

# International Journal of Advanced Research in Biological Sciences

ISSN: 2348-8069

www.ijarbs.com

Coden: IJARQG(USA)

## Research Article



SOI: <http://s-o-i.org/1.15/ijarbs-2-12-34>

## Iron status, Haemoglobin and C-Reactive protein levels in pregnant women with malaria Parasitaemia in Owerri

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## Abstract

Iron Status, Haemoglobin and C - reactive protein levels in pregnant women with malaria parasitaemia were investigated at the Federal Medical Centre Owerri, Imo State. One hundred and fifty (150) pregnant women infected with malaria and one hundred and fifty (150) pregnant healthy (Malaria-free) women (control) between the ages of 22-40 years were studied. The results showed that the mean values of iron level ( $27.50 \pm 6.17$ ), Haemoglobin ( $9.80 \pm 0.64$ ) and human ferritin level ( $20.24 \pm 4.66$ ) were significantly ( $P < 0.05$ ) reduced in pregnant women with malaria parasitaemia when compared with the controls ( $75.60 \pm 10.730$ ) ( $13.07 \pm 0.63$ ) ( $61.60 \pm 18.32$ ). There was no significant difference in the mean values of serum total iron binding capacity (transferrin) ( $363.84 \pm 42.06$ ) ( $363.08 \pm 44.77$ ) in pregnant women with malaria parasitaemia in their 1st and 2nd trimesters when compared with the control ( $359.22 \pm 31.09$ ) ( $360.70 \pm 36.187$ ) subjects respectively. But a significant difference ( $p < 0.05$ ) was observed in the 3rd trimesters ( $340.62 \pm 31.80$ ) of malaria infected pregnant women and the control ( $356.70 \pm 28.32$ ) subjects. Furthermore, a significant difference ( $P < 0.05$ ) was observed in the serum C-reactive protein levels ( $6.86 \pm 8.74$ ) in pregnant women with malaria parasitaemia with respect to their trimesters when compared with the serum C-reactive protein levels ( $3.77 \pm 1.32$ ) in pregnant healthy women. The findings of this study show that malaria prevention and iron supplementation may likely improve maternal and infant outcomes.

**Keywords:** Iron status, Haemoglobin, C-reactive protein, Pregnant women, Malaria parasitaemia.

## Introduction

Pregnancy is associated with normal physiological changes that assist the nurturing and survival of the foetus. Biochemical parameters reflect these adaptive changes in most organ system and are clearly distinct from the non-pregnant state (Tran, 2005). However, these changes become very important in the event of complications as a result of malaria parasitaemia. These complications may include anaemia, hyperlipidaemia, hypertension, diabetes and

preeclampsia (Mathai, 2005; Goonewardene and Shehata, 2011; Okojie et al., 2011). Globally, the most common cause of anaemia is believed to be iron deficiency due to inadequate dietary iron intake, physiologic demands of pregnancy and rapid growth and iron losses due to parasitic infections. However, iron deficiency is not the only cause of anaemia. Other prevalent causes include malaria parasitaemia, chronic infections and nutritional deficiencies of Vitamin A,

Folate and Vitamin B12. The relative contributions of these causes of anaemia and iron deficiency vary by sex, age and population and are not well described in many populations. During pregnancy, Iron requirements exceed storage iron for most women (Bothwell and Charlton, 2004). The increased need by the body for iron is due to increases in the red cell mass, iron needs of the foetus and iron losses during delivery (Bothwell and Charlton, 2004). Although haemodilution from expansion of the plasma volume leads to a "physiologic pregnancy anaemia" (DeLeeuw et al., 2006), inadequate iron supply can limit red cell mass expansion and lead to further deterioration in Iron status during pregnancy (Viteri, 2004) that may pose risks for the pregnant woman and her infant (Alien, 2007). Recently some acute phase reactants have emerged as biomarkers in malaria infection in addition to chemokines and cytokines. In particular reactive protein (CRP) and Nitric oxide (No) have been identified as important inflammatory biomarkers (McGuire et al., 1996; Conroy et al., 2011). CRP is an acute phase protein that is involved in activation of complement, acceleration of phagocytosis and detoxification of substances released from the damaged tissue. Measurement of serum CRP is most frequently used for the evaluation of injury in the body tissue or for the detection of inflammatory event somewhere in the body. In malaria, CRP secretion is induced by pro-inflammatory cytokines that are secreted by host mononuclear cells and strong correlations have been found between CRP levels and parasitaemia (Harpaz et al., 1992). In fact, CRP levels have proven to be valuable in assessing the severity of malaria among pregnant women and as a prognostic tool in the follow up response to treatment (Gillespie et al., 2013). Successful outcome of pregnancy requires monitoring of biochemical and haematological parameters to avoid complications caused by malaria parasitaemia throughout the trimesters of pregnancy. Several authors have reported disturbances in Carbohydrate and Lipid metabolism (Salisu and Atiku, 2009), protein profile (Buul et al., 2000; Imoru and Emeribe, 2010), Liver function (Tran, 2005), Kidney Function (Dennem, 2011) and Specific records on the haematological and biochemical changes such as iron status and CRP levels in malaria parasitaemia among pregnant women in Owerri Municipality have been poorly documented and not widely reported in this regard. Therefore, the present study seeks to investigate alterations in some haematological and biochemical indices in malaria parasitaemia among pregnant women in Owerri Municipality.

## Materials and Methods

### Study area:

The study was conducted at the Federal Medical Centre Owerri, the Imo State Capital. Owerri the Capital of Imo State is strategically located in the Eastern states of Nigeria sharing boundaries with and being a gateway to Umuahia and Aba in Abia State, River State, the Eastern Nigerian part cities and Anambra State. It lies on latitude 5°27' N, and longitude 6°55' - 7° 03' E. It has a population of over 127,213 (National Population Commission, 2005) and is cosmopolitan being home to many non-indigenes apart from the ethnic Igbo. The Federal Medical Centre is a Federal government owned tertiary healthcare institution. The subjects were selected amongst those attending antenatal care at the Department of Obstetrics and Gynaecology, of the hospital.

The vegetation of the study area consists of tropical rain forest belt. The area experiences two annual climatic conditions of a short, hot, dry season (November to March) and a longer wet season (April to October). The mean annual precipitation is 160mm, relative humidity 71±3.0 and mean annual temperature 30°C. Two major water bodies, the larger Otamiri and minor Nworie Rivers provide all-season sources of water for the inhabitants. In addition, these water sources provide breeding sites for the mosquito vectors and increase the humidity - conditions which enhance the rapid proliferation and survival of various mosquito species some of which are vectors of plasmodium malaria. The inhabitants are mostly civil servants and traders.

### Study Population

A total of 150 pregnant women between the ages of 22-40 years who were confirmed to be infected with *P.falciparum* malaria through peripheral thick and thin blood film examination constituted the study population. And 150 pregnant healthy (Malaria-free) women were enrolled as the control group. The subjects were selected from those attending ante-natal care at the department of Obstetrics and Gynaecology, Federal Medical Centre, Owerri, between August to November 2014.

### Inclusion Criteria

- Subjects of gestational age between 12 and 35 weeks.
- Absence of recent history of convulsion.
- Absence of hyperthermia.
- Subjects that tested malaria parasite positive.

### exclusion criteria

Renal disease, diabetes mellitus, hypertension, obesity, and women over 40 years because pregnancy in this age is considered to be high risk. Again, those who showed skepticism to the essence of the study and those from whom informed consent could not be obtained were also excluded.

### Sample Collection

With a hypodermic syringe, 7ml of blood was collected from each subject from the antecubital or dorsal vein using the standard clean venepuncture technique between the hours of 9am and 12.30pm. About 2ml of the blood was dispensed into an EDTA bottle (container) labelled and used for the determination of the haemoglobin levels at the same day of blood collection. About 5ml of the blood was dispensed into a dry plain plastic tube and allowed to clot. The clotted blood samples were centrifuged at 3,000 revolutions per minute (rpm) for 5 minutes to separate the serum. The sera were stored at -20°C prior to use for the determination of Total Serum Iron, Ferritin, and Transferrin under study as well as the c-reactive protein levels.

### Laboratory Procedures

All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly followed,

### Results

Table 1: Mean Values of Serum Iron Levels Among Malaria Infected Pregnant Women According to their Trimesters and Control

Trimesters of pregnancy	Control (n)	$\bar{x} \pm SD$	t-value	P-value	t-test
n)					
1st	1st	-48.04 + 11.288	-30.092	0.000	0.000<0.05
2nd	2nd	-46.620 + 10.80	-30.512	0.000	0.000<0.05
3rd	3rd	-49.32 + 11.92	-29.252	0.000	0.000<0.05

### Parasitological Examination

The presence and relative parasite count of *Plasmodium falciparum* in each blood sample was determined from Giemsa stained Thick and Thin Film. The identification of the *Plasmodium falciparum* parasite in the blood films was carried out according to WHO method (WHO, 1980). A slide was scored as negative when 100 high power fields (at 100x magnification) had been examined for about 5 minutes without seeing any malaria parasite. The amount of relative parasite count in positive smears was done using a simple code from one to four crosses (++++).

according to Brace-Chwat (1980) as stated below + : 1-10 parasites per 100 thick film fields, ++: 11-100 parasites per 100 thick film fields, +++: 1-10 parasites per one thick film field, ++++: more than 10 parasites per one thick film field.

### Haematological and Biochemical Analysis.

Haemoglobin estimation was carried out using Cyanmethaemoglobin method. Iron status, Ferritin, Transferrin and C-reactive protein levels were estimated using the Enzyme Immunoassay technique method reported Tietz (1995).

### Statistical Analysis

The computational analysis was done using statistical package for Social Sciences (SPSS Version 17). Test of significance was by student t-test, and ( $P < 0.05$ ) was considered as statistically significant.

Table 1 shows the mean value of serum iron levels in pregnant women with malaria Parasitaemia with respect to their various trimesters and pregnant healthy women (control). The mean value of serum Iron for those in the 1st trimester ( $27.42 \pm 8.112$ ), 2nd trimester ( $27.50 \pm 6.17$ ), 3rd trimester ( $26.28 \pm 5.459$ )

in pregnant women with malaria parasitaemia (subjects) decreased significantly ( $P < 0.05$ ) when compared to the mean values of serum Iron for 1st trimester ( $75.46 \pm 1.290$ ), 2nd trimester ( $74.12 \pm 9.23$ ) and 3rd trimester ( $75.60 \pm 10.730$ ) in pregnant healthy women (controls).

Table 2: Mean Values of Serum Total Iron Binding Capacity (Transferrin) among malaria infected pregnant women according to their trimesters and control.

Trimesters of pregnancy (n)	Control (n)	x $\pm$ SD		t-value	P-value	t-test
1st	1st	4.62 + 51.545	0.634	0.529	0.529 > 0.05	
2nd	2nd	2.38 + 53.78	0.313	0.756	0.756 > 0.05	
3rd	3rd	-16.08 $\pm$ 37.51	-3.031	0.004	0.004 < 0.05	

Table 2 shows the mean values of serum total iron binding capacity (serum transferrin) among pregnant women with malaria parasitaemia with respect to their trimesters and pregnant healthy women (control). The mean values of serum transferrin for 1st trimester ( $363.84 \pm 42.06$ ), 2nd trimester ( $363.08 \pm 44.77$ ) in pregnant women with malaria parasitaemia showed no significant differences ( $P > 0.05$ ) when compared to the mean values of serum transferrin for 1st trimester ( $359.22 \pm 31.09$ ) and 2nd trimester ( $360.70 \pm 36.187$ ) in pregnant healthy women respectively (control). While the mean value of serum transferrin for the 3rd trimester ( $340.62 \pm 31.80$ ) statistically showed a

significant difference ( $P < 0.05$ ) in pregnant women with malaria parasitaemia when compared to the mean value of serum transferrin for those in the 3rd trimester ( $356.70 \pm 28.32$ ) in pregnant healthy women (controls).

pregnant women with malaria parasitaemia (subjects) decreased significantly ( $P < 0.05$ ) when compared to the mean values of serum human ferritin in 1st trimester ( $61.60 \pm 18.32$ ), 2nd trimester ( $58.94 \pm 13.77$ ), and 3rd trimester ( $60.06 \pm 15.47$ ) in pregnant healthy women (controls).

Table 3: Mean Values of Serum Human Ferritin Among Malaria infected pregnant women according to their trimesters and control.

Trimesters of pregnancy (n)	Controls (n)	X $\pm$ S.D-		t- value	P- value	t-test
1st	1st	-43.98 $\pm$ 18.019	-17.26	0.000	p-value < 0.05	
2nd	2nd	-40.30 $\pm$ 14.154	-20.13	0.000	p-value < 0.05	
3rd	3rd	-39.820 $\pm$ 15.634	-18.01	0.000	p-value < 0.05	

Table 3 shows the mean values of serum human ferritin among pregnant women with malaria parasitaemia with respect to their trimesters and pregnant healthy women (controls). The mean values

of serum human ferritin in the 1st trimester ( $17.62 \pm 1.63$ ), 2nd trimester ( $18.64 \pm 1.19$ ), 3rd trimester ( $20.24 \pm 4.66$ ) in

Table 4: Mean Values of Serum O reactive Protein Among malaria infected pregnant women according to their trimesters and control.

Trimesters of pregnancy (n)	Controls (n)	X ± S.D		t- value	P- value	t-test
1st	1st	1.44 + 2.05	4.96	0.000	0.000 < 0.05	
2nd	2nd	1.85 + 2.04	6.41	0.000	0.000 < 0.05	
3rd	3rd	3.09 + 8.96	2.44	0.000	0.000 < 0.05	

Table 4 shows the mean values of serum C-reactive protein levels among pregnant women with malaria parasitaemia with respect to their trimesters and pregnant healthy women (control). The mean values of serum C-reactive protein level for those in 1st trimester ( $5.09 \pm 1.99$ ), 2nd trimester ( $5.60 \pm 1.69$ );

3rd trimester ( $6.86 \pm 8.74$ ) in pregnant women with malaria parasitaemia showed a significant difference ( $P < 0.05$ ) when compared to the mean values of serum C-reactive protein levels in 1st trimester ( $3.66 \pm 0.85$ ), 2nd trimester ( $3.76 \pm 1.35$ ) and 3rd trimester ( $3.77 \pm 1.32$ ) in pregnant health women (controls).

Table 5: Mean Values of Haemoglobin Levels of Pregnant Women with Malaria Parasitaemia According to Their Trimesters and Control.

Trimesters of pregnancy (n)	Controls (n)	x ± S.D		t-value	P-value	t-test
1st	1st	-3.34 + 1.00	-23.569	0.000	0.000 < 0.05	2nd
-3.10 + 0.966	-22.705	0.000	0.000 < 0.05	3rd	3rd	-2.96 + 0.79
26.397	0.000	0.000 < 0.05				-

Table 5 shows the mean values of the haemoglobin level in pregnant women with malaria parasitaemia with respect to their trimesters and pregnant healthy women (control). The mean values of haemoglobin in those in the 1st trimester ( $9.724 \pm 0.75$ ), 2nd trimester ( $9.80 \pm 0.64$ ), 3rd trimester ( $9.70 \pm 0.54$ ) for pregnant women with malaria parasitaemia (subjects) decreased significantly ( $P < 0.05$ ) when compared to the mean value of haemoglobin in 1st trimester ( $13.07 \pm 0.63$ ) and trimester ( $12.90 \pm 0.61$ ), 3rd trimester ( $12.66 \pm 0.60$ ) in pregnant healthy women (controls).

## Discussion

In this study, decreases in the serum Iron levels in pregnant women with malaria parasitaemia with respect to their trimesters were significant ( $P < 0.05$ ) when compared with serum Iron levels in pregnant healthy women. This decrease was also observed in serum human Ferritin levels when their mean values in

pregnant women with malaria parasitaemia were compared with those of the control subjects. These findings are consistent with that previously reported by Emmanuel et al.(2011) and Chitra et al.(2004). Decreasing of Serum Iron and Ferritin in the present study highlights the importance of these substances in the mechanism of erythropoiesis in the foetus and mother. These marked iron metabolism disturbance in the pregnant women with malaria parasitaemia, may be due to the destructive effect of the parasite on the erythrocytes or as a result of increased metabolic demands for Iron. These findings are also consistent with the report of Donald and Richard (2001) which stated that serum ferritin concentration declines transferrin level is unaffected by pregnancy or inflammation and Wheeler (2008) who reported that evidence of how pregnancy affects concentrations of transferrin is lacking and extent to which transferrin is affected by pregnancy will determine the appropriateness of using transferrin as a biomarker



for detecting iron deficiency in pregnancy, especially in malaria endemic settings.

Decreases in the haemoglobin level in pregnant women with malaria parasitaemia with respect to their trimesters were significant ( $P < 0.05$ ) when compared with the haemoglobin levels in pregnant healthy very early in the development of Iron deficiency and that it serves as a very sensitive Indicator of Iron deficiency. This report contradicts the findings of Sangare et al. (2014), which stated that serum ferritin is increased in the presence of Malaria infection in pregnant women. The differences in the mean values of serum total Iron binding capacity (transferrin) in pregnant women with ind malaria parasitaemia in their 1 and 2 trimesters and the control subjects were not significant But a significant difference ( $p < 0.05$ ) was observed in the 3rd trimesters of malaria infected pregnant women and the control subjects. This may occur because iron binding capacity is a measure of an iron that serum protein can combine. Nearly all the binding capacity is due to transferrin, so that serum transferrin is considered as reserve iron binding capacity, hence increased iron binding capacity appears in Iron deficiency anaemia (Scholl et al., 1992). This therefore contradicts the report of carriaga et al. (1991) who suggested that women. The pathogenesis of anaemia in plasmodial parasitized subjects is complex, multifactorial and is thought to result from haemolysis of parasitized red cells, combination of haemolytic mechanism and accelerated removal of both parasitized and non parasitized red blood cells, depressed and ineffective erythropoiesis (Weatherall et al., 2002). Furthermore, because of hemodilution and increasing needs of Iron for both the mother and the foetus, haemoglobin (Hb) levels may decrease progressively in pregnancy. This finding is in agreement with the report of (Deleeuw et al., 2006) which stated that although haemodilution from expansion of the plasma volume leads to a "physiologic pregnancy anaemia", inadequate Iron supply can limit red cell mass expansion and lead to further deterioration in Iron status and haemoglobin level during pregnancy that may pose risks for the correlates with the severity of infection particularly due to *P. falciparum* (Maina et al., 2010). There was a significant difference ( $P < 0.05$ ) in the serum, C-reactive protein levels in pregnant women with malaria parasitaemia with respect to their trimesters when compared with the serum C-reactive protein levels in pregnant healthy women. Iron deficiency (ID) was defined as serum ferritin  $< 12\mu\text{g/l}$  or serum ferritin between 12 and  $70\mu\text{g/l}$  in the context of inflammation defined as a positive CRP (ie CRP concentration  $> 5\text{mg/ml}$ ). Studies by Paul et al. (2012)

and Israelsson et al. (2009) indicated that elevated level of CRP are associated with malaria morbidity and mortality. According to Ansar et al. (2006), CRP is one of the most widely used acute phase inflammatory proteins because of its early rise and rapid kinetics. It binds damaged host cells including erythrocyte infected by *P. falciparum* resulting in their clearance by both humoral and cellular Immune mechanism. Investigations carried out by pregnancy especially in areas where anaemia due to malaria parasitaemia is highly prevalent to be considered and it would also be important to ensure that individual women receiving iron pregnant woman and her infant. This observation is also consistent with a previous report that plasmodium infection is one of the commonest cause of Haemoglobin degradation in pregnant women resulting in anaemia and MC Guire et al. (1996) stated that CRP is less prone to rapid changes and may therefore be better suited to assess morbidity in malarious subjects. This contradicts the findings by O'Donnell et al. (2009) which reported that reduced levels of C-reactive protein may be an important pathological mechanism in severe malaria among pregnant women.

In conclusion, anaemia is a great public health issue in pregnant women in Owerri, when almost 50% of women are anaemic throughout gestation. *P. falciparum* infection and nutrient deficiencies seemed to be the main preventable causes of anaemia. However, the effectiveness of daily iron supplements to prevent iron deficiencies as well as maternal anaemia was less perceptible in this population, and a lower compliance to this strategy seems to be the most likely explanation. It is therefore pertinent that international guidelines which recommended universal iron supplementation throughout supplementation are indeed protected by an insecticide treated net and are screened for malaria at each scheduled visit until they can receive IPTp as part of routine antenatal care.

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## Conclusion

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**How to cite this article:**

**Nwosu, D.C, Obeagu, Emmanuel Ifeanyi, Ikeh, K.O, Nwanjo, H.U., Nwangwu, S.A., Oluh, C.C., Okorie, H., Obioma-Elemba, J.E., Nwankpa, P., Ezenwuba, C. and Ozims, S.J. (2015). Iron status, Haemoglobin and C-Reactive protein levels in pregnant women with malaria Parasitaemia in Owerri Int. J. Adv. Res. Biol. Sci. 2(12): 316–323.**