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Relationship Between Malaria Parasite Density And The Concentration Of Iron In Pregnant Women In Nnewi (South East Nigeria)

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Abstract: *Malaria during pregnancy continues to be a major health problem in endemic countries with clinical consequences including death of both mother and child and attendant derangement in trace elements. This study is aimed at evaluating the relationship betweentrace element iron and malaria density in pregnant women with malaria. The patients are pregnant women attending ante natal clinic of NnamdiAzikiwe University Teaching Hospital Nnewi, Anambra, South East, Nigeria. The controls are pregnant women without malaria, non-pregnant women with malaria and non-pregnant women without malaria. Iron level was determined by atomic absorption spectrophotometry while the malaria density was determined by counting the parasites against white cells. From the results Iron showed a significant increase in pregnant women with malaria 14.50±7.984µmol/L compared to pregnant women without malaria 14.30±8.32µmol/L, non-pregnant women with malaria 10.16±2.59µmol/L and non-pregnant women without malaria 10.17±5.09µmol/L (F =15.45; p<0.05). Iron levelshowed a moderate positive correlation with parasite density (r=0.31; p=0.002).*

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I. Introduction

Malaria is an important public health problem in developing countries.

Plasmodium falciparum a pathogenic agent remains a major cause of morbidity and mortality to mother and child (Jeffrey and Pia, 2012).

There are about 300 million cases of malaria each year, 9 of 10 cases occur in Africa. Women and children are most at risk (WHO, 2011).

About 30 million African women are pregnant yearly, for these women; malaria is a threat both to themselves and their babies (Menendez *et al.,* 2000; WHO, 2011). 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 (WHO, 2011).

Pregnant women are particularly vulnerable to malaria as pregnancy reduces a woman's immunity to malaria infection and increasing the risk of illness, severe anemia and death for the unborn child. Maternal malaria increases the risk of spontaneous abortion, still birth, premature delivery and low birth weight (WHO, 2003). Pregnancy is a period of increased metabolic demands with changes in a woman's physiology and requirements of a growing feotus (Broughton, 2007).

Insufficient supplies of essential vitamins and micronutrients can lead to a state of biological competition between the mother and conceptus which can be detrimental to the health status of both (King, 2003).

Some authors have associated malaria acquisition and its severity to the concentration of micronutrients in pregnant mothers, the protection against acute infection through a moderated deficiency in iron (Nyakeriga*et al.,* 2004); the reduction of risk of fever and clinical malaria episodes through a zinc supplementation (Zeba*et al.,* 2008) and the copper associated with zinc, which the reduction of the ratio copper/zinc is an increasing factor of the oxidative stress (Mezzetti*et al.,* 1998).

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 (Fall *et al.,* 2003), pre eclampsia and associated risk of diseases in adulthood, including cardiovascular diseases and type 1 diabetes (Lykke*et al.,* 2009).

Another consequence of oxidative stress resulting from antioxidant deficiency is the development of malaria anemia (Kremsner*et al.,* 2000).

Micronutrients are known to be integral part of antioxidants and have been found to influence host cellular and humoral immunological functions (Spallhoiz*et al.,* 1990). Cell mediated immunological response to malaria is found to decrease during pregnancy (Riche *et al.,* 2000). These antioxidants have been shown to provide protection against oxidative stress induced by malaria (Adelekan*et al.,* 1997).

Iron plays an important role in the production of haemoglobin, oxygenation of red bloods cells and lymphocytes. It improves the function of enzymes in protein metabolism and enhances the function of calcium and copper. Pregnant women have increased need of iron because of the high demand and this accounts for the recommendation of iron supplement in pregnancy (Okochi and Okpuzor, 2005).

Background of study

Malaria during pregnancy continues to be a major health problem in endemic countries with clinical consequences including death of both mother and child. Research shows that maternal mortality is twice in pregnant women with malaria than among non-pregnant patients with severe malaria. 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 iron and malaria density in pregnant women.

Aim and Objectives

To determine the relationship between Iron level and malaria density in pregnant women in Nnewi. The objectives are as follows:

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

II. Materials And Methods

Materials and Methods

Study Site

- This study was conducted at NnamdiAzikiwe University Teaching Hospital, Nnewi.

Ethical clearance

- Ethical approval for this study was issued by the ethics committee of NnamdiAzikiwe University Teaching Hospital, Nnewi.

Subjects

- Four Hundred and sixty women were used for the study, out of this, One Hundred and Sixty pregnant women served as the test subject, 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 (Melaine*et. al.,* 2010).

Exclusion Criteria

- These are women with established medical risk factors for oxidative stress such as AIDS, diabetes, tuberculosis, smoking and alcohol consumers.

Sample Collection

A volume 6ml of venous blood was collected from each of the participants. An aliquot 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 iron. The thick film was left to air dry before staining.

Statististical Analysis

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

III. Results

1 Iron in Pregnant Women with Malaria and Control subjects (Mean±SD)

The results showed thatironlevel in pregnant women with malaria, pregnant women without malaria, non-pregnant women with malaria and non-pregnant women without malaria have mean serum level of 14.50±7.984µmol/L, 14.30±8.32µmol/L, 10.16±2.59µmol/L and 10.17±5.09µmol/L respectively. The result also shows a statistically significant difference between the means ($F = 15.45$; $p<0.0001$). Further analysis shows no significant difference in iron level in pregnant women with malaria compared to pregnant women without malaria ($p>0.05$), significant higher level in pregnant women with malaria when compared with non-pregnant women with malaria ($p<0.0001$) and a significant higher level in pregnant women with malaria when compared to non-pregnant women without malaria ($P<0.0001$), (Table 1).

Table 1 Iron in Pregnant Women with Malaria and Control subjects (mean±SD)

	IRON µmol/l
Pregnant Women With Malaria n=160	14.50±7.98
Pregnant Women Without Malaria n=100	14.30±8.32
Non-Pregnant Women With Malaria n=100	10.16±2.59[a,b]
Non Pregnant Women Without Malaria n=100	10.17±5.10[a,b]
F-Value	15.45
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

2. Iron level and Parasite Density in Pregnancy (Mean±SD)

Parasite Density of <2000/µl, between 2000-10000/µl and >10000/µl corresponded to iron levels of 15.97±8.54µmol/L, 13.53±8.11µmol/L and 17.54±5.23µmol/L respectively. The result shows no significant difference between the means ($F=2.893$; $p>0.05$), (Table 2).

Table 2: Iron levels and parasite density in pregnancy (Mean±SD)

	IRON µmol/l
<2000/µl n=44	15.97±8.542
2000-10000/µl n=96	13.53±8.11
>10000/ µl n=20	17.54±5.232
F-Value	2.893
P-Value	0.0584

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

3 Iron levels And Parasite Density in Non-Pregnancy (Mean± SD)

Parasite Density of <2000/µl, between 2000-10000/µl and >10000/µl corresponded with iron levels of 9.373±3.73µmol/L, 10.25±2.18µmol/L and 10.70±1.94µmol/L respectively. The result shows no significant difference between the means though there is a slight progressive increase in iron level as the malaria parasite density increases (F=1.693; p>0.05), (Table 3).

Table 3: Iron levels and parasite density in non-pregnancy (Mean±SD)

	IRON µmol/l
<2000/µl	
n=23	9.37 ± 3.73
2000-10000	
n=51	10.25±2.18
>10000	
n=26	10.70±1.94
F-Value	1.693
P-Value	0.1894

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

4 Iron levels and Parasite Density in Pregnant and Non Pregnant Women (Mean±SD)

Pregnant women with parasite density <2000/µl showed iron level of 15.97±8.54µmol/L while non-pregnant women showed 9.373±3.734µmol/L. There is a statistically significant higher level of iron in pregnant women than in non-pregnant (P<0.0001). At parasite density level between 2000-10000/µl, pregnant women (13.53±8.107µmol/l) has a statistically significant higher level of iron compared to non-pregnant women (10.25±2.18µmol/L), (P<0.005). At parasite density level >10000/µl, pregnant women (17.54±5.232µmol/L) has a statistically significant higher level compared to non-pregnant women (10.70±1.939µmol/L), (P<0.0001). (Table 4)

Table4: Iron levels 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
Iron	15.97± 8.54	9.37 ± 3.73	13.53 ± 8.11	10.25± 2.18	17.54± 5.23	10.70± 1.94
P-Value	<0.0001**		0.0003**		<0.0001**	

*P Value Significant.

5 Correlation between Iron levels and Parasite Density in Pregnancy

Iron has a moderate positive correlation with parasite density (r=0.31; p=0.002), (table 4.5). This implies that iron level increase with an increase in parasite density.

Table 5: Correlation between Iron levels and parasite density in pregnancy

	R	p
Iron	0.31	0.002

Correlation is significant at the 0.05 level

IV. Discussion And Conclusion

Iron plays an important metabolic role particularly in electron transfer reaction. Much of the iron in human body is in circulating red cells. Neither bacteria nor nucleated cells proliferate when the supply of iron is insufficient (Martin, 2006). Also, there is a significant increase in level of iron in pregnant women compared with non-pregnant women. Arinola*et al.*,2004) reported an increased number of circulating blood cells in pregnant women resulting in increased iron level. From the present, there is no significant difference in iron level of pregnant women with malaria compared with pregnant women without malaria. These are contrary to the study by Asaolu and Igbaakin (2009) who reported a significant lower level of iron in pregnant women with malaria compared with pregnant women without malaria. Also from the present study, a significant higher serum level of iron was seen in pregnant women with malaria compared to non-pregnant women with malaria. Furthermore, it was observed that serum iron level increases with an increase in parasite density. Study by Raimi*et al.,* 2012 shows that a high dose iron supplementation of children timing in tropics has been associated with increased risk of clinical malaria and other infections like pneumonia. Studies in cell culture and animal suggest that the survival of infectious agents that spend part of their life cycle within the host cells such as plasmodium and mycobacteria may be enhanced by iron therapy. Again, damage of red blood cells by the developmental stages of malaria parasite and reactive oxygen species generated by increased oxidative in pregnancy leads to leakage of iron into blood circulation. This will in turn increase plasma level of iron (Arinola*et al.*, 2008). This implies that the more the parasite density, the more red cells are destroyed and in turn more iron leak into the circulation. This is also contrary to outcome of the study by Saira*et al.,* 2013 who reported a significant decrease in iron level in malaria.

V. Conclusion

An increased level of iron was observed in pregnancy and malaria which resulted from increased haemolysis due to higher burden of oxidative stress and malaria during pregnancy. Since the presence of higher malaria parasite density causes more haemolysis of red cells, severe malaria in pregnancy can result in anaemia in pregnancy that cannot be treated with iron supplementation which can lead to maternal death.

Recommendation

From the results of this study and the conclusion derived therefrom, the following recommendationis necessary for consideration;

The increase in serum iron in this study means that iron status should be evaluated before iron supplementation is undertaken to avoid iron overload during pregnancy.

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