg/dL	89.40±18.13	119.00±33.36	0.004
Copper, µg/dL	69.00±16.53	73.00±13.50	0.13
Iron, reported units*	1.53±0.478	1.93±0.59	0.05

Maternal–cord comparisons

Cord zinc concentrations were higher than maternal zinc concentrations in both preterm and term groups ($p=0.003$ and $p=0.001$, respectively). Cord copper concentrations were lower than maternal concentrations in both groups ($p=0.001$ for each). Cord iron concentrations were higher than maternal concentrations in both groups ($p=0.001$ for each). These within-group comparisons were reported in the source dissertation; the appropriate paired test and confidence intervals should be verified against the raw data before publication.

DISCUSSION

Study rationale and principal findings

The neonatal period is a nutritionally critical stage of life. This study therefore evaluated maternal and umbilical cord serum concentrations of zinc, iron, and copper and examined relationships between maternal and cord levels. It also assessed whether these trace elements were associated with preterm birth and gestational age. Preterm neonates are anatomically and physiologically immature and face increased risks of morbidity and mortality. The mechanisms initiating normal labour remain incompletely understood, and the pathways responsible for labour before term are even less clearly defined. Trace elements may be relevant because zinc, iron, and copper participate in enzymatic activity, oxygen transport, cellular growth, and antioxidant defence [1-7].

Maternal trace elements

Maternal serum zinc was significantly lower among mothers of preterm neonates than among mothers of term neonates. The mean maternal zinc concentration was 77.12 ± 23.22 µg/dL in the preterm group compared with 89.28 ± 29.09 µg/dL in the term group ($p=0.02$). This finding is consistent with observations reported by Jeswani and Vani in India and Wasowicz *et al.* in Poland [8,9]. However, Iqbal *et al.*, Perveen *et al.*, and Bro *et al.* did not find a significant relationship between maternal zinc concentration and gestational age [9-11]. Maternal zinc concentrations are influenced by fetal and placental uptake, expansion of maternal plasma volume, albumin binding, inflammation, timing of collection, and laboratory methodology [5,7,9]. These factors may explain some of the variation between studies.

Maternal copper and iron

Maternal copper concentrations did not differ significantly between preterm and term deliveries. The

mean maternal copper concentration was 98.74 ± 22.37 µg/dL in the preterm group and 105.78 ± 28.34 µg/dL in the term group ($p=0.08$). This is similar to reports by Perveen *et al.* and Bro *et al.* [11,12]. Pregnancy-related mobilisation of tissue copper and increased ceruloplasmin synthesis may influence maternal serum copper. Copper deficiency has been associated with anaemia, neutropenia, impaired glucose and cholesterol metabolism, and increased susceptibility to infection, but ceruloplasmin was not measured in this study. Maternal serum copper has also been associated with fetal growth; Bermúdez *et al.* reported relationships between trace elements and birth weight [13]. In this study, maternal copper was associated with neonatal morbidity, mortality, and birth weight in exploratory correlation analyses. Maternal serum iron was lower in preterm deliveries (0.74 ± 0.143 versus 0.87 ± 0.197 ; $p=0.05$). Haemoglobin alone is not a complete measure of iron status; ferritin, transferrin saturation, soluble transferrin receptor, and inflammatory markers would provide stronger evidence [14-17].

Cord trace elements

Cord serum zinc was lower in preterm neonates than in term neonates (89.40 ± 18.13 versus 119.00 ± 33.36 µg/dL). Similar findings were reported by Perveen *et al.* and Wasowicz *et al.*, whereas other studies reported no association or different directions [9-12]. Zinc is actively transferred across the placenta, and fetal requirements increase during late gestation. The positive relationship between maternal and cord zinc in this study supports the importance of maternal zinc availability, although serum zinc remains sensitive to albumin, inflammation, haemodilution, and timing of collection. Cord copper did not differ significantly according to gestational age and was lower than maternal copper in both groups. Similar maternal–cord differences have been reported by Algerwie and Khatri, Iqbal *et al.*, Wasowicz *et al.*, and Upadhyaya *et al.* [9,10,18,19]. Cord iron was lower in preterm neonates than in term neonates (1.53 ± 0.478 versus 1.93 ± 0.59 ; $p=0.05$). Cord iron was higher than maternal iron in both groups, possibly reflecting active placental transfer.

Ferrous-sulphate intake and neonatal outcomes

The study reported associations between ferrous-sulphate tablet intake and maternal and cord zinc concentrations. Iron and zinc can interact during intestinal absorption, and previous studies have reported effects of iron supplementation on zinc absorption or



circulating zinc [6,10]. However, dietary zinc intake, total iron dose, duration of supplementation, adherence, albumin, inflammation, and baseline zinc were not measured. Therefore, the observed association cannot establish that ferrous-sulphate supplementation caused zinc deficiency. Maternal copper was associated with neonatal morbidity, mortality, and birth weight in exploratory analyses; Alimonti A *et al.* also reported an association between maternal copper and low birth weight [20]. The mechanism remains uncertain, and further investigation is required.

Other determinants and overall interpretation

No significant association was found between trace-element concentrations and the recorded causes of prematurity. Among the 50 preterm neonates, no identifiable cause was documented in 25 cases. In the remaining cases, previous prematurity, maternal anaemia, and pregnancy-induced hypertension were the most frequent recorded causes, each accounting for 12%; premature rupture of membranes, antepartum haemorrhage, multiple pregnancy, and other obstetric illnesses were less frequent. No significant associations were observed with order of pregnancy, socioeconomic status, neonatal sex, maternal age, BMI, or maternal haemoglobin. These negative findings should be interpreted in the context of the small sample size and restricted inclusion criteria. Overall, lower maternal and cord zinc and iron concentrations were associated with preterm birth, whereas copper did not differ significantly. Because samples were obtained at delivery, the findings do not establish whether altered trace-element concentrations preceded preterm labour or resulted from it.

Strengths and limitations

Strengths include prospective recruitment, a term comparison group, paired maternal and cord specimens, measurement of three trace elements, and recruitment from a rural tertiary-care hospital. Limitations include the small single-centre sample, restriction of maternal age to 21–30 years, exclusion of women with BMI <18 kg/m², absence of antenatal serial samples, and lack of dietary assessment, ferritin, transferrin saturation, albumin, ceruloplasmin, inflammatory markers, and adjusted regression models.

CONCLUSION

Lower maternal and cord zinc and iron concentrations were associated with preterm birth in this study of 100 mother–neonate pairs. Maternal and cord copper concentrations did not differ significantly between preterm and term groups. These findings are hypothesis-generating and should not be interpreted as evidence that zinc or iron supplementation prevents preterm birth. Larger multicentre studies should obtain antenatal samples, measure validated biomarkers of micronutrient status and inflammation, document dietary and supplement intake, and use multivariable models to account for clinical and socioeconomic confounding.

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Conflicts of Interest- Nil.

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Ethical statement: Approval was obtained from the Institutional Ethics Committee. Written informed consent was obtained from the parent or guardian according to the dissertation consent form.

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