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Original Article

# Serum Iron, Total Iron Binding Capacity and Malaria Parasite Density of Pregnant Women with Asymptomatic Malaria Parasite Infection Attending Ante-Natal Clinic at A Tertiary Hospital in Calabar, Nigeria.

Ifeyinwa Maryann Okafor[1], Oko John Odey[1], Valerie Esame Njar [1,2], and Emechebe Cajethan[3]

[1]Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, College of Medical Sciences, University of Calabar, Nigeria, [2]Department of Medical Laboratory Science, Faculty of Medicine and Pharmaceutical Sciences, Kampala International University, Tanzania, [3]Department of Obstetrics and Gynaecology, Faculty of Clinical Sciences, College of Medical Sciences, University of Calabar, Nigeria.

## Abstract

**Objective:** Asymptomatic malaria refers to malarial parasitemia of any density, in the absence of fever or other acute symptoms, in individuals who have not received recent antimalarial treatment. This study was aimed at providing information on the serum iron, total iron binding capacity and malaria parasite density of pregnant women with asymptomatic malaria parasite infection attending ante-natal clinic at University of Calabar Teaching Hospital. **Method:** This was a cross-sectional study, involving fifty (50) pregnant women positive for malaria parasites, without any malaria-related symptoms and attending antenatal clinic at the University of Calabar Teaching Hospital, and 50 non-pregnant uninfected women. Blood samples were collected from the participants and analyzed. Malaria was diagnosed using Microscopy method, haematocrit was determined using the automated haematology analyzer, while serum iron and total iron binding capacity (TIBC) were determined using a modification of the automated AAII-25 colorimetric method. **Results:** Results obtained showed the mean haematocrit and serum iron was significantly lower ($p \leq 0.05$) in pregnant women with asymptomatic malaria than in non-pregnant uninfected women, while TIBC was significantly higher in pregnant women with asymptomatic malaria in comparison with non-pregnant uninfected women. A strong negative correlation was observed between the mean parasite densities of infected pregnant women, their haematocrit and serum iron while a positive correlation was observed between TIBC and malaria parasite density in the infected pregnant women. **Conclusion:** This study has shown a significantly lower value of haematocrit and serum iron among pregnant women with asymptomatic malaria. The study also shows that malaria parasite density of pregnant women with asymptomatic malaria is significantly high, therefore the burden of asymptomatic malaria and iron reduction in pregnancy is still a major health issue among pregnant women in developing malaria—endemic countries. The study therefore recommends that healthcare interventions for pregnant women in malaria-endemic regions should include regular monitoring of iron status.

**Keywords:** Serum Iron, TIBC, Asymptomatic malaria, Pregnant Woman

**Correspondence:**

Ifeyinwa Maryann, Okafor
Phone Number +2348080680620
Email address is okaforify12@ gmail.com;
okaforify@unical.edu.ng

## Introduction

Malaria remains a substantial public health challenge that disproportionately affects sub-Saharan Africa (SSA): in 2018, 213 million (93%) malaria cases worldwide and 405,000 of all malaria-related deaths (94%) worldwide occurred in SSA[1]. There are five well-established malaria parasite species that infect humans, namely *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. *P. falciparum* accounts for 99·7 % of infections in sub-Saharan Africa, while *P. vivax* accounts for 75% of infections in the Americas[2]. Pregnant women are considered to be at higher risk of malaria and adversely affected by the disease[3]. According to the WHO, there were 229 million cases of malaria in 2019. More than 90% of these cases were located in the WHO African region[1].

Iron is the second most abundant metal on earth, comprising about 5% of the earth's crust[4]. Its significance to humans is paramount, as it is a vital micronutrient for human existence. Being a d-block transition metal, it interchanges between various oxidation states, which

empowers it to participate in electron transfer and also bind to several biological ligands. The two most common iron states are the divalent ferrous ($Fe^{2+}$) and the trivalent ferric ($Fe^{3+}$). Within the human body, iron is required as a cofactor for many haemoproteins and non-haem iron-containing proteins. Haemoproteins include haemoglobin and myoglobin that are responsible for oxygen binding and transport, catalase and peroxidase enzymes, which take part in oxygen metabolism, and cytochromes, which are involved in electron transport and mitochondrial respiration. Non-haem iron-containing proteins also have crucial functions, as these are used in DNA synthesis, cell proliferation and differentiation, gene regulation, drug metabolism, and steroid synthesis[5]. In the plasma, iron is predominantly bound to TF, and TIBC depends on the concentration of this globulin.The fraction of TF to which iron is not actually bound is known as the "unsaturated iron-binding capacity" (UIBC). The sum of serum iron concentration and the UIBC gives the TIBC[6].

Micronutrient deficiencies are a major public health burden, especially in low-income countries, and accurate prevalence estimates are important to guide planning and monitoring of nutritional interventions[7]. However, prevalence of micronutrient deficiencies can be incorrectly estimated because certain micronutrient biomarkers are affected by inflammation and infections such as malaria[8]. Iron deficiency is the most common micronutrient deficiency in the world and consequently anemia during pregnancy is most commonly caused by iron deficiency. In 2020, the worldwide maternal mortality ratio was estimated at 152 deaths per 100 000 live births, although the true number may be higher. The World Health Organization (WHO) has previously estimated the global burden of deaths attributed to anemia in women of reproductive age, with an estimated incidence of 16,800–28,000 deaths annually[9].

Although there is no standard definition for "asymptomatic" malaria infections, it is generally accepted to be malarial parasitemia of any density, in the absence of fever or other acute symptoms, in individuals who have not received recent antimalarial treatment[10]. It is the detection of Plasmodium species in peripheral blood, an axillary temperature <37.5°C, and an absence of malaria-related symptoms, given the lack of standard definition[11]. In moderate and high malaria transmission areas, a significant proportion of asymptomatic malaria infections are caused by P. falciparum, while few infections are caused by other species of Plasmodium[12]. In pregnancy, malaria affects not only pregnant women but also the fetus and newborn child[13]. Malaria during pregnancy is characterized by sequestration of infected erythrocytes within the placenta with local inflammation and infiltration of immune cells (such as macrophages, monocyte, and lymphocytes). Such condition results in adverse consequence such as miscarriage, stillbirths, low birth weight, and infant mortality[14].

Asymptomatic malaria, if left untreated, can also progress to chronic infection leading to decreased erythropoiesis' precursors, and increased erythrophagocytosis. These changes contribute to maternal anemia and its associated complications such as iron deficiency anaemia which may lead to maternal mortality during pregnancy or postpartum, and may also result in increased infant/fetal mortality[15]. Despite the significant impact of asymptomatic malaria parasite infections on disease transmission, limited research has investigated the relationship between haematocrit, serum iron, total iron binding capacity (TIBC), and malaria parasite density in pregnant women without clinical symptoms. Therefore, there is a need to explore the dynamics of serum iron, TIBC, and malaria parasite density in pregnant women with asymptomatic malaria infections to address this research gap and provide insights for malaria control efforts.

## Materials and Method

This study was conducted in University of Calabar Teaching Hospital (UCTH). University of Calabar Teaching Hospital is a Tertiary Health Institution established in 1979 to provide tertiary health care through the training of medical students of the College of Medical Sciences of the University of Calabar, resident Doctors for the different Post Graduates as well as other healthcare providers in different specialties of medicine and its allied professions. The facility is located at Calabar in Calabar Municipality Local Government Area of Cross River State, South-South, Nigeria, and is affiliated with University of Calabar, Calabar.

*Study Population and Design:* This is a cross sectional study. A total of fifty (50) pregnant women positive for malaria parasites, but without any malaria-related symptoms and attending antenatal clinic at the University of Calabar Teaching Hospital were enrolled for this study.

*Ethical Consideration/Informed Consent:* Ethical clearance was obtained from Research and Ethical Committee of the University of Calabar Teaching Hospital (UCTH/HREC/33/VOL111/150).. Detailed explanation of the purpose, objectives, risks, and benefits to the study subject and the confidentiality of responses was given to participants, after which verbal, consent was obtained. The respondents' right to refuse or withdraw from participating in the interview was fully maintained. Data was collected after obtaining informed consent and agreement from the

patients under study. Sample collection was performed following ethical steps and procedures.

*Inclusion Criteria and Exclusion Criteria:* Only pregnant women attending antenatal clinic at UCTH who are positive for malaria parasites upon microscopy, and have been confirmed to be without associated malaria symptoms, and give informed consent were co-opted into the study. Those who do not meet the above-stated criteria study were excluded from the study.

*Administration of questionnaire:* After a detailed explanation, the participants' consent was asked for their willingness to enroll in the study. Then, before blood sample collection, responses of participants was taken using a structured questionnaire.

The questionnaire contained data on socio-demographic parameters. Each respondent's age group, marital status, educational level, occupation, previous malaria infection, time of last malaria episode, and 'use of bed net the previous night' was covered in the socio-demographic section.

*Collection, Handling, And Storage of Samples:* Three millilitres (3ml) of venous blood samples were drawn aseptically from each volunteer participants via venepuncture using a disposable plastic syringe into 5ml plain containers and labelled with the name, gender, and unique identification number of each participant. Thick and thin blood films were made and labelled appropriately.
The samples were temporarily stored in a cold flask packed with ice prior to their transfer to the laboratory for analysis.

*Sample Analysis:* Standard microscopy technique was used for detection of malaria parasite with thick and thin films stained with 3% Giemsa and examined microscopically with the x100 objective lens.

*Quantification of Parasites:* After detection of malaria parasite using thick blood film, the number of malaria parasites observed in the fields were quantified using this method;

$$\frac{\text{Number of parasites counted} \times 8000}{\text{Number of WBCs counted}} \quad \frac{}{1}$$

Unit= value gotten/µl of blood

*Reporting:* The parasite density was reported as the number of parasites per µL of blood. Serum iron and total iron binding capacity (TIBC) were determined using a modification of the automated AAII-25 colorimetric method.

*Data processing and software used in statistical analysis* Data was entered and analyzed using Statistical Package for Social Sciences (SPSS), version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics like frequency, median, and percentage were used to describe the study population characteristics. Results were also expressed as means ± standard deviation, while comparisons were made between different groups using the Students t-test. The level of statistical difference was set at p value ≤ 0.05, at 95% confidence interval.

## Results

Table 1 shows the socio-demographic characteristics of pregnant women attending antenatal centre at the University of Calabar Teaching Hospital. Out of the 50 respondents, those belonging to the age group of 35 – 39 years had the highest participation, 18 (36.0%), closely followed by those belonging to the age group of 30 – 34years (32.0%). Majority of the respondents were Christians 42(84.0%), while the remaining 2(4.0%) and 6(12.0%) were Muslims and Traditional worshippers respectively.

Nearly all of the respondents were married 47(94.0%) with only 3(6.0%) being singles. Participants with tertiary education constituted the majority 21(42.0%), while those with no formal education, primary education, and secondary education constituted the remaining 5(10.0%), 12(24.0%), and 12(24.0%) respectively. Most of the respondents were business-women 18(36.0%), while civil servants, hairdressers, and tailors made up the remaining 9(18.0%), 14(28.0%), and 9(18.0%) respectively. All the respondents reported having previous malaria infections, while majority reported suffering from malaria infection within the last 6 months.

Table 2 shows the mean haematocrit (HCT), serum iron and total iron binding capacity in the test group (pregnant women) and in the control group respectively. The pregnant women had mean HCT, serum iron, and TIBC values of 32.51±5.71%, 95.70±19.91µg/dl and 328.58±58.78µg/dl respectively, and the controls had mean values of 38.15±2.38%, 328.58±58.78µg/dl and 289.14±71.59µg/dl respectively. Significant variations were observed in serum iron and total iron binding capacity among the two groups studied; pregnant women with asymptomatic malaria and control group ($p \leq 0.05$).

Table 3 shows the malaria parasite density (MPD) of pregnant women with asymptomatic malaria and non-pregnant controls. Pregnant women with asymptomatic malaria had mean values of 692.16±151.95parasites/µl,

while the controls had mean values of 0.00±0.00 parasites/µl. Significant variation was observed in the malaria parasite density (MPD) of the pregnant women and the non-pregnant controls (p≤0.05).

Table 4.1: Socio-Demographic Characteristics of Pregnant Women Attending Antenatal Centre at the University of Calabar Teaching Hospital.

| Variables | Frequency (n = 50) | Percentage (%) |
|---|---|---|
| Age (Years): | | |
| 20 - 24 | 4 | 8.0 |
| 25 - 29 | 8 | 16.0 |
| 30 - 34 | 16 | 32.0 |
| 35 - 39 | 18 | 36.0 |
| 40 and above | 4 | 8.0 |
| Religion: | | |
| Christianity | 42 | 84.0 |
| Islam | 2 | 4.0 |
| Traditional | 6 | 12.0 |
| Marital status: | | |
| Married | 47 | 94.0 |
| Single | 3 | 6.0 |
| Education: | | |
| No formal education | 5 | 10.0 |
| Primary education | 12 | 24.0 |
| Secondary education | 12 | 24.0 |
| Tertiary education | 21 | 42.0 |
| Occupation: | | |
| Business | 18 | 36.0 |
| Civil servant | 9 | 18.0 |
| Hairdressing | 14 | 28.0 |
| Tailoring | 9 | 18.0 |
| On iron supplementation: | 47 | 94.0 |
| Yes | 3 | 6.0 |
| No | | |
| Previous malaria infection: | 50 | 100.0 |
| Yes | 0 | 0.0 |
| No | | |
| Period of last malaria infection: | | |
| Within the last 6 months | 44 | 88.0 |
| About a year ago | | |
| More than a year ago | 4 | 8.0 |
| Use of bed net: | 2 | 4.0 |
| Yes | 48 | 96.0 |
| No | 2 | 4.0 |

Table 2: HCT, Serum Iron and Total Iron Binding Capacity of Pregnant Women with Asymptomatic Malaria and Non-Pregnant Controls.

| Parameter | Groups: Pregnant women with asymptomatic malaria (n = 50) | Groups: Non-pregnant controls (n = 50) | t | p –value |
|---|---|---|---|---|
| HCT (%) | 32.51±5.71 | 38.15±2.38 | -6.446 | 0.001* |
| Serum iron (µg/dl) | 95.70±19.91 | 103.04±17.31 | -1.967 | 0.052* |
| TIBC (µg/dl) | 328.58±58.78 | 289.14±71.59 | 3.011 | 0.003* |

Values are expressed as Mean±SD; HCT = Haematocrit; TIBC = Total Iron Binding Capacity; * = Significant at P≤0.05

Table 3: MPD of Pregnant Women with Asymptomatic Malaria and Non-Pregnant Controls.

| Parameter | Groups: Pregnant women with asymptomatic malaria n = 50 | Groups: Non-pregnant controls n = 50 | t | p – value |
|---|---|---|---|---|
| MPD (parasites/µl) | 692.16±151.95 | 0.00±0.00 | 32.209 | 0.001* |

Values are expressed as Mean ± SD; MPD = Malaria Parasite Density; * = Significant at p≤0.05

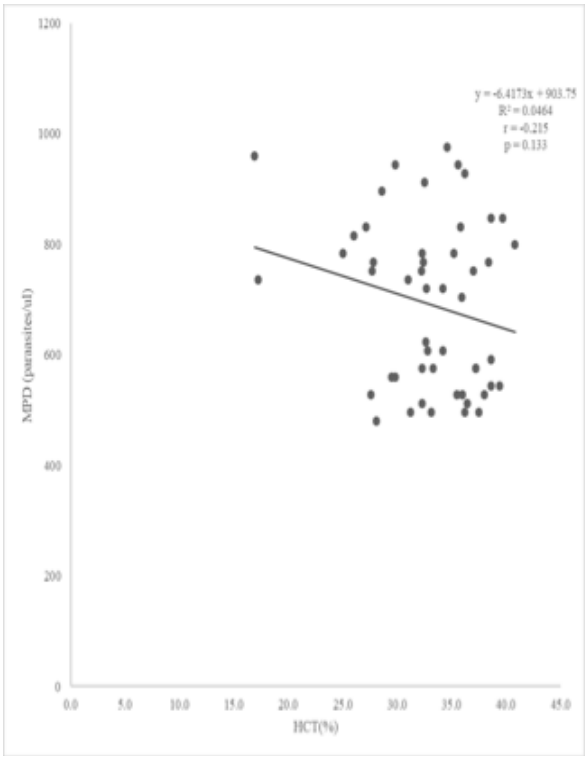


Fig. 1 shows a correlation plot of malaria parasite density (MPD) against Haematocrit (HCT)

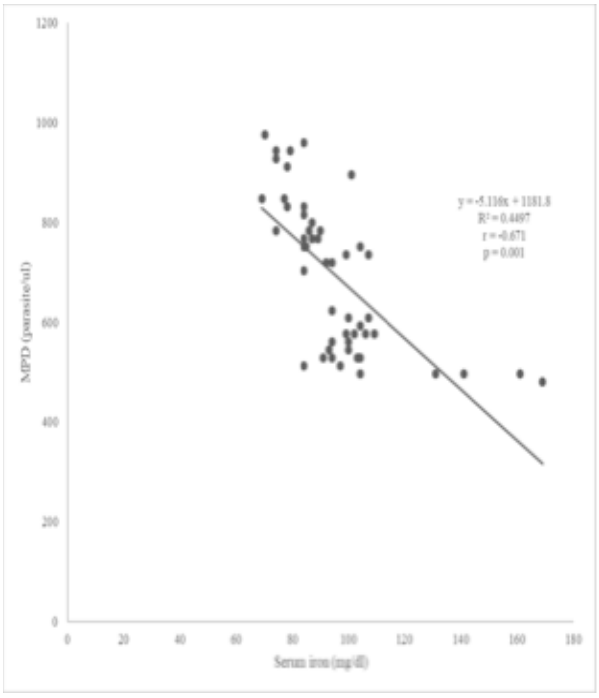


Fig 2 shows a correlation plot of malaria parasite density (MPD) against serum iron.

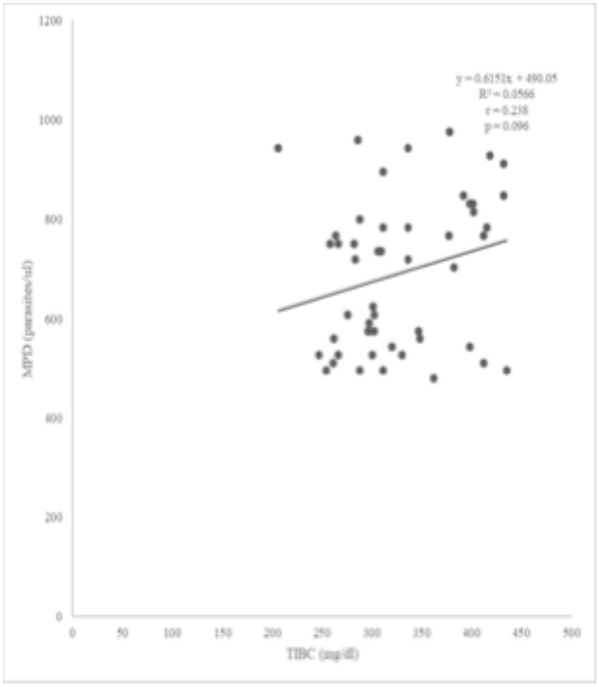


Fig 3 shows a correlation plot of malaria parasite density (MPD) against Total Iron Binding Capacity (TIBC).

Fig 1 shows a correlation plot of malaria parasite density (MPD) against Haematocrit (HCT). A non-significant negative correlation ($r = -0.215$, $p = 0.133$) was observed between MPD and HCT in the test groups. Fig. 2 shows a correlation plot of malaria parasite density (MPD) against serum iron. A significant negative correlation ($r = -0.671$, $p = 0.001$) was observed between MPD and serum iron in the test groups while Fig. 3 shows a correlation plot of malaria parasite density (MPD) against Total Iron Binding Capacity (TIBC). A non-significant positive correlation ($r = 0.238$, $p = 0.096$) was observed between MPD and HCT in the test groups.

## Discussion

Iron is an essential element required for many biochemical processes in the body. It plays a role in internal respiration and haemoglobin synthesis. This study compared the haematocrit, serum iron and total iron binding capacity of pregnant women with asymptomatic malaria to non-pregnant uninfected women.

Among the 50 pregnant women with asymptomatic malaria, the age range of 35-39 years exhibited the highest involvement (36.0%), closely followed by the 30-34 years age group (32.0%). This trend is contrast to the findings of Kahn *et al.,*[16] who reported that younger women below 25 years were more likely to receive antenatal care than older women above 31years of age.
The increased participation by the age group of 35-39 years in the study could be influenced by several factors.

One possible explanation is that women in their late thirties may be more likely to have planned pregnancies or be more conscious of the importance of regular check-ups during this stage of life. The majority identified as Christians (84.0%), while smaller percentages identified as Muslims (4.0%) and Traditional worshippers (12.0%). Nearly all participants were married (94.0%), with a small fraction being singles (6.0%). Regarding education, those with tertiary education constituted the largest segment (42.0%), while participants with no formal education, primary education, and secondary education comprised 10.0%, 24.0%, and 24.0%, respectively. In terms of occupation, a significant portion of respondents were engaged in business (36.0%), followed by civil servants (18.0%), hairdressers (28.0%), and tailors (18.0%). Notably, all respondents reported a history of previous malaria infections, with a majority indicating recent experiences of malaria within the last 6 months. This finding is a reflection of the endemicity of malaria in the study area.

Findings from the study revealed significant reductions in the haematocrit and serum iron levels of the pregnant women with asymptomatic malaria in comparison to the controls. This is consistent with the findings of

Okafor *et al.*[17] who also reported significantly lower values of haematocrit and serum iron among pregnant women when compared to their non-pregnant counterparts. Another study conducted in Calabar by Okafor *et al.*[18] also reported similar findings. This is also in line with the findings of a study in Ondo by Obeagu *et al.*[19] who reported significant reduction in packed cell volume and serum iron in pregnant women. The findings of this study are also in consonance with the findings of Vaucher *et al.*[20] and Arif *et al.*[21] who both reported significant reductions in serum iron and haemaatocrit of pregnant women. One possible reason for this finding might be due to pregnancy-induced haemodilution, where plasma volume increases disproportionately in comparison with the increase in red cell mass. Valerie et al.[30] reports several remarkable differences exist between pregnant women who have an infection and the negative counterparts. Another reason for the significant reductions in haematocrit and serum iron witnessed in the study might be the progressive haemolysis that results from chronic infections such as asymptomatic malaria, which can contribute to long term alterations in iron metabolism.

The study also revealed significantly higher total iron binding capacity in pregnant women with asymptomatic malaria than in non-pregnant women. A similar finding was reported by Okafor *et al.*[17] and Amaihunwa *et al.*[22]. The reports of this study also agree with the findings of previous studies by Ogbu *et al.*[23] and Onochie and Egwunyenga.[24] who also reported significantly elevated levels of total iron binding capacity in pregnant women. The elevated levels of total iron binding capacity in the pregnant women with asymptomatic malaria could be due to the body's compensatory mechanism in which the body produces more transferrin to enhance iron transport in response to low iron stores.

The study also reported significantly increased malaria parasite density in the pregnant women with asymptomatic malaria than in the non-pregnant uninfected controls. This value is in line with those reported by a study in Ghana by Adu-Gyasi *et al.*[25]. The finding of the study also agrees with the study conducted by Ogbu *et al.*[23] and Emiasegen *et al.*[26] who found the high prevalence of malaria in Nigeria. The high malaria density obtained in this study in spite of the high number of pregnant who are using insecticide treated mosquito nets (96.0%) shows that the women may not be using the insecticide treated mosquito nets correctly, therefore, there is need for increased education and awareness.

The study also revealed a strong negative correlation between malaria parasite density (MPD) and serum iron. Possible reasons for this finding could be red cell haemolysis in which iron is also broken down and utilized by the malaria parasite, or due to the inflammation-induced increase in hepcidin (a key regulator of iron homeostasis) brought on by malaria infection. Elevated hepcidin levels can reduce intestinal iron absorption by 40% and promote iron sequestration within cells, resulting in lowered serum iron[27].

## Conclusion

In conclusion, this study has shown a significantly lower value of haematocrit and serum iron among pregnant women with asymptomatic malaria. The study also shows that malaria parasite density of pregnant women with asymptomatic malaria is significantly high, therefore the burden of asymptomatic malaria and iron reduction in pregnancy is still a major health issue among pregnant women in developing malaria—endemic countries.

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