SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Gynaecology-Obstetrics

Fluctuations in Trace Elements During Pregnancy in Pregnant Women with Preeclampsia in Kisangani, Democratic Republic of Congo

Likilo Osundja Jérémie[1]*, Tshodi Bulanda Arsene[2], Lemalema Litanga Benjamin[1], Komanda Likwekwe Emmanuel[1], Juakali Sihalikyolo Jean-Jeannot[1], Buhendwa Mirindi Victor[3], Katenga Bosunga Gédéon[1]

[1]Department of Gynaecology-Obstetrics, Faculty of Medicine and Pharmacy, University of Kisangani
[2]Department of Gynaecology-Obstetrics, Faculty of Medicine and Pharmacy, University of Mbujimayi
[3]Department of Human Nutrition, Faculty of Medicine and Pharmacy, University of Kisangani

DOI: https://doi.org/10.36347/sasjm.2025.v11i06.009 | **Received:** 30.04.2025 | **Accepted:** 04.06.2025 | **Published:** 13.06.2025

***Corresponding author:** Likilo Osundja Jérémie
Department of Gynaecology-Obstetrics, Faculty of Medicine and Pharmacy, University of Kisangani

Abstract **Original Research Article**

Introduction: The concentration of various trace elements that are metabolically bound undergoes significant variation throughout pregnancy. This study aimed to determine variations in serum trace element concentrations in pregnant women who developed preeclampsia in Kisangani. ***Methods:*** From January 9th to September 9th, 2023, we conducted a prospective cohort study of pregnant women attending first-trimester antenatal clinics at eight health facilities in Kisangani. The sample size was 636 people. Concentrations of trace elements in sera were analysed using an Agilent 7700X inductively coupled plasma mass spectrometer (ICP-MS). The data were entered into a computer using Microsoft Excel 2021. Statistical analysis was performed using R software, version 4.3.0. ***Results:*** 73.9% of pregnant women were aged between 20 and 34, with an average age of 26.5 ± 6.3 years. The Trace element averages were as follows: for calcium in the first, second and third trimesters: 2.1±0.9, 1.8±0.6 and 1.8±0.6 mmol/l, and 1.4±0.6 mmol/l at diagnosis. For copper in the first, second and third trimesters: 9.6±2.5, 12.8±1.2 and 12.8±1.2 micromol/l and 12.9±1.3 micromol/l at diagnosis. For selenium and zinc: 0.7±0.3 and 11.2±3.1 micromol/l in the first trimester, 0.6±0.2 and 10.0±2.8 micromol/l in the second trimester, 0.5 ± 0.2 and 9.6 ± 2.5 micromol/l in the third trimester and 0.5±0.2 and 8.7±2.5micromol/l at the time of diagnosis. ***Conclusion****:* Fluctuations in serum trace element concentrations vary according to the period of pregnancy, in line with the progressively increasing micronutrient requirements for maternal-fetal well-being and the maintenance of a successful pregnancy.

Keywords: trace element, zinc, copper, selenium, pregnant woman, concentration, Kisangani.

INTRODUCTION

During pregnancy, women undergo a wide range of physiological and physical changes. Both mother and fetus undergo rapid growth and cellular differentiation; this state is highly sensitive to micronutrient intake, especially when maternal nutrition is deficient [1].

The demand for trace elements during pregnancy is increased to support optimal fetal growth, resulting in a decrease in their concentrations in maternal blood and tissues [2]. In particular, the physiological adaptations of a pregnant organism create a unique demand for essential trace elements. This demand is not identical throughout pregnancy [3].

Nutritional status, including the bioavailability and concentration of trace elements, is thought to be influenced by numerous physiological and dietary factors [4-6]. During pregnancy, physiological and dietary changes influence the bioavailability of trace elements as they are digested, absorbed and utilized in the body's biochemical circuit [3]. Moreover, little information is available on variations in trace element concentration in pregnant women during the different trimesters, or on how these changes are affected by varied and/or minimal nutritional intakes [7-10].

Nutrient stores and the maternal diet provide all the macronutrients and trace elements required for optimal fetal growth, essential for a successful pregnancy [11]. Not surprisingly, maternal deficiencies in key trace

elements can have profound effects on fetal development and pregnancy outcome [12].

Pregnancy complications, including pre-eclampsia, gestational hypertension, gestational diabetes, spontaneous preterm delivery and fetal growth retardation, together affect 25% of first pregnancies and predispose to both maternal and fetal morbidity and mortality. 85% of these pregnant women have deficiencies in one, two or more trace elements, and most are of low socio-economic status [13].

Serum trace element levels are an important indicator of the mother's nutritional status during pregnancy [14]. Early pregnancy is a period of particular exposure to the influences of maternal trace element levels. These may affect both maternal health and fetal development [15-18].

Pre-eclampsia still occurs in 5-10% of all pregnancies and remains one of the leading causes of maternal and fetal mortality in both developed and developing countries [19]. Despite extensive research, the etiology of pre-eclampsia remains one of the major unsolved mysteries in obstetrics.

Placental and maternal systemic oxidative stresses lead to a generalized inflammatory process that occurs in pre-eclampsia and is a major event in this multi-system disorder [20,21]. The placenta itself harbors certain antioxidant defenses, including selenium-dependent glutathione peroxidases, thioredoxin reductases, selenoprotein-P and copper/zinc and manganese superoxide dismutases (Cu/Zn and Mn SOD), which prevent the placenta from undergoing necessary damage. These antioxidant enzymes are rich in trace elements, which enable them to perform their functions (actions) optimally. This is the link between trace elements and other antioxidants in the pathogenesis of preeclampsia [22].

In low-income countries, preeclampsia has contributed between 20% and 80% to the increase in maternal mortality. It has increased perinatal mortality fivefold, accounting for 15% of premature births in high-income countries [23].

The severity and high prevalence of pre-eclampsia in low-income countries have led researchers to firmly believe in the role of nutrition, particularly trace elements, in the etiopathogenesis of pre-eclampsia [24-26]. Its epidemiology has shown that it is more prevalent in indigent women, which has further strengthened the possibility that nutrients may be involved in this disease [27, 28].

Various trace elements are metabolically linked, and their concentration undergoes significant fluctuations throughout pregnancy. Increased demand for these trace elements in an impoverished environment could affect pregnancy, childbirth and the postpartum period, with morbidities that can be difficult to manage and put the fetomaternal prognosis at risk [29].

In our environment, there is no doubt that nutritional deficiencies are common during pregnancy. However, little attention has been paid to trace element concentrations during pregnancy for the prevention of hypertensive disorders. In an attempt to improve the situation in the prevention of pre-eclampsia, we conducted this research to determine the pattern of trace elements (including calcium, copper, magnesium, selenium and zinc) from the first trimester of pregnancy through to delivery and the onset of pre-eclampsia.

To carry out this research, the main question was to determine the oscillations of trace elements in the serum of pregnant women in Kisangani.

The specific aims of this research were to determine trace element fluctuations during the three trimesters of pregnancy in pregnant women in Kisangani, and to determine the correlation between serum trace element levels and the onset of pre-eclampsia.

METHODS

1. Site, population and study period

This study was carried out in the town of Kisangani, located in the north-east of the Democratic Republic of Congo, capital of the Tshopo province. The choice of this city was justified by its technical facilities capable of carrying out the serum determination of trace elements useful for this research, and the presence of a HUB KISANGANI deconcentrated depot with a hyper-cold chain (from -50 to -120°C) capable of preserving sera in a stable state at -80°C.

This multi-site study was carried out in 8 health facilities in Kisangani, including the Kisangani University Hospital de, the Kabondo general referral hospital, the Foyer referral health center, the Saint Joseph health center, the Matete referral health center, the Mangobo general referral hospital, the Lubanga general referral hospital and the Makiso-Kisangani general referral hospital. All these health facilities have prenatal consultation and maternity wards supervised by doctors and/or nurses; medical biologists able to take venous blood samples.

The study population consisted of all pregnant women attending antenatal clinics in the first trimester, living in the city of Kisangani. The study ran from January 09, 2023 to September 09, 2023.

2. Sample and Sampling

To calculate our sample size, we used the article “First trimester microelement and their relationships with pregnancy outcomes and complications” published by Malgorzata Lewandowska *et al*. in Poland in 2020 [30]; we used the α threshold of 0.05 and a power of 95%. The minimum sample size was calculated using G-Power

software version 3.1.9.7. It was 636 individuals, including 318 with low trace element levels and 318 with normal trace element levels.

Our sampling was non-probability convenience sampling.

3. Selection criteria

a. Inclusion criteria

- Pregnant women living in the city of Kisangani;
- Being in apparent good health at the start of pregnancy or in the first trimester of pregnancy;
- To have voluntarily consented to participate in the study by signing the informed consent form;
- To have accepted that blood sampling be carried out during the three trimesters of pregnancy, and that personal data be recorded.

b. Non-inclusion criteria

- Pregnant women not living in the city of Kisangani;
- In- or outpatient Pregnant woman;
- Pregnant women taking multiple trace element supplements;
- Pregnant women with morbidities such as diabetes, hypertension, etc,
- Pregnant women who have not consented to participate in research.

c. Exclusion criteria

- Pregnant woman with poorly recorded personal data and/or hemolyzed blood sample,
- Pregnant woman whose sample (serum) had not been frozen at -80°c.
- Pregnant women who gave birth outside the health facility selected for this study.

d. Case selection criteria and control group

* Case: Pregnant woman with low serum trace element levels in the first trimester of pregnancy;
* Control group: Pregnant women with normal or high serum trace element levels in the first trimester of pregnancy.

4. Study design: We conducted a prospective cohort study.

5. Data collection procedure

Data collection was prospective in the prenatal consultation and maternity wards of the selected hospitals. After a 24-hour training session with the research team (doctors, head nurses of antenatal and maternity wards, laboratory technicians and a community liaison officer).

Activities (from reception to sampling) took place every working day from 8:00 to 12:00, depending on the arrival of pregnant women. The principal investigator visited at 11:00 a.m. to assess the day's activities, fill in the forms and check the status of the blood samples.

Once the respondent had finished with the doctor and nurse, she was received for sampling. Using a 5ml syringe, venipuncture was performed on the forearm or back of the hand, where 4-5ml of blood was drawn. From the syringe, the blood was injected into a dry vacutainer tube, which was then placed vertically in an isothermal box with an internal temperature of 2-8°c.

Once the samples had been taken, they were sent to the laboratory of the Kisangani University Hospital for analysis. Tubes containing blood were centrifuged at 5,000 rpm for 5 minutes. Then the sera were collected and divided by 2, placed in 2 different cryotubes, marked (each cryotube had a case identifier code in our file, i.e. each woman had two cryotubes bearing her identifier or code). The cryotubes were stored in the Hub-Kisangani at -80°c. Half of these cryotubes were sent to the large biochemistry laboratory of the University of Strasbourg's Faculty of Life Sciences, in collaboration with the laboratory of the Chemistry and Biochemistry Section of the University of Geneva's Faculty of Science, for comparative assays of trace elements. The sera were transported in a liquid nitrogen container to the Strasbourg University laboratory.

Trace element concentrations in the sera were analyzed using an inductively coupled plasma mass spectrophotometer (ICP-MS Agilent 7700X).

24-hour urine collection: Pregnant women with above-normal blood pressure (≥ 140/90 mmHg) were given dry, sterile containers in which to collect their 24-hour urine. If they receive the containers today at 9:30 am. Urine collection would begin the following day at 6am or upon waking. The urine would be collected until waking up or at 6am the next day, to be taken to the hospital to the nurses in charge of receiving the urine samples. The nurses would inform the principal investigator directly to arrange for the urine to be collected by 9:30 am. The urine was then sent to the university clinic laboratory for 24-hour proteinuria assays.

6. Data processing and analysis plan

a. Manual data processing and categorization;
b. Data analysis.

Data were entered on computer using Microsoft Excel 2021. Statistical analysis of the data was carried out using R software version 4.3.0.

We used percentage calculations for qualitative variables, mean, median, standard deviation, variance, minimum, maximum, first and third quartile, and mode for quantitative variables. We used the Kolmogorov-Smirnov and Shapiro-Wilk tests to test the normality

hypothesis; then, as our sample did not have a normal distribution, we used the Friedman test to compare means between different periods. We performed univariate and multivariate logistic regressions to calculate the Relative Risks.

7. Study variables

a. Dependent variable: Serum level of trace elements (Calcium, Zinc, Selenium, Magnesium and Copper), Blood pressure, 24-hour Proteinuria.
b. independent variables: Age of respondent; Marital status of respondent; Professions or occupation of woman and/or her spouse; Municipality of residence; Educational level; Socio-economic level; Weight of woman; Parity; Gestity; Age of last child; Medical history (malaria, intestinal parasitosis...); Surgical history; Complains; Clinical signs.

8. Operational definitions

The socio-economic levels were determined using the household economic well-being as constructed by Tchamda and Nkabkob [31]. This index takes into account households' possession of certain durable goods and housing characteristics. For our study, in relation to household durable goods, we looked for whether or not they own: television, radio, car, refrigerator and mobile phone cell phones; and with regard to housing characteristics, we asked about the availability of electricity, drinking water supply, use of modern toilets, construction of walls in durable materials and the use of embers or stoves as cooking fuel.

First, we assigned a score to each property or housing feature: 1 for “yes” and 0 for “no”. We then assigned each household a total score corresponding to the sum of all the scores obtained for each property or housing feature. In this way, households were ranked in ascending order of total score and divided into 5 categories called quintiles: poorest (total score = 1-2), second or poor (total score =3-4), average (total score = 5-6), fourth or rich (total score =7-8) and richest (total score = 9-10).

Private sector civil servant: Non-governmental organization employee, independent contractor.

- Preeclampsia: Association of BP≥ 140/90 mmHg + 24h Proteinuria: +++ or ≥ 300mg + Gestational age ≥ 20 Weeks of amenorrhea [32].
- Gestational hypertension: BP≥ 140/90 mmHg + Gestational age ≥ 20 Weeks of amenorrhea [32];
- Positive 24h proteinuria: 24-hour urine collected, tested positive by dipstick or at a value ≥ 300 mg by spectrophotometry.

9. Ethical considerations

The ethics committee of the University of Kisangani gave us its approval by letter N° of Approval: UNIKIS/CER/025/2022 dated 26/12/2022 to start with the actual data collection. Anonymity was guaranteed during data collection and analysis. Data were only collected once the woman had signed the informed consent form in the language she spoke, or after explanation in the local language (Kiswahili or Lingala).

10. Expression of Interest: We have no conflicts of interest to declare for this work.

RESULTS

1. Socio-demographic characteristics of respondents

Table 1: Distribution of respondents by socio-demographic characteristics

Socio-demographic characteristics	Frequency N=628	Percentage
Age (years)		
< 20	77	12.3
20 to 34	464	73.9
≥ 35	87	13.9
Address		
Mangobo	175	27.9
Makiso	164	26.1
Kabondo	104	16.6
Tshopo	100	15.9
Kisangani	47	7.5
Lubunga	38	6.1
Occupation		
Housewife	339	54.0
Tradeswoman	62	9.9
Government employee	46	7.3
Student	35	5.6
Dressmaker	34	5.4

Socio-demographic characteristics	Frequency N=628	Percentage
Teacher	27	4.3
Private sector employee	27	4.3
Farmer	19	3.0
Pupil	18	2.9
Nurse	17	2.7
Military	4	0.6
Marital status		
Married	542	86.3
Single	86	13.7
Educational level		
Primary	21	3.3
Secondary	465	74.0
Higher or university	142	22.6

The table shows that 73.9% of pregnant women were aged between 20 and 34, with an average age of 26.5 ± 6.3 years. The extremes of age were 15 and 46. Respondents living in the Mangobo and Makiso communes accounted for 27.9% and 26.1% respectively. Housewives accounted for 54.0% and married women 86.3%. 74.0% of pregnant women had secondary education.

2. Fluctuations in trace element levels among respondents

Table 2: Fluctuations in trace elements among respondents

Characteristics	First trimester	Second trimester	Third trimester	At diagnosis moment	During post-partum	p-value
	M (ST)	M (SD)	M (SD)	M (SD)	M (SD)	
Calcium (mmol/l)	2.1 (0.9)	1.8 (0.6)	1.8 (0.6)	1.4 (0.6)	1.7 (0.5)	<0.001
Copper (micromol/l)	9.6 (2.5)	12.8 (1.2)	12.8 (1.2)	12.9 (1.3)	12.3 (1.5)	<0.001
Magnesium (mmol/l)	0.6 (0.2)	0.6 (0.3)	0.4 (0.2)	0.5 (0.2)	0.3 (0.1)	<0.001
Selenium (micromol/l)	0.7 (0.3)	0.6 (0.2)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	<0.001
Zinc (micromol/l)	11.2 (3.1)	10.0 (2.8)	9.6 (2.5)	8.7 (2.5)	9.3 (2.6)	<0.001

M= Mean; SD= Standard deviation.

This table shows that there was a statistically significant difference between the levels of all trace elements over the different periods.

3. Fluctuations in trace element levels in pregnant women with preeclampsia

1. Fluctuating serum calcium levels

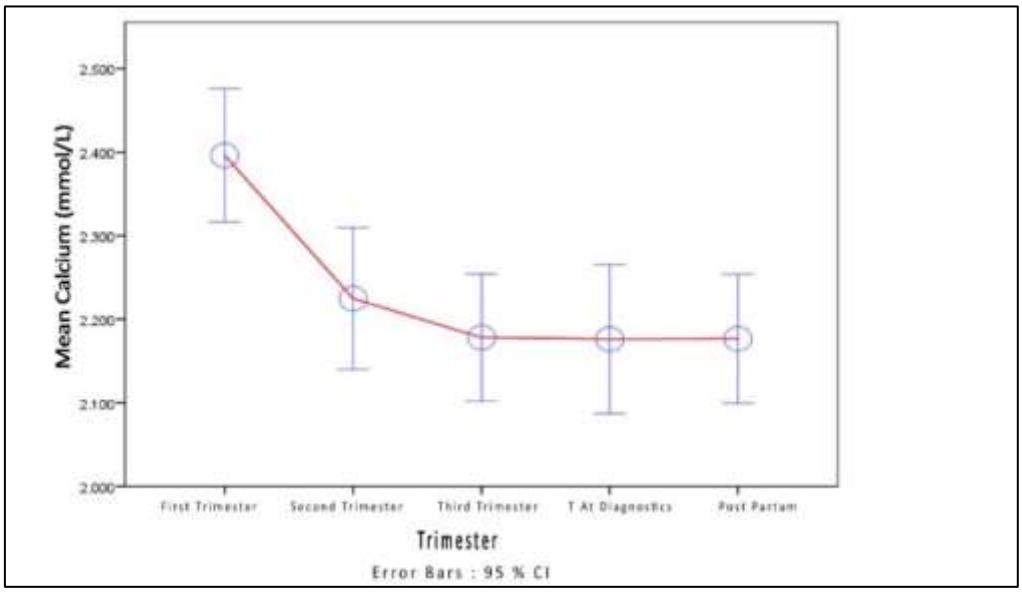

Figure 1: Serum calcium fluctuation in pre-eclamptic pregnant women with normal status

2. Serum copper fluctuation

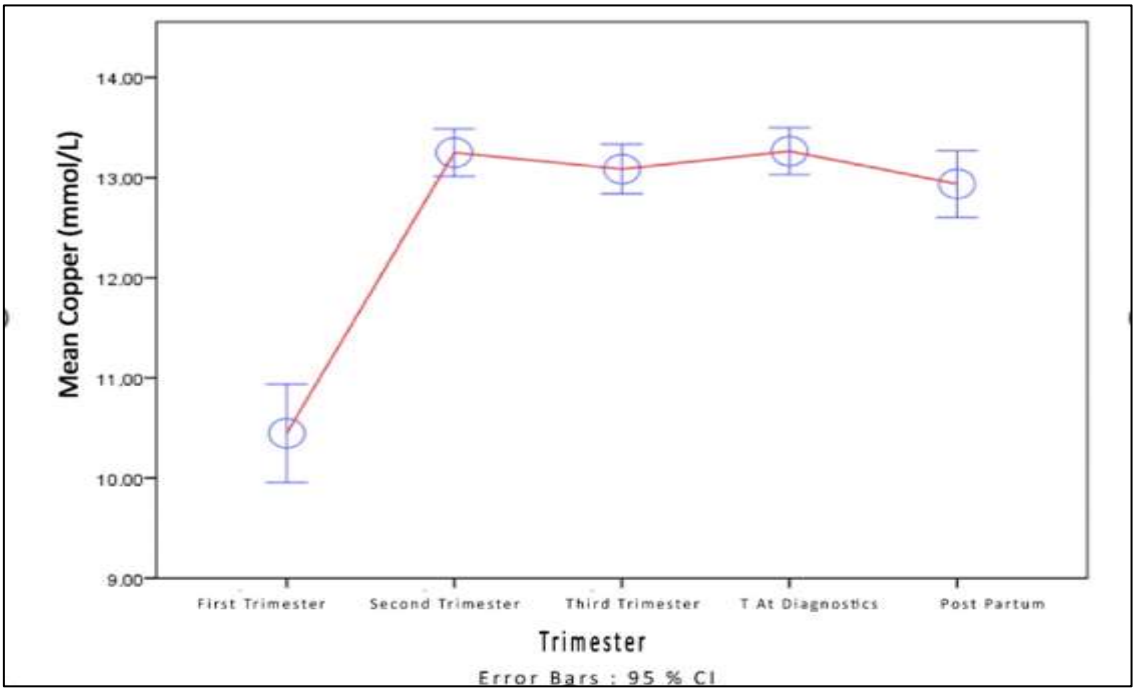

Figure 2: Serum copper fluctuation in pre-eclamptic pregnant women with normal status

This figure shows that blood calcium levels stabilized from the third trimester of pregnancy to the postpartum period.

This figure shows a rise in cupremia from the first to the second trimester of pregnancy, stabiling and remaining within the normal range until the postpartum period.

3. Magnesium evolution

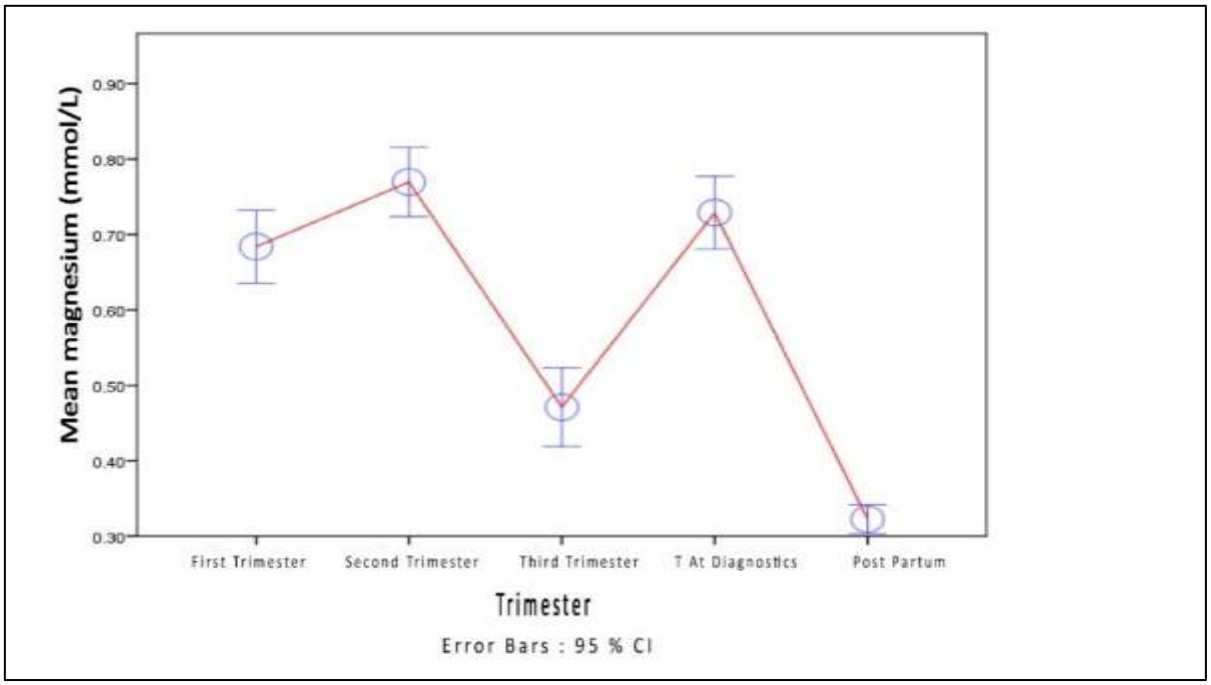

Figure 3: Changes in magnesium levels in pregnant women with normal trace element status who developed preeclampsia

This figure shows that high magnesium levels were observed in the second trimester and at the time of diagnosis

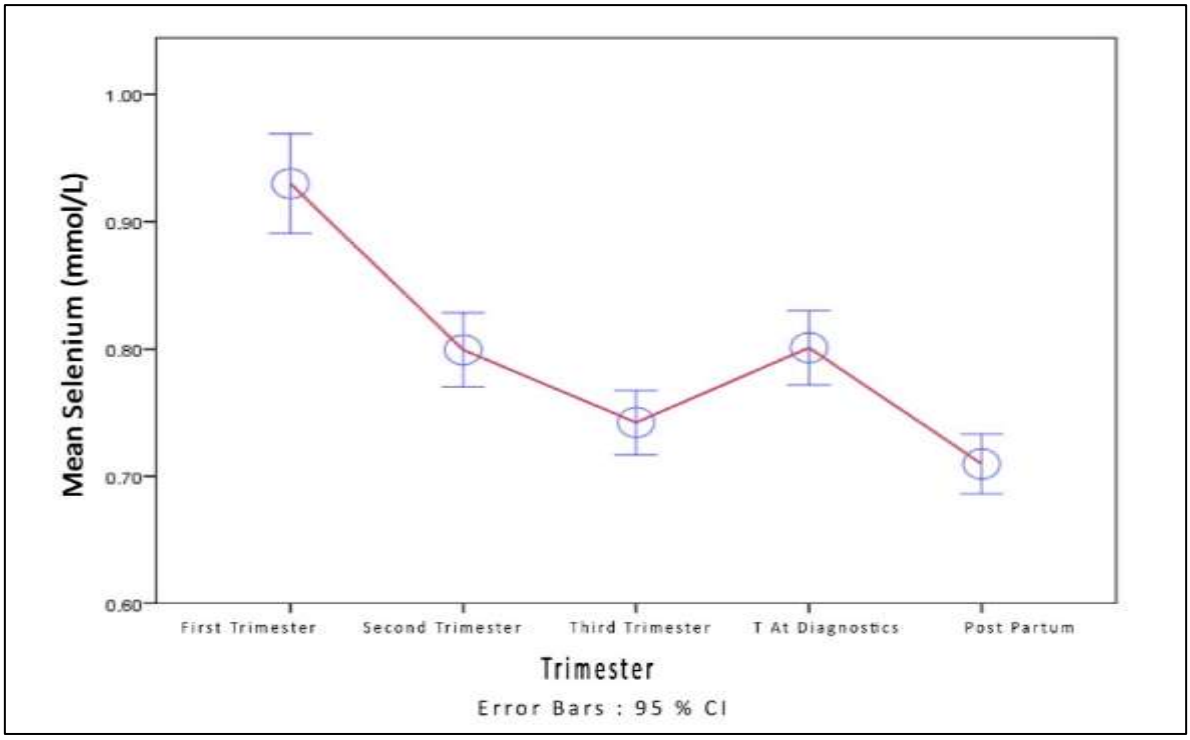

Figure 4: Serum selenium fluctuations in pregnant women with normal trace element status

This figure shows that selenium fluctuations remained within norms despite the decrease in values.

4. Selenium fluctuation

5. Serum zinc levels

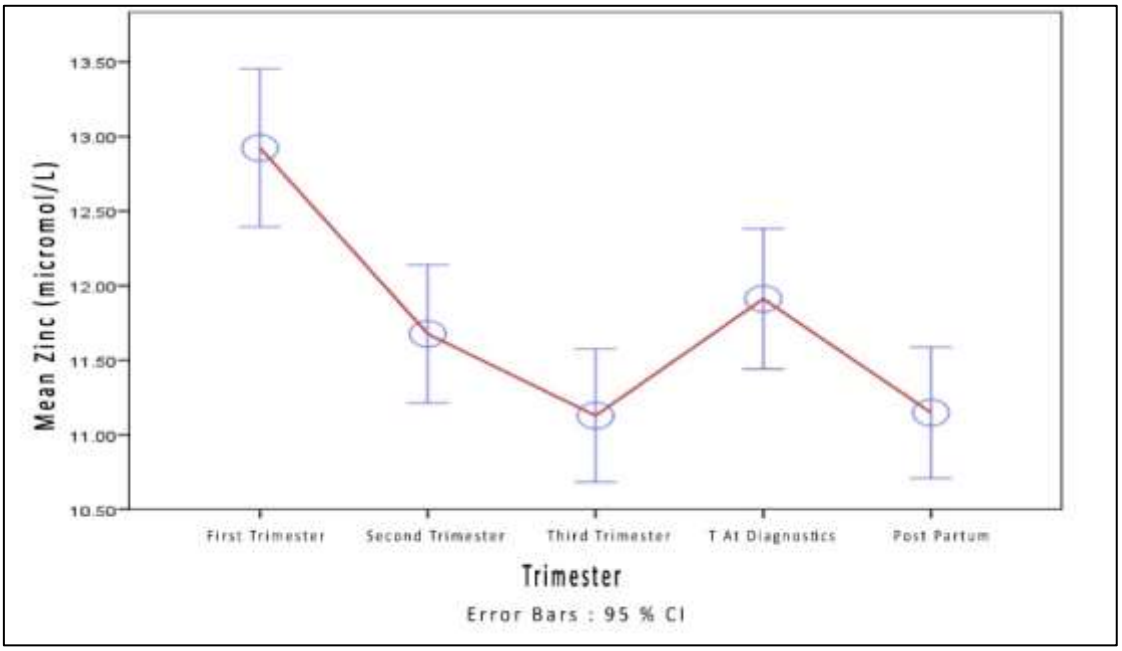

Figure 5: Fluctuation in serum zinc in pregnant women with normal trace element status
Analysis of this figure shows that zinc levels were within normal limits at the time of diagnosis and even in the post-partum period

6. Association of age, gestational age, trace element status and preeclampsia

Table 3: Association between age, parity, gestation and trace element status and preeclampsia

Characteristics	Total N = 628	Preeclampsia (n = 294)	RR	IC 95%	p-value
Age(years)					
< 20	107	53 (49.5%)	—	—	
20 to 34	447	204 (45.6%)	0.92	0.74 – 1.15	0.5
≥ 35	74	37 (50.0%)	1.01	0.74 – 1.37	>0.9
Parity					
Nulliparuous	175	80 (45.7%)	—	—	
Primiparous	110	49 (44.5%)	0.97	0.75 – 1.26	0.8
Secondiparous	130	69 (53.1%)	1.16	0.92 – 1.47	0.2
Multiparous	182	79 (43.4%)	0.95	0.76 – 1.19	0.7
Grand multiparous	31	17 (54.8%)	1.2	0.80 – 1.73	0.4
Gestity					
Primigravida	159	75 (47.2%)	—	—	
Secondgravida	105	46 (43.8%)	0.93	0.71 – 1.21	0.6
Multigravida	288	139 (48.3%)	1.02	0.83 – 1.26	0.8
Large multigravida	76	34 (44.7%)	0.95	0.70 – 1.27	0.7
Trace elements status					
Normal	322	77 (23.9%)	—	—	
low	306	217 (70.9%)	2.97	2.46 – 3.60	<0.001

Table 3 shows that low trace element status in our pregnant women was associated with the onset of preeclampsia. This risk was 2.97 times higher in deficient pregnancies (RR: 2.97; CI: 2.46-3.60; p-value < 0.001).

Table 4: Multivariate Analysis

Characteristics	ARR	IC 95%	p-value
Age (years)			
> 20	1	—	
20 to 34	0.89	0.67 – 1.18	0.4
≥ 35	1.04	0.67 – 1.59	0.9
Gestity			
Primigravida	1	—	
Secondgravida	0.63	0.30 – 1.15	0.2
Multigravida	0.58	0.24 – 1.27	0.2
Large multigravida	0.45	0.17 – 1.08	0.088
Parity			
Nulliparous	1	—	
Primiparous	1.62	0.86 – 3.46	0.2
Secondiparous	1.69	0.77 – 4.13	0.2
Multiparous	1.78	0.80 – 4.36	0.2
Grand multiparous	2.08	0.80 – 5.82	0.15
Trace element status			
Normal	1	—	
low	2.99	2.47 – 3.63	**<0.001**

After elimination of confounding factors, Table 7 shows that the ARR was 2.99. In other words, pregnant women with low trace element status were 2.99 times more likely to develop preeclampsia than those with normal status.

DISCUSSION

1-Fluctuation of trace elements during pregnancy

Calcium (in mmol/l): from 2.1±0.9 at T1, to 1.8±0.6 at T2, to 1.8±0.6 at T3 and 1.7±0.5 postpartum. Copper (in micromol/l): from 9.6±2.5 at T1, to 12.8±1.2 at T2, to 12.8±1.2 at T3 and 12.3±1.5 postpartum. Magnesium (in mmol/l): from 0.6±0.2 at T1, to 0.6±0.3 at T2, to 0.4±0.2 at T3 and 0.3±0.1 postpartum. Selenium (in micromol/l): from 0.7±0.3 at T1, to 0.6±0.2 at T2, to 0.5±0.2 at T3 and 0.5±0.2 postpartum. Zinc (in micromol/l): from 11.2±3.1 at T1, to 10.0±2.8 at T2, to 9.6±2.5 at T3 and 9.3±2.6 postpartum.

The results of current study are similar to those observed by Chun-mei Liang *et al* in China [33], Seema Jain *et al*.in Agra (India) [34], and Iwona Lewicka *et al.*

in Poland [35]. Those authors observed that only maternal serum copper levels fluctuated in the direction of increase among the different trimesters of pregnancy, returning to pre-pregnancy values in the post-partum or post-delivery period. Meanwhile, levels of trace elements such as calcium, magnesium, selenium and zinc decreased as pregnancy progressed.

However, Fahimeh Khoushabi *et al* [36], Fatemeh Moghaddam *et al* [37] in Iran; and Jinhao Liu *et al* [38] in China, had observed a constancy in the variation of trace elements during pregnancy where the levels of different trace elements did not decrease significantly to be noticed hence this serum stability until the postpartum period.

Our results show that copper levels increase during pregnancy and decrease after delivery, remaining within normal limits. Copper, a trace element, is involved in the function of several life-sustaining cooper related enzymes. Copper deficiency affects many enzymes, leading to defects in ATP production, lipid peroxidation, hormone activation, angiogenesis, and abnormalities of the vasculature, skeleton and lungs [39].

The increase in copper levels as pregnancy progresses may be partly linked to the synthesis of ceruloplasmin, a major copper-binding protein, due to high maternal estrogen levels. Another reason may be the decrease in biliary copper excretion induced by hormonal changes, typical during pregnancy [40], similarly copper absorption increases during pregnancy due to the increased need for maternal copper-containing enzymes, such as cytochrome c oxidase, required for aerobic respiration, and superoxide dismutases, the enzymes that catalyze the dismutation of superoxides into oxygen and hydrogen peroxide [33]. The increase in copper during pregnancy interferes with zinc absorption, thus explaining the low zinc concentration [37].

In our study, there was a decline in maternal serum zinc levels as pregnancy progressed, although there was no statistical significance in early pregnancy. The decline may be explained by a disproportionate increase in maternal plasma volume, as well as by maternal-fetal transfer of this trace element. Other reasons may be reduced binding of zinc to certain proteins [41], low dietary bioavailability [42], or very high dietary copper or iron levels competing with zinc at absorption sites [43]. There are also many different points of view: Nynke de Jong [44] found that there was no decrease in serum zinc measured at 24- and 36-weeks' amenorrhea, as did other researchers [41,45].

Hambidge *et al* [45] reported that Zn absorption clearly increases towards the end of pregnancy. This may perhaps explain the absence of any significant difference between mid- and late-pregnancy zinc concentrations in the present study. Zinc is widely recognized for its essential roles in cell division, differentiation and function, which are critical for tissue growth. Consequently, zinc is an essential nutrient during embryogenesis, fetal growth and development, increasing the mother's zinc requirements during pregnancy [22].

Although some studies have found that serum calcium levels do not change throughout pregnancy [46,47], our research and several other studies have proven the contrary. Calcium exists in the blood in three forms: ionic, protein-bound and complexed in physiological equilibrium with each other, and varies according to physiological, biochemical and pathological variations [48].

The decrease shown in the present study may be the result of an increased demand for calcium and inorganic phosphate for fetal development and the consequence of maternal hemodilution. This drop largely reflects the fall in serum albumin and the albumin-bound fraction of total calcium [49]. During pregnancy, the very high circulating concentrations of estrogen and progesterone alter the concentration of many substances, including calcium, in maternal blood [48]. Studies of calcium homeostasis responses during pregnancy have shown an increase in intestinal calcium absorption and urinary calcium excretion during pregnancy, and an increase in the rate of bone turnover during pregnancy [50,51]. Another factor affecting blood calcium levels is nutritional intake. In addition, increased maternal 1,25-dihydroxyvitamin D3 production is an independent phenomenon that leads to a marked increase in intestinal calcium absorption and may allow the maternal skeleton to store calcium ahead of peak fetal demands later in pregnancy [52]. All these elements may explain the serum zinc fluctuations during the different weeks of pregnancy in the present study.

As shown in the present study, serum magnesium levels had decreased with increasing gestational age, without returning to normal after delivery, revealing concordances with some earlier reports [53]. But there was no significant difference between mid- and late-pregnancy magnesium concentrations. In general, hypomagnesemia may be associated with hemodilution, renal clearance during pregnancy and mineral consumption by the growing fetus [53-55]. In addition, serum magnesium fluctuations are partly related to maternal estrogen concentration [56].

Selenium plays a vital role in the course and outcome of pregnancy. It is therefore doubly solicited for the metabolism of the maternal-fetal and placental pair. This is why, more often than not, we observe a progressive decrease in its serum concentration during pregnancy, with no tendency to return to normal pre-pregnancy levels without supplementation or adequate dietary intake after delivery. This pattern was observed

in this research and in those carried out by Ebba H *et al* [57], and Rihwa Choi *et al* [58].

2-Correlation between serum trace element levels and the onset of preeclampsia

Our research shows that there is a correlation between low trace element status and the risk of developing preeclampsia (RR: 2.97; CI: 2.46-3.60; p-value: < 0.001). Pregnant women with low trace element status had a 2.97 times greater risk of developing preeclampsia than those with normal status. After eliminating confounding factors, this risk increased to 2.99 times (ARR: 2.99; CI: 2.47-3.63; p-value: 0.001).

Our results are similar to those of Lisa SH *et al* [59], Sarwar MS *et al* [60] in Iran, and Naim Uddin S. M. *et al* in Bangladesh [61].

On the other hand, Pyla KK *et al* [62] found a correlation between micronutrients, the age of pregnant women and the onset of pre-eclampsia; and Noura Al-Jameil *et al* [63] found no correlation between the onset of pre-eclampsia and micronutrient status or level in pregnant women in Riyadh.

This difference may be due to the selection criteria, which differ from one study to another, as do the case selection sites and the environment.

In general, there is a physiological balance between antioxidants and free radicals. When antioxidants are deficient or depleted in the body, the increased level of lipid peroxidation damages blood vessels, leading to the alteration of polyunsaturated fatty acids, which destroy the mechanism and formation of the capillary endothelial cell; thus, endothelial cell damage or injury may be the main element in triggering the pathophysiological events of pre-eclampsia [61].

Copper, zinc and manganese are essential components of the antioxidant enzyme superoxide dismutase, which is involved in the destruction of free radicals/ROS and thus protects cells from damage. Their deficiency can lead to insufficiency of the superoxide dismutase enzyme system, thus exposing women to free radical/ROS accumulation and oxidative stress and cell damage, which leads to oxidative stress, playing a key role as a possible mediator of endothelial cell dysfunction,[64] hypertension, and thus clinical manifestations of preeclampsia [65].

Preeclampsia, a multifactorial disease, results from the generation of oxidative stress in pregnant women. Increased production of free radicals and reduced levels of certain trace elements required for antioxidant defense mechanisms are the main contributors to this stress.

The correlations between low levels of trace elements and the occurrence of preeclampsia in this study could be explained by the fact that these reduced or low serum levels of trace elements (calcium, selenium, zinc, and magnesium) may have contributed to the inefficiency of the antioxidant defense system, allowing for the free production of free radicals (oxidative stress), increased vascular resistance, and high blood pressure in deficient pregnant women.

CONCLUSION

At the end of this research, we observed that the fluctuations of various trace elements did not occur uniformly throughout pregnancy, at the time of diagnosis of preeclampsia, and in the postpartum period. Pregnant women with a low status of trace elements had 2.97 to 2.99 times more risk of developing preeclampsia than those with normal trace element status.

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