

# **Original Research Article**

# Effect of trace elements in the immune system of pregnant women attending antenatal clinic in alex ekwueme federal university teaching hospital

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# ABSTRACT

Copper, iron, selenium and zinc are important trace elements during pregnancy. They function as antioxidants and play major roles in strengthening the immune system. This study evaluates the importance of these trace elements in the immune system and their concentrations during pregnancy. These trace elements were evaluated using 75 pregnant women attending antenatal clinic in Alex Ekwueme Federal University Teaching Hospital, Abakaliki as Test participants and 75 non-pregnant women were used as control participants. The analysis was done using Atomic Absorption Spectrophotometer. The sociodemographic, Obstetrics and Gynaecological characteristics of the participants were obtained using questionnaire. A cross-sectional comparative study design was adopted for this. The results showed that the mean and standard deviation ( $M\pm SD$ ) in mg / l of the test and control of trace elements (copper, iron, selenium and zinc) are; 0.13±0.03 and 0.38±0.03, 0.48±0.07 and 0.82±0.09, 0.86±0.11 and 0.94±0.07, 0.52±0.07 and 0.52±0.05 respectively. Also mean levels of Cu and Fe were significantly decreased in the pregnant women than in control (p=<0.001; 0.002) respectively. Nevertheless, there was no statistically significant differences between selenium and zinc levels in the pregnant women when compared to their controls (p=0.575; 0.982) respectively. Low concentration of iron is suggestive of iron deficiency and this study revealed 65.3% non-compliance to routine iron supplement given during antenatal. Trimester, age and parity appeared not to have any significant effect on the level of these trace elements. Hence, there is need to ensure the intake well-balanced meal to optimize copper levels during pregnancy.

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# 1. Introduction

Pregnancy is a period of increased hormonal and metabolic demands associated with changes in a woman's physiology

and the requirements of a growing foetus.<sup>1,2</sup> These changes during pregnancy help to facilitate the handling of nutrients by the body tissues of the mother as well as their transfer to the foetus. From conception to birth, foetal organ synthesis and development stems from the maternal nutrient intake and hence, adequate supplies of

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https://doi.org/10.18231/j.jchm.2021.040 2394-272X/© 2021 Innovative Publication, All rights reserved. vitamins and trace elements are critical in maintaining health and development of humans.<sup>3–5</sup> Deficiency of trace elements during pregnancy can lead to a state of biological competition between the mother and fetus which can be detrimental to the health status of both.<sup>6</sup> Deficiencies of specific antioxidant activities associated with the trace elements; selenium, copper and zinc can result in poor pregnancy outcomes; including suppressed immunity, fetal growth restriction, preeclampsia and the associated risk of diseases in adulthood; including cardiovascular diseases and type 2 diabetes.<sup>7</sup>

Trace elements are inorganic molecules found in human and animal tissues in milligram - per-kilogram amounts or less and are essential for life. They are essential because a deficient intake of these elements can cause an impairment of function and only physiological amounts of that element can alleviate the impairment.<sup>8</sup> They are also components of regulatory enzymes and hormones essential to the division and differentiation of fetal cells and their further development.<sup>9</sup> Some of the trace elements include; iodine, iron, cobalt, chromium, copper, manganese, magnesium, molybdenum, selenium and zinc. Elements such as iron, zinc and selenium are essential components of enzymes where they attract or subtract molecules and facilitate their conversion to specific end products.<sup>8</sup> Iron is involved in normal metabolism of the cells, the iron requiring enzymes include; the xanthine oxidase, cytochrome C reductase, acyl - Co A dehydrogenase, NADH - reductase. This group uses riboflavin as co enzyme. Other enzymes in this group that requires iron as a cofactor include succinate dehydrogenase, aconitase and ribonucleotide reductase.<sup>6</sup> Zinc forms an integral part of several enzymes (metallo enzymes) in the body. They include superoxide dismutase, carbonic anhydrase, leucine amino peptidase and carboxyl peptidase 'A' others include alcohol dehydrogenase, retininereductase, alkaline phosphatase, glutamate dehydrogenase. Selenium is a major component of the enzyme, glutathione peroxidase which is present in cell cytosol and mitochondria and functions to reduce hydrogen peroxide. Few trace elements donate or accept electrons in redox reactions which results in generation and utilization of metabolic energy and have an impact on the structural stability of certain biological molecules; iron is involved in normal metabolism as a constituents of enzymes such as xanthine oxidase, cytochrome reductase.<sup>8</sup> Some of the trace elements; control important biological processes either by facilitating the binding of molecules to their receptor sites on cell membrane or inducing gene expression resulting in the formation of protein involved in life processes.<sup>10</sup> Trace elements are required for the immune system to function especially in pregnancy and their deficiency suppresses immunity during pregnancy by affecting innate T cell mediated and adaptive antibody responses leading to increase susceptibility to infections with increased

morbidity and mortality. Also, infections aggravate trace element deficiencies by reducing nutrient intake, increasing losses and interfering with utilization by altering metabolic pathways.<sup>10</sup> Furthermore, the increase rate of adverse pregnancy outcomes in Nigeria has been associated with oxidative stress and anaemia. Also with the global concern in the reduction of maternal mortality and morbidity during pregnancy, there is need to ascertain the levels of selected trace elements; such as iron, copper, selenium and zinc which have antioxidant and immunological properties as this will aid in adequate antenatal care. Although some other authors have investigated some other aspects of parameters in pregnant women,<sup>11,12</sup> this study intends to summarize the immunologic roles of these trace elements; copper, iron, selenium and zinc, during pregnancy.

#### 2. Materials and Methods

# 2.1. Research design

Cross-sectional comparative study was adopted for this study to analyse the status of selected trace elements in pregnant and non-pregnant women. This design is considered appropriate for this study because it allows for collection of data from a group of people for the purpose of describing a phenomenon under study. The design has been successively used by other authors such as Igbal et al. to conduct similar quantitative studies that permitted the description and documentation of aspect of a situation as it occurred.<sup>13</sup>

# 2.2. Population of the study

The population for this study consisted of new cases of pregnant women who were attending antenatal clinic in Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) within January to March, 2019. The antenatal clinic runs from Monday to Friday of every week with an average of twenty-one (21) patients in a day totalling one thousand, four hundred and ninety-five (1,365) pregnant women. The non-pregnant women population consisted of the women that have gynaecology cases within the same period. The gynaecology clinic runs from Monday to Friday of every week with an average of six (6) women in a day totalling three hundred and ninety (390) women. Therefore, the population of the study was one thousand, seven hundred and fifty-five (1,755) women.

#### 2.3. Study area

The study area chosen for this research work was the Obstetrics and Gynaecology Department of the Alex-Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA), Ebonyi State. The hospital was established by the British Colonial Administration in 1930. The name changed from Casualty Control Centre to Abakaliki General Hospital and to Federal Medical Centre (FMC). On 7<sup>th</sup> December, 2011, FMC was upgraded to a Federal Teaching Hospital (FETHA). Ebonyi State University Teaching Hospital (EBSUTH) was also absorbed into the new mega Teaching hospital, Comprising of FETHA 1 (Former FMC) and FETHA 11 (Former EBSUTH). Currently in April 2019, the name was changed to AE-FUTHA. As the only referral Teaching Hospital in the State, most women come for ante- natal care in this institution.

#### 2.4. Sample size determination

The sample size was determined using formula for sample size calculation for comparison between two groups when endpoint is quantitative data.<sup>14</sup>

$$n = \frac{2\left(Z_{\alpha/2} + Z_{\beta}\right)^2 \sigma^2}{d^2}$$

Where n is the desired sample size

 $Z_{\alpha /2}$  is the critical value of the Normal distribution at  $\alpha/2$  (e.g. for a confidence level of 95%,  $\alpha$  is 0.05 and the critical value is 1.96),

 $Z_{\beta}$  is the critical value of the Normal distribution at  $\beta$  (e.g. for a power of 80%,  $\beta$  is 0.2 and the critical value is 0.84),

 $\sigma$  is the standard deviation from previous study of Igbal et al., (2020) = 1.09 $\mu$ mol/L for Selenium

d is the difference between mean values you would like to detect =  $0.5\mu$ mol/L for Selenium

$$n = \frac{2 \times (1.96 + 0.84)^2 \times 1.09^2}{0.5^2}$$

$$n = \frac{2 \times (2.8)^2 \times 1.09^2}{0.5^2}$$

 $n = 74.5 \approx 75$ 

A total of seventy-five (75) samples was collected from pregnant women aged between 18 to 45years, attending antenatal clinic for the analyses. Also a total of seventyfive (75) control samples were collected from non-pregnant women attending gynaecology clinic.

#### 2.5. Sample collection

5ml venous blood was collected from subjects using sterile disposable syringes into sterile plain tubes. Samples for the non – pregnant group was collected on the 5<sup>th</sup> day of their menstrual cycle after an early morning negative pregnancy test. Every sample collected was screened for HBsAg and HIV I and II before analyses. The samples stood for 30minutes to clot, then centrifuged for 5minutes at 3500rpm. The serum was transferred into metal free plain tube and stored frozen at -20°C until analysed.

#### 2.6. Sample analysis

The serum was analysed for the level of trace elements (copper, iron, selenium and zinc). These analyses were done using the atomic absorption spectrophotometer (AAS) technique.

# 2.7. Principle of AAS technique

This procedure is based on flame absorption rather than flame emission. Metal atoms absorb strongly at discrete characteristic wavelengths which coincide with the emission spectra of the metal in question. A solution of the sample was converted into an aerosol which is injected into a flame which then converts the sample into an atomic and molecular vapour. The atomic vapour absorbs radiation from a hollow cathode lamp at specific wavelengths. This beam then transverses the flame and is focused on the entrance slit of a monochromator, which is set to read the intensity of the chosen spectra line. Light with this wavelength is absorbed by the metal in the flame and the degree of absorption is a function of the concentration of the metal in the sample.<sup>15</sup>

# 2.8. Inclusion and exclusion criteria

- 1. Every sample was screened for HIV I and II and Hepatitis B surface Antigen and the reactive participants were excluded in this study while the nonreactive participants were included in the analyses.
- 2. Every sample from the Non-pregnant participants was tested to rule out pregnancy.
- 3. The age range for Test and Control participants were between 18 45 years.
- 4. The study participants were residing within Abakaliki metropolis

## 2.9. Method of data analysis

The Statistical Package for Social Sciences software version 20 (SPSS Inc, Chicago, IL, USA) was used for data analysis. Frequency counts and percentages were used for the demographic characteristics and obstetrics and gynaecology characteristics of the study participants. Student t-test and Analysis of Variance (ANOVA) were used to test the significance difference of the trace elements in the immune system of the participants as P<0.05 was considered significant. Receiver Operating Characteristics (ROC) curves was used to predict the level of the trace elements in the immune system of the pregnant women.

#### 2.10. Ethical consideration

The ethical approval for this study was obtained from the Hospital Research Ethical Committee of Alex-Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State (FETHA/REC/VOL. 2/2018/060). Also written and verbal informed consent was gotten from every participant.

#### 3. Results

The result in Table 1 shows the demographic characteristics of the respondents in the study. The mean age of the participants who were pregnant and non-pregnant were  $30.3 \pm 4.8$  years and  $31.1 \pm 5.1$  years respectively. Almost half of them were in age group 26-34, while those in age 18-25 were the least, which was not significant between the two groups (P>0.05). About 30% of them were civil servants, closely next were the self-employed; however, this was not significant between the two groups (P>0.05). About 50% of them had tertiary education, while those with primary education were very few; this is not significantly different in both groups (P>0.05). Most of the study participants had their spouse to be civil servants, which was followed by trader and self-employed but not significantly different between the two groups (P>0.05). Their social economic class showed that they were more of middle class, while the high class was the least. The participants were predominantly Igbos with close to 90% of them, while others were from different tribes.

The obstetric and gynaecological characteristics of the participants showed that most of them were in their third trimester, while those in their first trimester were the least. Most of the study participants had children between 1 to 4, while very few of them had no children; this is not significantly difference between the two groups (P>0.05). Majority of the study participants had no history of miscarriage, while most of the pregnant women (about 65.3%) were not consistent with their antenatal routine drugs. See Table 2.

The level of trace elements in the immune system of the pregnant women and the control as shown in Table 3 revealed that the copper and iron in the immune system of the pregnant women are significantly lower than that of the control (P<0.05). However, selenium and zinc in the immune system of the pregnant women are not significant with that of the control (P>0.05).

The level of trace elements in the immune system of the pregnant women is not significantly different with their age (P>0.05). In the same vein, the level of trace elements in the immune system of the non-pregnant women is not significantly different with their age (P>0.05). See Table 4.

The level of trace elements in the immune system of the pregnant women is not significantly different with their gestational age (P>0.05). See Table 5.

The level of trace elements, selenium and zinc in the immune system of the pregnant women is not significantly different with their social class (P>0.05) except for copper and iron which have a p value of less than 5% significance. In the same vein, the level of trace elements in the immune system of the non-pregnant women is not significantly different with their social class (P>0.05). See Table 6.

Receiver Operating Characteristics (ROC) curves were used to set the cut-off points of the test results using nonpregnant women as the standard. From the results obtained from the ROC curves, the cut-off point for Copper in pregnancy is Cu<0.234, that of Iron is Fe<0.551, Selenium is Se<1.077, and Zinc is Zn<0.601. See Figure 1.

The prediction of the level of copper in the immune system of pregnant women to be Cu<0.234mg/dl had the highest accuracy of 78.5%, while that of Zinc is the least with Zn<0.601mg/dl and accuracy of 64.3%. See Table 7.



Fig. 1: ROC curves for Cu, Fe, Se, and Zn measured for pregnant women and non-pregnant women as control

#### 4. Discussion

It was observed in this study that there were significant variations in the level of copper and iron level in pregnant (test) and non-pregnant (control) participants. No significant variation was observed in the levels of Se and Zn.

The Immune system requires Cu to perform several functions. The mean Cu level of pregnant women showed a significant decrease (0.13±0.03mg/l for Test and  $0.38\pm0.03$  mg/l for control with a P < 0.001). This decrease in the level of copper during pregnancy when compared with non-pregnant women is not consistent with the findings of Kilinc et al., Nwagha et al, Mistry and Pala; Spenser et al and Skalnaya et al. who reported that plasma Cu concentrations significantly increases during pregnancy and returns to non-pregnant values after delivery.<sup>6,16-19</sup> Cu is an essential cofactor for antioxidant enzymes including copper/zinc superoxide dismutase (SOD) and cytochrome c oxidase. Cu plays essential role in combating the oxidative stress associated with pregnancy through its ability in preventing damage due to free radical effects on lipids, proteins and DNA molecules; without which there will be increase in oxidative stress and decrease immune function. The decrease immune function is as a result of reduction in

 Table 1: Socio-demographic characteristics of the participants

Socio-demographic Variables	Test (n=75)	Control (n=75)	χ2	P- value	
Age (years)					
18-25	7 (9.3%)	4 (5.3%)	1.713	0.425	
26-34	35(46.7%)	42(56.0%)			
35-45	33(44.0%)	29(38.7%)			
Mean $\pm$ SD	$30.3 \pm 4.8$	31.1±5.1			
Occupation					
Civil Servant	19(25.0%)	27(36.0%)	4.757	0.575	
Trading	16(21.3%)	10(13.3%)			
Self-employed	20(26.7%)	15(20.0%)			
Housewife	5 (6.7%)	7 (9.3%)			
Student	7 (9.3%)	5 (6.7%)			
Teaching	6 (8.0%)	9(12.0%)			
Others	2 (2.7%)	2 (2.7%)			
Educational Level					
Primary	6 (8.0%)	7 (9.3%)	3.376	0.185	
Secondary	36(48.0%)	25(33.3%)			
Tertiary	33(44.0%)	43(57.3%)			
Spouse Occupation					
Civil Servant	20(26.7%)	32(42.7%)	4.645	0.200	
Trading	24(32.0%)	21(28.0%)			
Self-employed	24(32.0%)	18(24.0%)			
Others	7 (9.3%)	4 (5.3%)			
Social Economic Class					
High	14(18.7%)	23(24.7%)	2.910	0.233	
Middle	36(48.0%)	31(41.3%)			
Low	25(33.3%)	21(28.0%)			
Tribe					
Igbo	66(88.0%)	63(84.0%)	0.498	0.480	
Others	9 (12.0%)	12(16.0%)			

\*Statistically significant at p<0.05.

# Table 2: Obstetric and gynaecological characteristics of the participants

Obstetric and Gynaecological Variables	Test (n=75)	Control (n=75)	χ2	P- value
Gestational age (weeks)				
1-14	15(20.0%)			
15-27	24(32.0%)			
>27	36(48.0%)			
Number of children				
0	11(14.7%)	8 (10.7%)	1.492	0.474
1-4	47(62.7%)	54(72.0%)		
>4	17(22.7%)	13(17.3%)		
History of Miscarriage				
Yes	11(14.7%)	14(18.7%)	0.432	0.511
No	64(85.3%)	61(81.3%)		
Compliance with antenatal routine drugs				
Yes	26(36.7%)			
No	25(33.3%)			
Sometimes	24(32.0%)			

\*Statistically significant at p<0.05.

Trace elements (mg/l)	Test (n=75) Mean ± SEM	Control (n=75) Mean ± SEM	t-test	P-value	
Copper	$0.13 \pm 0.03$	$0.38 \pm 0.03$	6.035	< 0.001*	
Iron	$0.48 \pm 0.07$	$0.82 \pm 0.09$	3.089	0.002*	
Selenium	$0.86 \pm 0.11$	$0.94 \pm 0.07$	0.563	0.575	
Zinc	$0.52 \pm 0.07$	$0.52 \pm 0.05$	0.022	0.982	

Table 3: T	The level of	trace elements in	n the immu	ne system of	the pregnant	women and the control
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SEM – Standard Error of Mean; \*statistically significant at p<0.05.

Table 4: The level of trace elements in the immune system of the pregnant women and the control according to age
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Group	Trace	Age (years)	No	Mean ± SEM	F-ratio	P-value	
-	Elements						
	Cu	18-25	7	$0.15 \pm 0.06$	0.797	0.455	
		26-34	33	$0.16 \pm 0.06$			
		35-45	35	$0.09 \pm 0.07$			
	Fe	18-25	7	$0.19 \pm 0.07$	1.144	0.324	
		26-34	33	$0.55 \pm 0.10$			
Dragnant woman		35-45	35	$0.48 \pm 0.10$			
Fleghant wonten	Se	18-25	7	$0.67 \pm 0.11$	0.488	0.616	
		26-34	33	$0.78 \pm 0.14$			
		35-45	35	$0.98 \pm 0.20$			
	Zn	18-25	7	$0.25 \pm 0.06$	2.305	0.107	
		26-34	33	$0.67 \pm 0.12$			
		35-45	35	$0.43 \pm 0.08$			
	Cu	18-25	4	$0.32 \pm 0.11$	0.427	0.654	
		26-34	29	$0.35 \pm 0.05$			
		35-45	42	$0.41 \pm 0.04$			
	Fe	18-25	4	$1.09 \pm 0.45$	2.023	0.140	
		26-34	29	$1.01 \pm 0.18$			
Non-pregnant		35-45	42	$0.67 \pm 0.09$			
women	Se	18-25	4	$0.77 \pm 0.26$	1.010	0.369	
		26-34	29	$1.07 \pm 0.13$			
		35-45	42	$0.87 \pm 0.09$			
	Zn	18-25	4	$0.45 \pm 0.23$	3.078	0.052	
		26-34	29	$0.38 \pm 0.07$			
		35-45	42	$0.63 \pm 0.07$			

\*Statistically significant at p<0.05.

**Table 5:** The level of trace elements in the immune system of the pregnant women according to gestational age

Group	Trace Elements	Gestational age (week)	No	Mean ± SEM	F-ratio	P-value
	Cu	1-14	15	$0.09 \pm 0.02$	0.187	0.830
		15-27	24	$0.14 \pm 0.06$		
		>27	36	$0.13 \pm 0.04$		
	Fe	1-14	15	$0.42 \pm 0.17$	0.509	0.603
		15-27	24	$0.58 \pm 0.15$		
Descenant warman		>27	36	$0.44 \pm 0.07$		
Pregnant women	Se	1-14	15	$0.93 \pm 0.21$	0.199	0.820
		15-27	24	$0.93 \pm 0.22$		
		>27	36	$0.79 \pm 0.16$		
	Zn	1-14	15	$0.45 \pm 0.10$	1.715	0.187
		15-27	24	$0.70 \pm 0.13$		
		>27	36	$0.43 \pm 0.10$		

\*Statistically significant at p<0.05.

Group	<b>Trace Elements</b>	Social Class	No	Mean ± SEM	F-ratio	P-value
	Cu	High	14	$0.19 \pm 0.03$	3.358	0.045
		Middle	36	$0.10 \pm 0.05$		
		Low	25	$0.19 \pm 0.04$		
	Fe	High	14	$0.64 \pm 0.05$	3.553	0.031
		Middle	36	0.33±0.10		
Pregnant		Low	25	0.34±0.13		
women	Se	High	14	1.17±0.33	1.432	0.245
		Middle	36	$0.90 \pm 0.16$		
		Low	25	0.63±0.16		
	Zn	High	14	$0.42 \pm 0.14$	1.332	0.270
		Middle	36	$0.45 \pm 0.07$		
		Low	25	0.67±0.16		

 Table 6: The level of trace elements in the immune system of the pregnant women according to social class

\*Statistically significant at p<0.05.

Tab	le	7:	Accuracy	of	trace e	lements	in	pregnant	women
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Trace Elements	AUC	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Copper (<0.234mg/l)	0.837	73.1	81.9	73.1	82.2	78.5
Iron (<0.551mg/l)	0.698	76.8	68.9	62.3	81.6	72.1
Selenium (<1.077mg/l)	0.606	59.7	75.2	61.7	73.6	70.0
Zinc (<0.601mg/l)	0.539	29.8	87.4	61.4	65.0	64.3

interleukin 2 which is responsible for T cell proliferation thereby decreasing the number of circulatory neutrophils and its ability to kill ingested microorganism which may contribute to poor pregnancy outcomes such as reproductive failure, early embryonic death and preeclampsia.<sup>20</sup> The research work of Tabrizi and Pakdel also reported a low prevalence of Cu deficiency in India and China.<sup>21</sup> The research works of Ugwuja et al<sup>22</sup> and Akinloye et al<sup>23</sup> in Abakaliki, Ebonyi State and Oshogbo, Osun State respectively reported low serum Cu level among pregnant women with pre-eclampsia. Furthermore, this study is consistent with the study done by Ugwuja et al.<sup>24</sup> among pregnant women in Abakaliki, South-eastern Nigeria. The decrease could be as a result of frequent intakes of carbohydrates and cereal based foods and sparingly intakes of meat, dairy products, nuts and vegetables. Also a decrease in the activity of feroxidases of ceruloplasmin which is responsible for the metabolism of iron and copper can cause copper deficiency. Some other possible causes of decrease in Cu levels may include malnutrition, malabsorption, or menke's disease.

Iron is an important trace element necessary for haemoglobin synthesis and several other important functions in the body. Iron deficiency can result not only in reduced oxygen carrying capacity but can affect immunity, growth and development.<sup>25</sup> During pregnancy, iron needs are usually very high to meet the requirement for the foetus, placenta and maternal red cell expansion.<sup>26</sup> Globally, poor pregnancy outcome has been most commonly

associated with anaemia.<sup>21</sup> The result of this study showed that in comparison with the values of the non-pregnant participants, serum iron concentration significantly decreased in the pregnant women (0.48±0.07mg/l for Test and  $0.82\pm0.09$  mg/l for control p = 0.002). Age, Parity and gestational age did not affect this concentration. This reduction is consistent with the findings of Saeed et al.<sup>27</sup> who attributed the cause to haemodilution. This study also reveals that about 65.3% of the participants do not comply with their routine drugs. Abnormal Fe status can lead to impaired immune function. This is because the proliferative phase of lymphocyte activation is an iron-requiring step.<sup>27</sup> Again, Fe is also linked with the regulation of cytokine production and activation of protein kinase C which is indispensable for the phosphorylation of factors regulating cell proliferation.<sup>28</sup> Fe is necessary for myeloperoxidase activity which is involved in the killing process of bacteria by neutrophil through the formation of highly toxic hydroxyl radical.<sup>28</sup> Therefore, any alteration in cellular Fe homeostasis due to deficiency especially in pregnancy can lead to an unfavourable consequence in the immune system.

Furthermore, pregnancy is associated with a progressive reduction of Se concentration.<sup>6</sup> Reduced Se concentration results in reduced glutathione peroxidase activity resulting in reduced anti-oxidant protection of biological membranes and DNA during the early stages of embryonic development. The reduction may be due to haemodilution and other factors that cause complications in pregnancy.<sup>27</sup>

Selenium also has effect on neutrophil function. Neutrophils produce superoxide derived radicals that take part in killing of microbes. Selenium deficiency does not affect neutrophil numbers but its function is affected.<sup>29</sup> In this study, serum Se concentration was the same as non-pregnant level irrespective of the haemodilution that occurs during pregnancy (0.86±0.11mg/l for Test and 0.94±0.07mg/l for Control p = 0.575). This result agrees with research work of Nwagha et al.<sup>17</sup> and Eze et al.<sup>30</sup> It however, disagrees with the works of Alvarez et al<sup>31</sup> and Kilinc et al.<sup>16</sup> that reported a decrease in selenium concentration. A work by Beck et al. reported that Se deficiency enhances viral virulence.<sup>32</sup> Selenoproteins are an important component of the antioxidant host defence system affecting leucocytes and NK cell function. Hence Se is a critical trace element in host defence against viral infection.<sup>27</sup>

The requirement of zinc during the third trimester is approximately twice as high as that in non-pregnant women.<sup>27</sup> This is because of high uptake of Zn by the foetus and associated tissues. Alteration in zinc homeostasis may have devastating effects on pregnancy outcome, including prolonged labour, premature rupture of membrane, foetal growth restriction and preeclampsia.<sup>6</sup> The lower serum Zn concentrations have been associated partly due to reduced oestrogen and zinc binding protein levels.<sup>6</sup> No significant variation was observed in this study (0.52±0.07mg/l for Test and  $0.52 \pm 0.05$  mg/l for control, p = 0.982). This agrees with research work of Erhabor et al.<sup>9</sup> and inconsistent with the findings of Alvarez et al.<sup>31</sup> and Kilinc et al.<sup>16</sup> that proposed a decrease in zinc level during pregnancy. A work by Ugwuja et al. reported a low Zn level among pregnant women with pre eclampsia.<sup>22</sup> Severe Zn deficiency is rare and isolated to several geographic regions.<sup>27</sup> Zn is also known to play a role in immune system. It is required for the regeneration of new CD4+ T cell; it also causes a decline of NK cell lytic activity.<sup>33</sup> Zn deficiency may cause increased susceptibility to a variety of pathogens because the deficiency causes atrophy of the thymus of which in turn impairs T cell function.34

# 5. Conclusion

This study has demonstrated a significant variation in the concentration of iron and copper among pregnant women and non-pregnant controls. It also showed significant increase in serum copper in economically advantaged groups, however, the study could not demonstrate a significant variation in selenium and zinc levels among pregnant women and non-pregnant controls. Trimester, age, parity appears to have no significant effect on these trace elements among pregnant women residing in Abakaliki metropolis. Pregnancy is a period of increased metabolic demand for the maternal wellbeing and the developing fetus. Trace element deficiency in pregnancy can have a widespread effect on almost all the components of immune response resulting in increase susceptibility to infection

and adverse pregnancy outcome. Therefore, maintaining adequate and balanced nutrition during pregnancy will ensure optimal utilisation of these trace elements and in turn boost the immune system and reduce adverse pregnancy outcome.

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None.

#### 7. Conflict of Interest

The authors declare no conflict of interest.

#### References

- Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. *Cardiovasc J Afr.* 2016;27(2):89–94.
- Zeng Z, Liu F, Li S. Metabolic adaptations in pregnancy: a review. *Ann Nutr Metab.* 2017;70(1):59–65.
- Marangoni F, Cetin I, Verduci E, Canzone G, Giovannini M, Scollo P, et al. Maternal Diet and Nutrient Requirements in Pregnancy and Breastfeeding. An Italian Consensus Document. *Nutrients*. 2016;8(10):629.
- Burtis AC, Ashwood RE, Bruns ED. Tietz Textbook of clinical Chemistry and Molecular diagnostics; 2014. p. 404–21.
- Cheatham CL. Nutritional Factors in Fetal and Infant Brain Development. Ann Nutr Metab. 2019;75(suppl 1):20–32.
- Mistry DH, Pala JW. Oxidative Medicine and Cellular Longevity, the Importance of antioxidant micronutrients in pregnancy. *J Nutr.* 2011;5:138–44.
- Gernand AD, Schulze KJ, Stewart CP, West KP, Christian P. Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. *Nature Rev Endocrinol.* 2016;12:274–89.
- Prashanth L, Kattapagari KK, Chitturi RT, Baddam VR, Prasad LK. A review on role of essential trace elements in health and disease. *J NTR Univ Health Sci.* 2015;4(2):75–85.
- Erhabor O, Iwueke IP, Digban K. Serum zinc and copper levels among pregnant women of African descent attending antenatal clinic in Sokoto North Western Nigeria. J Clin Case Reports. 2020;2:2–117.
- Maggini S, Wintergst ES, Hornig DH. Selected vitamins and trace elements support immune function. *Br J Nutr.* 2007;98(Suppl 1):529– 35.
- Job EE, Ogbodo EC, Ogbu ISI, Analike RA, Onuora IJ, Obi-Ezeani CN. Determination of blood levels of lead in pregnant women in Nnewi, Anambra State, Southeastern Nigeria. Saudi J Med Pharmaceut Sci. 2018;4(11):1338–42.
- Ogbodo EC, Ezeugwunne IP, Eze BC, Njoku CM, Oguaka VN, Amah AK. The effect of malaria infection on some hematological parameters of pregnant women in Nnewi. *Int J Dev Res.* 2018;8(10):23353–8.
- Igbal S, Ali I, Rust P, Kundi M, Ekmekcioglu C. Selenium, Zinc, and Manganese Status in Pregnant Women and Its Relation to Maternal and Child Complications. *Nutrients*. 2020;12(3):725.
- Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian J Psychol Med.* 2013;35(2):121– 6.
- Baker FJ, Silverton RE. Introduction to Medical Laboratory Technology. 10th ed. Butterworth publishers; 2000. p. 71–3.
- Kilinc M, Coskun A, Bilge F, Imrek SS, Atli Y. Serum reference levels of selenium, zinc and copper in healthy pregnant women at a prenatal screening program in south Mediterranean region of Turkey. *J Trace Elem Med Biol*. 2010;24(3):152–6.
- Nwagha UI, Ogbodo SO, Nwogu-Ikojo EE, Ibegbu DM, Ejezie EE, Nwagha TU, et al. Copper and Selenium status of healthy pregnant women in Enugu, Southeastern Nigeria. *Niger J Clin Pract.*

2011;14(4):408-12.

- Spencer BH, Vanderlie JJ, Perkins AV. Essentiality of trace element Micronutrition in human pregnancy: A systematic Review. J Preg Child Health. 2015;2:157. doi:10.4172/2376-127X.1000157.
- Skalnaya MG, Tinkov AA, Lobanova YN, Chang JS, Skalny AV. Trace elements and infertility. J Trace Elements Med. 2019;56:124–30.
- Djoko KY, Ong CY, Walker MJ, Mcewan AG. The Role of Copper and Zinc toxicity in Innate Immune defence against bacterial pathogens. J Biol Chem. 2015;31:18954–61.
- Tabrizi FM, Pakdel FG. Serum levels of some minerals during the three trimesters of pregnancy in Iranian women and new born: A Longitudinal study. *Indian J Clin Biochem.* 2014;29(2):174–80.
- Ugwuja E, Akubugwo F, Ibiam U, Obodoa O, Ugwu N. Plasma copper and zinc among pregnant women with pre eclampsia in Abakaliki, Southeastern Nigeria. *Internet J Nutr Wellness*. 2009;10:1–8.
- Akinloye O, Oyewale O, Oguntibeju Y. Evaluation of trace elements in pregnant women with pre eclampsia. *Afr J Biotechnol.* 2010;32(4):5196–5202.
- Ugwuja EI, Akubugwo EI, Ibiam UA, Obidoa O, Ugwu N. Plasma copper and zinc among pregnant women in Abakaliki, Southeastern Nigeria. *Internet J Nutr Wellness*. 2010;29(2):156–62.
- Beard JL. Iron Biology in Immune function, Muscle metabolism and Neuronal functioning. J Nutr. 2001;131(2S-2):568–79S.
- 26. Sudha S. Textbook of Obstetrics. 3rd ed.; 2007. p. 45-62.
- Saeed F, Nadeem M, Ahmed RS, Nadeem MT, Arshad MS, Ullah A. Studying the impact of nutritional immunology and underlying the modulation of immune responses by nutritional compounds: A

Review. J Food Agric Immunol. 2016;27(4):205-9.

- Oppenheimer SJ. Iron and its relation to immunity and infectious diseases. J Nutr. 2001;131(2S-2):616S–33S.
- Arthur JR, Mckenzie RC, Beckette GJ. Selenium in the immune System. J Nutr. 2003;133(5):1457–9.
- Eze SC, Ododo NA, Ugwu EO, Enebe JT, Onyegbule OA, Eze IO, et al. Serum selenium level of pre eclamptic and normal pregnant women in Nigeria. *PLoS One.* 2020;15:e0238263. doi:10.1371/journal.pone.0238263.
- Alvarez SI, Castanon SG, Ruata SG, Aragues EF, Terraz PB, Irazabal YG. Updating of normal levels of copper, zinc and selenium in serum of pregnant women. J Trace Elem Med Biol. 2007;21(Suppl 1):49–52.
- Beck MA, Levander OA, Handy J. Selenium Deficiency and Viral Infection. J Nutr. 2003;133(5):1463–7S.
- Fraker PJ, King LE, Laakko T, Vollmer TL. The dynamic link between the integrity of the immune system and zinc status. J Nutr. 2000;130(3):1399–1406.
- Sherman AR. Zinc, Copper and Iron Nutriture and Immunity. J Nutr. 1992;122(3 Suppl):604–9.

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