Research article

Effects of maternal blood selenium and zinc levels on mitochondrial DNA copy number at term and in-turn their effect on the birthweight of the baby

Manjula R.[1], Rekha Udgiri[1], Ashalatha Mallapur[2], Sangappa V. Kashinakunti[3], Kavita Hiremath[3], Shailaja Patil[1]

[1]Department of Community Medicine, BLDE deemed to be University, BLDE Medical College, Bijapur, Karnataka, India
[2]Department of OBG, [3]Department of Biochemistry, S. Nijalingappa Medical College, Navanagar, Bagalkot 587 102, Karnataka, India

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Corresponding author: **Manjula R.** Email: drmanjulakashinakunti@gmail.com

ABSTRACT

Introduction and Aim: The micronutrients such as selenium and zinc have the antioxidant property. They are cofactors of antioxidant enzymes, regulate the inflammatory response by counterbalancing the oxidative stress. Reduced maternal selenium and zinc levels have been shown to be associated with early pregnancy loss and low birthweight. The aim is to study the effects of maternal blood selenium and zinc levels on mitochondrial DNA copy number at term and in-turn their effect on the birth weight of the baby.

Methods: An Institutional ethical clearance was obtained and the present hospital based cross sectional study was conducted in the OBG department of a medical college of North Karnataka between December 2019 to February 2022.

Results: A total of 150 term pregnant women participated in the study. There was a slightly increased serum level of zinc and selenium found in the term pregnant mothers who gave birth to normal birth weight babies when compared to low-birth-weight babies, though this difference was not statistically significant. Median (IQR) values of Delta CT values of Mitochondrial DNA copy number in low-birth-weight babies were 3.07(1.7-5.74) and in normal birth weight babies was 3.71 (0.83-4.4). The difference in median values was not statistically significant (p=0.57). We observed a positive correlation between the maternal zinc, selenium, and mitochondrial DNA copy number with birth weight of the baby. Though the correlation between Delta CT means and birthweight of the baby is found to be statistically significant.

Conclusion: In the present study, apparently healthy pregnant women participated. The serum selenium and zinc levels were found to be within normal limits according to lab reference values. There was an increase in Mitochondrial DNA copy number in the present study and it was positively correlated with the birthweight of the baby.

Keywords: Mitochondrial DNA copy number; maternal selenium; maternal zinc; birthweight.

INTRODUCTION

Nutrient intake is very crucial for the well-being of pregnant women and the fetus. Birth weight is an important indicator of the status of public health, maternal health, and nutrition. Globally low birth weight is an important risk factor contributing to infant mortality (40-60%; 1). Maternal nutrition is an important determinant for the birth weight of neonate (2).

In nutrition, Micronutrients are considered as an important factor for normal growth and development of the fetus. Micronutrient deficiencies have been seen to be associated with intrauterine growth retardation (IUGR) and small for gestational age (SGA) infants. Micronutrients have many functions: antioxidant process, interaction with intercellular signaling protein transcriptional regulation, cell proliferation etc., (3). The protein-energy undernourishment is clinically evident and acutely visible, whereas the health impacts of micronutrient deficiency are not always acutely visible; Hence this micronutrient deficiency is also synonymously called as hidden hunger (4).

Micronutrients such as selenium and zinc have antioxidant property. They are cofactors of antioxidant enzymes, regulate the inflammatory response by counterbalancing the oxidative stress (5). Selenium is a trace element which is bound and present in the seleno-proteins, which includes glutathione peroxidase, thioredoxin reductases and seleno-protein-P (5). Selenium is also an important part of the key antioxidative enzyme glutathione peroxidase and iodothyronine deiodinases D1, D2 and D3, at their active sites (6). Glutathione peroxidases have an impact on redox status and has a key role in regulating oxidative stress, while the iodothyronine deiodinases have key roles thyroid homeostasis by regulating circulating and intracellular levels of thyroid hormones (T3 and T4; 7) Reduced maternal selenium levels have been shown to be associated with abortions (early pregnancy loss) and low birth weight (LBW; 8,9).

Zinc is an important component of many anti-oxidative metallo-enzymes participating in protein and carbohydrate metabolism, nucleic acid synthesis, and antioxidant functions through the Cu/Zn superoxide dismutase (10). Changes in zinc homeostasis have been associated with various effects on pregnancy includes intrauterine growth restriction (IUGR) and LBW (11,12).

Mitochondria are intra-cellular organelles which are membrane-enclosed, contributing to the functions of pyruvate and fatty acid oxidation, nitrogen metabolism, heme biosynthesis and apoptosis regulation (13). This organelle functions either through transcription factors or through retrograde regulation in mitochondria itself. Transcription factors are influenced by environmental factors, nutrition or exercise. The stimulation of mitochondrial DNA (Mt DNA) biogenesis has been explained with oxidative stress conditions. Nutrition factors such as micronutrients which have antioxidant properties could regulate the mitochondrial DNA copy number (14).

METHODOLOGY

The present study was carried out in the outpatient department and Labor room of OBG Department at Tertiary Care Centre of North Karnataka, India. An Institutional ethical clearance (BLDE(DU)/IEC/409 /2019-20 dated 27th December 2019) was obtained. We included Apparently Healthy term Pregnant women of ≥37 weeks. Those pregnant women who give consent to participate in the study. Multiple pregnancies like twins have diagnosed anytime during pregnancy or after delivery. Chronic medical conditions such as hepatic, renal, cardiovascular diseases, women who are known to have HIV, hepatitis B infection, hypertension including preeclampsia and diabetes mellitus including gestational diabetes were excluded. Babies born with severe congenital anomalies were excluded during the time of analysis.

Sample size estimation was done using Openepi software version 2.3.1. At 95% confidence limits, and at 80% Power of the study, Zα= standard table value for 95% CI =1.96, $Z_{1-\beta}$ = Standard table value for 80% Power = 0.84 According to study conducted by Lili *et al.*, (15). The correlation coefficient between the Maternal zinc levels and birth weight of the baby=0.5. Formula used= $N = ([Z_\alpha + Z_\beta]/C)^2 + 3$, where C= 0.5*ln ([1 + *r*]/ [1 − *r*]) Sample size estimated is 120, which is rounded off to 150.

After obtaining ethical clearance, this study was conducted in the outpatient department (OPD) and Labor rooms of OBG Department of Tertiary care Centre of North Karnataka. Informed consents were obtained from the study subjects. All apparently healthy term pregnant women (≥37 weeks) who are coming to OPD for Antenatal care and those who are admitted in labor room for safe confinement, inclusion and exclusion criteria was considered for the study. The pregnant women who are willing to participate by giving the informed consent for the present study were selected for the study.

A pretested questionnaire for obtaining basic demographic characteristics, investigations done during (≥37 weeks): About 5 ml of venous blood was collected by venipuncture and following investigations will be done Whole blood is taken in EDTA bulb was stored at -20°C, and later selenium was analyzed inductively coupled plasma mass spectrometry (ICP-MS).

Zinc was estimated using Autolumo 1000, a fully automated analyzer which works based on the principle of Chemiluminescence (CLIA) method.

Quantification of Mitochondrial DNA Copy Numbers in Peripheral Blood. MtDNA in peripheral leukocytes was extracted from 1 mL of whole blood using the QIAamp Tissue Kit 250 (Qiagen Inc., Valencia, CA, USA). The relative mtDNA copy number was quantified by a real-time polymerase chain reaction (QPCR) and corrected by simultaneous measurement of the nuclear DNA (*β*-globin) using the method given by Wong and Cortopassi (16) and Liu *et al., (*17) Reactions were performed using a Lightcycler-Faststart DNA Master SYBR Green I kit. The forward and reverse primers of *β*-globin (used to amplify a 268 bp product) were 5'GAAGAGCCAAGGACAGGTAC- 3' and 5'-CAACTTCATCCACGTTCACC-3', respectively. The forward and reverse primers of the mitochondrial gene (ND1 gene) used to amplify a 153 bp product were 5'-AACATACCCATGGCCAACCT-3' and 5'-AGCGAAGGG-TTGTAGTAGCCC-3' respectively. The genomic DNA (50ng) was mixed with 10 µl SYBR Green I Master Mix that contained 10 pmol of forward and reverse primers in a final volume of 20µl. After denaturation at 95°C for 30 seconds, DNA samples were treated at 95°C for 0.1 seconds, 58°C for 6 seconds, and 72°C for 18 seconds for 40 cycles. A total of 50 ng of DNA was used and the number of PCR cycles to reach this amount of DNA was defined as the threshold cycle (Ct). The following equation was used to quantify the mtDNA copy number relative to *β*-globin: relative copy number = 2ΔCt (ΔCt = Ct*β*-globin − Ct ND1; 18).

Low birth weight is determined by the birth weight of less than 2500 g. IUGR/SGA as birth weight below the 10th centile for gestational age at delivery.

Statistical analysis

Data was analyzed statistically using SPSS package 'IBM SPSS Statistics for Windows, version 19 (IBM Corp., Armonk, N.Y., USA)'. Data was expressed as percentages and mean ±SD for qualitative and quantitative data respectively. Later it was

statistically analyzed statistical tests such as Chi-square test, odds ratio (95% CI), student unpaired t-test, Mann-Whitney U test and Pearson's correlation coefficient. The p value at 0.05 was considered as statistically significant.

RESULTS

In the present study 150 apparently normal pregnant women participated. In the present study, a total of 150 pregnant women participated in the study. Maximum of 83.3% were in the age group of 21-30 years. About 16.7 % were from rural areas. 6.7% were illiterate, whereas 54% of them had studied up to a degree. About 62% of them were *primigravida*. 65.3% of them delivered vaginally. The birth weight of 22% of neonates were below 2.49 kg (Table 1). There was an increased serum level of zinc and selenium was found in the term pregnant mothers who gave birth normal birth weight when compared to low birthweight babies, though this difference was not statistically significant (Table 2). Median (IQR) values of Delta CT values of Mitochondrial DNA copy number in low-birth-weight babies were 3.07(1.7-5.74) and in normal birth weight babies was 3.71 (0.83-4.4). The difference in median values was not statistically significant (p=0.57; Table 3 and Fig. 1).

Table 1: Demographic characteristics of the study subjects.

Variables		**Number**	**Percentage**
Age	<= 20	7	4.7
	21 - 30	125	83.3
	31+	18	12.0
Address	Rural	25	16.7
	Urban	125	83.3
Educational status	Illiterate	10	6.7
	Primary	11	7.3
	High school	10	6.7
	PUC	56	37.3
	Degree	54	36.0
	Post-graduation	4	2.7
	Professional	5	3.3
Gravida	1	93	62.0
	2	32	21.3
	3	22	14.7
	4	3	2.0
Mode of delivery	Vaginal	98	65.3
	Caesarean section	52	34.7
Birth weight of baby in kg	<= 2.49	33	22.0
	2.50+	117	78.0
	Total	150	100.0

Table 2: Comparison of maternal zinc and selenium levels with low birth weight and normal birth weight

Baby birth weight (Binned)		**Mean**	**Std. Deviation**	**t**	**p**
Zinc µg/dL	<= 2.49	101.552	55.2840	0.72	0.42
	2.50+	108.562	41.4530		
Selenium µg/l	<= 2.49	157.414	44.0907	0.31	0.75
	2.50+	161.427	66.4856		

Table 3: Comparison of maternal mitochondrial DNA copy number with normal birth weight and Low birth weight using Mann Whitney U test.

	Low birth-weight	**Normal birth-weight**
Sample size	33	117
Lowest value	-8.8500	-7.0100
Highest value	-0.1300	10.9800
Median	-3.0700	-3.7100
95% CI for the median	-4.5042 to -1.7658	-3.8400 to -3.0926
Interquartile range	-5.7400 to -1.7000	-4.4000 to -0.8300

Average rank of first group	71.7273
Average rank of second group	76.5641
Mann-Whitney U	1806.00
Test statistic Z (corrected for ties)	0.565
Two-tailed probability	P = 0.5720

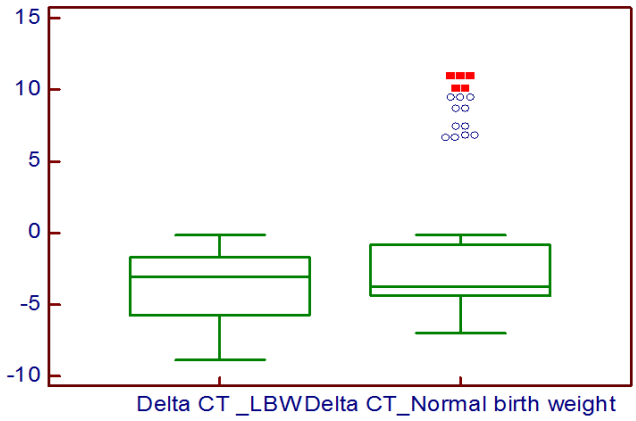

Fig. 1: Box and whisker plot diagram showing the Median values of Delta CT values of mitochondrial DNA copy number in low-birth-weight babies and Normal Birth weight babies

There was a positive correlation between the maternal zinc, selenium, and mitochondrial DNA copy number with birth weight of the baby. Though the correlation between Delta Ct means, and birth weight of the baby is found to be statistically significant (Table 4; Fig. 2).

Table 4: Correlation between the maternal zinc, selenium, and mitochondrial DNA copy number with birthweight of the baby

Variable	Baby birth weight	
	Pearson Correlation (r)	p
Zinc µg/dL	.049	.572
Selenium µg/l	.133	.107
CT	.025	.764
Delta Ct Mean	.250**	.002

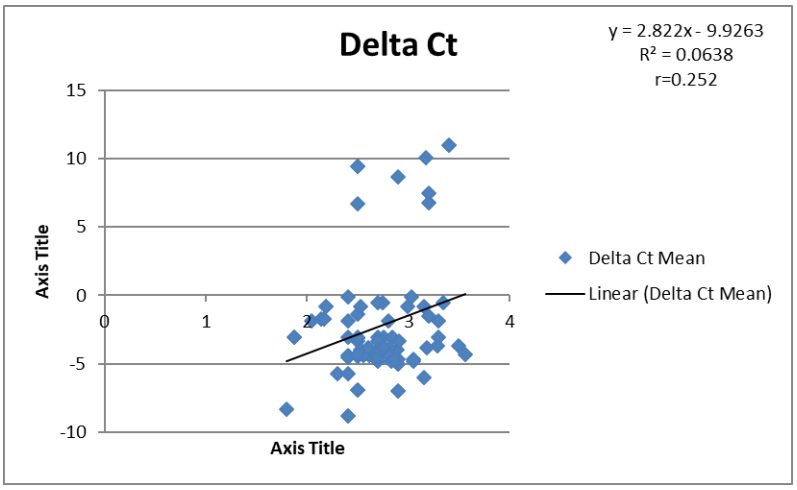

Fig. 2: Correlation between birth weight of the baby and the mitochondrial DNA copy number (Delta Ct values).

In the present study, almost 98.6 % of the women had zinc levels less than the recommended levels for pregnancy. Among them 81.9% of women gave birth to a normal birth weight baby. And this association was found to be statistically significant. Serum selenium was found to be <150 µg/l in 64.5% of the low-birth-weight babies. Odd ratio (95% CI) was 1.58(0.68-3.54), though it was not statistically significant (Table 5).

Table 5: Association between the maternal zinc and selenium levels with birth weight of the baby

Variables			Outcome		Chi square	p	Odds Ratio (95% CI)
			Normal	LBW cases			
Zinc µg/dL	<= 200.0	Count	109	24	4.05*	0.02	0.11(0.9-1.27)
		% within Zinc µ gm/dL (Binned)	82.0%	92.3%			
	200.1+	Count	0	2			
		% within Zinc µ gm/dL (Binned)	0.0%	7.7%			
Selenium	<150	Count	63	20	1.13	0.28	1.58(0.68-3.54)

µg/l		% within outcome	53.8%	64.5%			
	>151	Count	54	11			
		% within outcome	46.2%	35.5%			

*Yates corrected Chi square

DISCUSSION

In the present student, 150 term pregnant women participated. About 22% of the newborns were weighed<2.49 kg. Birth weight of the baby is determined by so many factors, in that maternal nutrition is foremost important. Dietary intake of balanced food and other micronutrients is very important for the birth weight.

Fetal growth is totally dependent on maternal nutrition through the placenta. The transport of small membrane permeable molecules such as oxygen (O_2) and carbon dioxide (CO_2) are influenced mainly by umbilical gradient blood flow and placental structure, whereas the larger nutrient molecules such as amino acids, fatty acids and glucose transport through placenta are dependent on nutrient transport proteins. The nutrient transport capacity of the placenta is influenced by numerous factors, including hormones, nutrient levels, and placental function. Furthermore, oxidative stress in the placenta has been shown to influence the transport of nutrients through altering the gene expression of different nutrient transporters (e.g., glucose and amino acid; 19).

In the present study, it was found that serum levels of Selenium were more among mothers who gave birth to normal weight babies, when compared to the low-birth-weight babies. In vitro studies have shown that selenium supplementation protects placental cells from oxidative stress through increased expression of selenium-containing antioxidants, such as glutathione and thioredoxin reductase. Hence, one of the leading hypotheses regarding how selenium may affect fetal growth is through the selenium-dependent anti-oxidative defense system (19,20). Other selenium-dependent proteins are the iodothyronine deiodinase (DIOs) that are involved in thyroid hormone metabolism (21). Thyroid hormones are essential in regulating placental nutrient transport, for example, hyperthyroidism is known to reduce circulating glucose in fetal tissues (22). Hence, another hypothesis on how selenium may influence fetal growth is through regulating the levels of thyroid hormones. In the animal study, wherein mice fed a diet low in selenium developed selenium deficiency. These mice had increased levels of both maternal and fetal plasma levels of the thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4; 19).

Mitochondria are both the culprit and victim of oxidative stress, being the major intracellular source and important target of oxidative stress. Investigators have observed that cells affected by oxidative stress synthesize more copies of their mtDNA and increase their mitochondrial density or abundance for compensating the damage, and to meet increased respiratory demand required for clearance of reactive oxidative species (23). Conversely, oxidative stress resulting from increased mitochondrial abundance may contribute to oxidative damage to mitochondria and other intracellular constituents including nuclear DNA, RNA proteins and lipids. Their preliminary investigation showed that placental mtDNA copy number is statistically significantly and positively associated with increased oxidative stress. But in the present study, MtDNA copy number was positively associated with the birthweight of the baby. This could be because of the optimum serum levels of selenium levels and zinc levels in pregnant women, who are apparently normal. The serum selenium levels were little less than the lab reference value, could be due to the hemodilution in the last trimester of pregnancy. The mechanism for the association of maternal serum concentration and increased risk of low birth weight could be explained due to the dual function of zinc, as an anti-oxidant as well as a pro-oxidant. The placenta is equipped with antioxidants inclusive of selenium-dependent enzymes of glutathione dismutase, thioredoxin reductases, seleno-protein-P and Cu/Zn superoxide dismutase which require the optimum levels of Selenium and Zinc. Free radical ions damage mitochondria and NADPH oxidases to produce Reactive oxygen species (ROS; 23).

Reactive oxygen species (ROS) increase has been documented in IUGR pregnancies. And ROS may have a role in altering the mtDNA copy number. It has been proposed that metabolic stress in mammalian cells, altering the expression of nuclear oxidative phosphorylation genes and mitochondrial biogenesis (24,25).

The increase of mtDNA copy numbers could be either due to the result of the feedback response that compensates for defective mitochondria bearing impaired respiratory chain or mutated mtDNA, caused by higher levels of ROS or due to the physiological increase in mitochondrial DNA copy numbers required for the fetal growth. Probably a vicious cycle results, when impaired mitochondria produce elevated levels of ROS, which further activate the nuclear response for the expression of oxidative phosphorylation genes (25).

There was an increase in Mitochondrial DNA copy number in the present study and it was positively

correlated with the birthweight of the baby. Though the selenium and Zinc levels were within the normal range in most of them, and not associated with the birthweight of the baby. This increase in mitochondrial DNA could have been raised to meet the increased demand by the developing fetus.

CONCLUSION

In the present study, apparently healthy pregnant women participated. The serum selenium and zinc levels were found to be within normal limits according to lab reference values. There was an increase in Mitochondrial DNA copy number in the present study and it was positively correlated with the birthweight of the baby. Hence further studies are required to study the actual interplay of micronutrients and MtDNA levels.

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CONFLICT OF INTEREST

There is no conflict of interest.

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