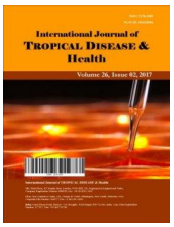

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Studies on the Level of Selenium in Pregnant Women with Malaria (A Case Study of Nnewi South East Nigeria)

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Authors' contributions

This work was carried out in collaboration between all authors. Author CPO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MOA managed the analyses of the study. Author SNE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Malaria in the tropics has continued to be a threat to public health despite all efforts curb it, more so in pregnancy. The clinical consequences of this disease as it concerns mother and child have always been grievous sometimes leaving death in its wake. This work is aimed at examining the relationship between the trace element selenium and malaria density in pregnant women with malaria. A total of 460 subjects were recruited for the study, comprising 160 pregnant women with malaria attending the antenatal clinic of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra, South East, Nigeria. The controls are 100 pregnant women without malaria attending the same clinic, 100 non-pregnant women with malaria and 100 non-pregnant women without malaria. The concentration of selenium was determined by atomic absorption spectrophotometry while the malaria density was determined by counting the parasites against white cells. From the results

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selenium showed a significant decrease in pregnant women with malaria 55.68±16.69 µg/L ($p<0.05$) compared to non-pregnant women without malaria 64.83±13.27 µg/L and a significant increase compared to pregnant women without malaria 32.94±14.41µg/L and non-pregnant women with malaria 36.44±9.59 µg/L (($F=42.91$; $p<0.05$). Selenium showed a weak positive correlation with parasite density ($r=0.27$; $p=0.004$).

Keywords: Studies; selenium; malaria; pregnancy.

1. INTRODUCTION

The malaria parasite has for long remained a threat to public health in tropical endemic countries. *Plasmodium falciparum* a very important malarial pathogenic agent remains a major cause of death to mother and child [1]. There are about 300 million cases of malaria each year, 9 of 10 cases occur in Africa. Women and children are most at risk [2]. About 30 million African women are pregnant yearly, for these women; malaria is a threat both to themselves and their babies [2]. In malaria endemic areas, malaria during pregnancy may account for up to 15% of maternal anemia, 5-14% of low birth weight, 30% of preventable low birth weight [2]. Pregnant women are especially susceptible to malaria as pregnancy reduces a woman's immunity to infection and increases the risk of illness, severe anemia and death for the unborn child. Maternal malaria increases the risk of spontaneous abortion, stillbirth, premature delivery and low birth weight [3].

Pregnancy is a period of increased metabolic demands with changes in a woman's physiology and requirements of growing foetus [4]. Insufficient supplies of essential vitamins and micronutrients can lead to a state of biological competition between the mother and concepts which can be detrimental to the health status of both [5].

Some authors have associated malaria acquisition and its severity to the concentration of micronutrients in pregnant mothers, the protection against acute infection through a moderate deficiency in iron; the reduction of risk of fever and clinical malaria episodes through a zinc supplementation and the copper associated with zinc, which the reduction of the ratio copper/zinc is an increasing factor of the oxidative stress.

Deficiencies of specific antioxidant activities associated with the micronutrients iron, selenium, copper, zinc and manganese can result in poor pregnancy outcomes including fetal growth restriction [6], pre-eclampsia and associated risk of diseases in adulthood, including cardiovascular diseases and type 1 diabetes [7]. Another consequence of oxidative stress resulting from an antioxidant deficiency is the development of malaria anemia [8]. Micronutrients are known to be integral part of antioxidants and have been found to influence host cellular and humoral immunological functions. Cell-mediated immunological response to malaria is found to decrease during pregnancy [9]. These antioxidants have been shown to provide protection against oxidative stress induced by malaria [10].

Selenium is incorporated into proteins to make selenoproteins including the glutathione peroxidase antioxidant enzymes. Selenium is essential for the production of active thyroid hormones and for normal thyroid function. Trace elements are known to be an integral part of antioxidant and have been found to influence host cellular and humoral immunological functions. These essential factors are very important in the body in order for the immune system to cope with the challenges imposed by infectious agents.

This study is therefore aimed at evaluating the relationship between the trace element selenium and malaria density in pregnant women.

1.1 Aims and Objectives

- To determine the relationship between selenium concentration and malaria density in pregnant women.
- To determine the level of selenium in pregnant women with malaria.

2. MATERIALS AND METHODS

This study was conducted at Nnamdi Azikiwe University Teaching Hospital, Nnewi. Ethical approval for this study was issued by the ethical committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Four Hundred and sixty women were used for the study, out of this, One Hundred and Sixty pregnant women served as the test subjects, One Hundred pregnant

women without malaria, One Hundred women without malaria, One Hundred women with malaria served as control. These women were selected using simple random sampling technique. The pregnant women among them were selected from their clinic while the non-pregnant women were apparently healthy women within Nnewi town. The scope, nature, aims, and objectives of the study were explained to the participants for their consent. Women with malaria were later grouped according to parasite density.

Women with established medical risk factors for oxidative stress such as AIDS, diabetes, tuberculosis, smoking, and alcohol consumers were excluded from the study.

A volume 6 ml of venous blood was collected from each of the participants, 2ml was dispensed into an EDTA container for total white cell count, a drop of blood from the syringe was placed on a clean grease free slide that has been labeled for a thick film while the remaining blood was dispensed into a plain tube. It was allowed to clot at room temperature for approximately one hour and then centrifuged at 2500 RPM for 10 minutes to separate the serum. The serum samples were analyzed for, selenium. The thick film was left to air dry before staining.

2.1 Methods

2.1.1 Determination of selenium using Atomic Absorption Spectrophotometer (AAS) principle of atomic absorption

Spectrophotometer: The element is dissociated from its chemical bonds and placed in an unexcited or ground state (neutral atom). Thus the neutral atom is at a low energy level in which it is capable of absorbing radiation at a very narrow bandwidth corresponding to its own line spectra. A hollow cathode lamp with a cathode made of the material to be analyzed is used to produce a wavelength of light specific for the material. Thus, if the cathode is made of selenium, selenium light at predominantly 196nm is emitted by the lamp. When the light from the hollow cathode lamp enters the flame, part of it is by the ground state atom in the flame, resulting in a net decrease in the intensity of the beam from the lamp.

Procedure: The serum Samples were diluted 1 in 4 with distilled water and then analyzed using AAS.

2.1.2 Determination of total white cell count

Principle: Whole blood is diluted 1 in 20 in acid reagent (Turk solution) which haemolyzes the red cells (not the nucleus of nucleated red cells) leaving the white cells to be counted. White cells are counted microscopically using improved Neubauer ruled counting chamber and the number of white blood cells per liter of blood calculated.

2.1.3 Estimating parasite numbers/µl of blood by counting parasite against white cells

- A small drop of blood was placed in the center of a pre-cleaned slide. Using the corner of another slide or an applicator stick, the drop is spread in a circular pattern until it is the size of a dime (1.5 cm^2).
- The thick film was stained with 3% Giemsa
- The parts of the thick film where white cells are evenly distributed and the parasites are well stained were selected.
- Using the oil immersion, 200 white blood cells were counted systematically and at the same time estimating the number of parasites in each field covered. An average of three counts was taken while the number of parasites per µl of blood was calculated as follows

$$\frac{\text{WBC count x Parasites counted against 200 WBC}}{200}$$

2.2 Statistical Analysis

This was done using graph pad prism version 5. The results were presented as a mean ± standard deviation. The statistical methods utilized for the analysis were a one-way analysis of variance, students "t" test, and correlation analysis.

3. RESULTS

3.1 Selenium in Pregnant Women with Malaria and Control Subjects (Mean±SD)

The mean serum level of selenium in pregnant women with malaria is 55.68±16.69 µg/L while that in pregnant women without malaria, non-pregnant women with malaria and non-pregnant women without malaria is 32.94±14.41 µg/L, 36.44±9.59 µg/L, and 64.83±13.27 µg/L respectively. The result shows a statistically

significant difference between the means (F =42.91; p<0.0001). Further analysis shows significantly increased level in pregnant women with malaria compared to pregnant women without malaria (p<0.0001), significant higher level in pregnant women with malaria when compared with non-pregnant women with malaria (p<0.0001) and a significant decrease in pregnant women with malaria when compared to non-pregnant women without malaria (P = 0.0151) (Table 1).

3.2 Selenium and Parasite Density in Pregnancy (Mean±SD)

Selenium in pregnant women with a parasite density of <2000/µl is 48.90±21.38µg/L, between 2000-10000/µl, is 53.46±23.17µg/L while pregnant women with a parasite density of >10000/µl have selenium level of 62.19±25.43. The result shows no significant difference between the means though there is a progressive increase in selenium level as the malaria parasite density increases (F=2.301; p> 0.05) (Table 2).

3.3 Selenium and Parasite Density in Non-Pregnancy (Mean±SD)

Selenium level in non-pregnant women with a parasite density of <2000/µl is 32.18±11.28 µg/L, between 2000-10000/µl, is 39.67±10.08 µg/L while non-pregnant women with a parasite density of >10000/µl have selenium level of 33.83±1.93. The result shows a significant difference between the means (F=6.850; P=0.0016). Parasite density between 2000-10000/µl has higher selenium level than <2000 and >10000 (Table 3).

3.4 Selenium and Parasite Density in Pregnant and Non Pregnant Women (Mean±SD)

Pregnant women with parasite density <2000/µl has selenium level of 48.90±21.38 µg/L while non-pregnant women have 32.18±11.28 µg/L. There is a statistically significant higher level of selenium in pregnant women than in non-pregnant (P<0.0001). At parasite density level between 2000-10000/µl, pregnant women (53.46±23.17 µg/L) has a statistically significant higher level of selenium compared to non-pregnant women (39.67±10.08 µg/L), (P<0.0001). At parasite density level >10000/µl, pregnant women (62.19±25.43 µg/L) has a statistically significant higher level compared to non-pregnant women (33.83±1.926 µg/L) (P<0.0001) (Table 4).

While the non-pregnant woman has 2.057±0.2874 µmol/L. There is a statistically significant higher level of manganese in pregnant women than in non-pregnant (P<0.0011). At parasite density level between 2000-10000/µl, pregnant women (9.218±4.23 µmol/l) has a

Table 1. Selenium in pregnant women with malaria and controls subjects (mean±SD)

	Selenium (µg/l)
Pregnant Women With Malaria n=160	55.68±16.69
Pregnant Women Without Malaria n=100	32.94±14.41a
Non-Pregnant Women With Malaria n=100	36.44± 9.59a
Non Pregnant Women Without Malaria n=100	64.83±13.27a,b,c
F-Value	42.91
P-Value	< 0.0001**

NB: a; p<0.05 compared with pregnant women with malaria, b; p<0.05 compared with pregnant women without malaria, c; p<0.05 compared with non-pregnant women with malaria

Table 2. Selenium and parasite density in pregnancy (Mean±SD)

	Selenium(µg/l)
<2000/µl	
n =44	48.90±21.38
2000-10000/µl	
n =96	53.46±23.17
>10000/ µl	
n =20	62.19±25.43
F-Value	2.301
P-Value	0.1035

*NB: **; significant difference between the means (p<0.05), a; p<0.05 compared with parasite density <2000/µl*

Table 3. Selenium and parasite density in non-pregnancy (Mean±SD)

	Selenium µg/l
<2000/µl	
n =23	32.18±11.28
2000-10000	
n =51	39.67±10.08a
>10000	
n =26	33.83±1.926 b
F-Value	6.850
P-Value	0.0016**

*NB: **; significant (P<0.05) difference between the means a; p<0.05 compared with parasite density <2000/µl b; p<0.05 compared with parasite density 2000-10000/µl*

statistically significant higher level of manganese compared to non-pregnant women (2.077±0.3027 µmol/L), (P<0.0048). At parasite density level >10000/µl, pregnant women (4.532±2.643 µmol/L) has a statistically significant higher level compared to non-pregnant women (2.331±0.7059 µmol/L), (P<0.0016) (Table 4).

At parasite density level >10000/µl, pregnant women (62.19±25.43 µg/L) has a statistically significant higher level compared to non-pregnant women (33.83±1.926 µg/L) (P<0.0001), (Table 4.).

4. DISCUSSION

Selenium is a major component of glutathione. Glutathione peroxidase is a major antioxidant defense system within the body. Wide variations in selenium levels occur in humans. Geographical location, soil content, intake of selenium in diet and its bioavailability significantly affect selenium status [11]. In this study, it was observed that there was a significantly higher level of selenium in non-pregnant women without malaria compared with pregnant women with and without malaria. There have been varying reports on the selenium status during pregnancy with various studies showing the lower level in pregnancy [12]. [13] also reported a lower selenium level in pregnancy. Active transfer of selenium from maternal blood to the tissues of the developing fetus has been advocated. Indeed, haemodilution due to increased plasma volume in pregnancy further depletes selenium concentration. Additionally, inadequate intake and storage in the maternal tissue and increased demand by the fetus invariably lead to low maternal level during pregnancy [14]. From this study, there was a significantly high level of selenium in malaria during pregnancy. Pregnant women with malaria have a significantly higher level of selenium compared with pregnant women without malaria. During pregnancy, there was also an increase in level of selenium with an increase in malaria parasite density though not statistically significant. [15] in their study reported a higher level of selenium in pregnant women with malaria infection. This is attributed to induction of oxidative stress by malaria parasites. The destruction of malaria parasites by white blood cells through oxygen-dependent mechanism and engulfing of kuppfer cells by larva stage of malaria parasite induces oxidative stress. his will require antioxidant for removal, thus the need for increased production of antioxidants. Selenium is an integral part of enzymatic antioxidants and this accounts for the increase of selenium in malaria infection [16].

Table 4. Selenium and parasite density in pregnant and non pregnant women (Mean±SD)

	<2000/µl		2000-10000/µl		>10000/µl	
	Pregnant women	Non pregnant women	Pregnant women	Non pregnant women	Pregnant women	Non pregnant women
Selenium	48.90±21.38	32.18±11.28	53.46±23.17	39.67±10.08	62.19±25.43	33.83±1.94
P-value	<0.0001**		<0.0001**		<0.0001**	

Table 5. Correlation between Selenium levels and parasite density in pregnancy

Trace element	R	P
Selenium	0.27	0.004

Correlation is significant at the 0.05 level

5. CONCLUSION

From this study, the serum level of selenium was reduced in pregnancy but increases in malaria infection during pregnancy. A further increase was observed with an increase in malaria parasite density.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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