# The effect of *Plasmodium falciparum* malaria on platelet counts in patients attending the University of Calabar Teaching Hospital, Nigeria.

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**Abstract**

This prospective cross-sectional study was aimed at assessing the effect of *Plasmodium falciparum* malaria on platelet count among various categories of hospital patients in the University of Calabar Teaching Hospital, Calabar, Nigeria. A total of six hundred and ten (610) patients participated in the study and three hundred and sixty (360) non-malaria infected patients were used as controls. The prevalence rate of *Plasmodium falciparum* malaria in this study was 40.98%. The mean platelet count of 113 ± 57 x $10^9$/L observed in the malaria infected patients was found to be significantly lower than the mean count of 168 ± 48 x $10^9$/L in the non-infected subjects (controls) ($t = 4.867$, $P < 0.001$). The highest prevalence rate of 68.10% occurred in children aged 1-12 years and their platelet counts of 110 ± 41 x $10^9$/L was significantly lower than the control value of 166 ± 85 x $10^9$/L ($t = 4.696$, $P < 0.001$). The platelet count of 103 ± 41 x 10/L observed in pregnant women was also statistically significant when compared with 168 ± 76 x $10^9$/L in the non infected pregnant women. ($t = 3.051$, $P < 0.01$). Mild significant depression also occurred in adults aged 18 years and above ($t = 2.575$, $P < 0.05$). The effect of *Plasmodium falciparum* malaria on platelet count was not significant in adolescents aged 13 – 17 years. ($t = 0.047$, $P > 0.05$). The mean haematocrit value obtained in this study was 0.35l/l. The mean haematocrit values of 0.33 ± 0.07l/l in a Pf 2+ infection and 0.35 ± 0.06l/l in a Pf 1+ infection was not found to be statistically different ($t = 0.33$, $P > 0.05$). The reduction in platelet count and haematocrit values was found to be independent of the degree of parasitaemia. Thrombocytopenia might therefore be one of the useful indicators of

*Plasmodium falciparum* malaria in children and pregnant women.

**Keywords:** *Plasmodium falciparum,* malaria, platelet count, thrombocytopenia, Calabar, Nigeria.

## Introduction

*Plasmodium falciparum* malaria remains a major cause of morbidity and mortality in tropical areas despite better knowledge of the pathogenesis and management of severe malaria[1]. In Nigeria, malaria is holoendemic and causes severe morbidity among the population of young and old and high mortality among the infants [2]. Greater than 80% of malaria infection are caused by *Plasmodium malaria* and less than 5% are caused by *P ovale*. By school age, when a considerable degree of immunity has developed, asymptomatic parasitaemia can be as high as 75%[1,2].

Thrombocytopenia has been reported to be frequent in patients with acute malaria and is sometimes profound in cases of severe disease [3-5]. Most of the reports on the effect of malaria on platelet count are centred on children as the subjects for investigation with little or no consideration for other patient categories in the hospitals used for the studies [2,4,6,7]. This has led to the scarcity of information on this subject in the South-South region of Nigeria. This study was therefore designed to (1) Examine the effect of *Plasmodium falciparum* malaria on platelet count on a cross-section of hospital patients. (2) To establish a normal range for platelet count in the study population using the controls. (3) To determine which category of these patients are more vulnerable to the *Plasmodium falciparum* malaria attack. It is hoped that information gathered from this study will assist in the management of the patients affected and provide a database for future research.

## Materials and methods

*Subjects:*

A total of six hundred and ten (610) patients attending the University of Calabar Teaching Hospital (UCTH) were enrolled into the study after obtaining informed consents. Patients were classified as follows: (1) Children – In this group, a total of 110 children aged 1-12 years participated. They were all drawn from the children emergency room (CHER). (2) Adolescents (n = 45) aged 13-17 years and drawn from the general Outpatient Department (GOPD). (3) Adults, aged 18 years and above constituted this group. Three hundred and five (305) adults participated and were all drawn from the General Outpatient Department (GOPD). (4) Pregnant women: In this group a total of one hundred and fifty (150) pregnant women attending the antenatal clinic (ANC) of UCTH, Calabar, and aged 15-29 years participated in this study. In this preliminary study, the level of trimesters and disease conditions except malaria were not considered critical for the study. (5) Controls – A total of three hundred and sixty (360) non-malaria

infected patients (i.e. 59.02% of the study population) served as controls in this study. All the subjects admitted into this study were all examined by the clinician in their respective clinics and a provisional diagnosis of plasmodiasis (malaria) made before blood samples were collected from them. Malaria was defined in this study by the presence of *Plasmodium falciparum* in the thick blood films.

***Methods:***

Thick-blood films from the six hundred and ten (610) EDTA blood samples were made, giemsa-stained and microscopically examined. Total parasite count per micro litre was quantitated by counting the number of parasites per 100 white blood cells (WBC), assuming a total WBC count of 8000/l [8]. A thick film was declared negative after viewing high power fields containing 500 WBCs. Haematocrit values were estimated on all samples for the purpose of screening for anaemia, using the microhaematocrit centrifuge, HEMLE, 230H, BHG at the speed of 10,000 rpm for 5 minutes. Results were read with microhaematocrit reader and expressed in SI unit (l/l). Platelet count was done manually using the method approved by the International Committee for Standardisation in Haematology (ICSH)[9]. A 1 in 20 dilution of blood was made by adding 0.02ml of blood to 0.38 ml of 1% ammonium oxalate, then a haemocytometer (Improved Neubauer) was charged with the diluted blood. After allowing it to settle down for 20 minutes in wet chamber, platelets were counted in the five central squares of $0.02mm^2$ using x 40 objective with reduced light. Calculation was done from first principle and the results expressed as the number of platelets x $10^9$/L. (SI units.)

***Statistics:***

The student's t-test for coupled data was adopted for comparison of means. A $P < 0.05$ was considered as statistically significant.

**Results**

Table 1 shows the prevalence of *Plasmodium falciparum* malaria and the mean platelet counts of different categories of patients in the University of Calabar Teaching Hospital. Of the six hundred and ten (610) samples analysed, two hundred and fifty (250) were positive for *Plasmodium Falciparum* malaria giving a prevalence rate of 40.98%. Their mean platelet count of 113 ± 57 x $10^9$/L was significantly lower than the mean count of 168 ± $10^9$/L from those not infected with the parasite ($t = 4.867$, $P < 0.001$). The highest prevalence rate of 68.18% occurred in children 1-12 years. Student's t-test analysis revealed that the platelet counts in children, pregnant women and adults above 18 years were significantly reduced. Platelet value of 110 ± 43 x $10^9$/L in the infected children differed significantly when compared with values of 166 ± 85 x $10^9$/L in non-infected children. ($t= 4.696$, $P < 0.001$). Values of 103 ± 41 x $10^9$/Lin infected pregnant women also differed significantly when compared with the

platelet count of 168 ± 76 x $10^9$/L in the non-infected pregnant women. ($t = 3.051$, $P < 0.01$). The effect of the *Plasmodium falciparum* malaria was also noticed in adults aged 18 years and above. The value of 122 ± 44 x $10^9$/L in these adults was significantly lower than the mean value of 156 ± 60 x $10^9$/L in non-infected adults. There was no statistically significant difference between the mean platelet count of 144 ± 51 x $10^9$/L obtained in infected adolescents aged 13 – 17 years and a mean value of 146 ± 43 x $10^9$/L in the non-infected adolescents ($t = 0.047$, $P > 0.05$).

Also, there was no statistically significant difference between the mean platelet count of 99 ± 73 x $10^9$/L obtained in patients with Pf 2+ infection and the mean count of 121 ± $10^9$/L in patients with a Pf 1+ infection of the malaria parasite ($t = 0.719$, $P > 0.05$) as shown on Table 2. The mean haematocrit values of .33 ± 0.07L/L obtained in a Pf 2+ infection and .35 ± 0.06L/L in a Pf 1+ infection was not found to be statistically different ($t = 0.33$, $P > 0.05$). The haematocrit value obtained in this study was 0.35L/L (Table 2).

Table 1. Prevalence of *Plasmodium falciparum*(Pf) malaria and mean platelet counts in 610 participants

| Category of Patients | Pf Infected | | | Non-Pf Infected (Control ) | | | |
|---|---|---|---|---|---|---|---|
| | Number (%).x ± SD x $10^9$/L | | Observed Range | Number (%) x ± SD x 109/L | | Observed Range | SE |
| Children 1-12 years | 75 (68.8) | 110 ± 43 | 67 – 153 | 35 (31.2) | 166 ± 85 | 120 – 216 | 33. |
| Adolescents 13-17 years | 10 (22.2) | 144 ± 51 | 93 – 195 | 35 (77.8) | 146 ± 43 | 103 – 189 | 42. |
| Adults 18 years above | 115 (37.7) | 122 ± 47 | 75 – 169 | 190 (62.3) | 156 ± 60 | 96 – 216 | 13. |
| Pregnant women | 20 (33.3) | 103 ± 41 | 62 – 144 | 100 (66.7) | 168 ± 76 | 92 – 244 | 21. |
| Total | 250 (40.98) | 113 ± 57 | 56 – 170 | 360 (59.02) | 168 ± 48 | 120 – 216 | 11. |

x - mean values,
SD - standard deviation
SED - standard error difference
t Value - students t-test value

Table 2: Effect of degree of *Plasmodium falciparum* parasitaemia on platelet count and haematocrit values.

| Degree of Parasitaemia | Platelet count | Haematocrite |
|---|---|---|
| | $\overline{x}$ + SD x $10^9$/L x $\overline{+}$ SD (%) | |
| Pf 1+ | 121 ± 40 | 0.35 ± 0.06 |
| Pf 2+ | 99 ± 73 | 0.33 ± 0.07 |
| Pf 3+ | 0 | 0 |
| t value | 0.719 | 0.666 |
| p value | ns | ns |

ns = not significant (P > 0.05)
Pf = plasmodium falciparum
$\overline{x}$ = means,
SD = standard deviation
1+ = parasite density of 2800/ l. Average if 35 parasites were counted per 100 WBC; 35 /100 x 2800. 8000 = fixed value for patients WBC
2+ = Average 280 parasites were counted per 100 WBC. 180/100 x 800 = 22,400
3+ = Not encountered in the study
(The method of Trape [8] was used as a reference for this standard)

**Discussion**

With respect to platelets, it was observed in this study that the mean platelet count in *Plasmodium falciparum* malaria infected subjects, 113 ± 57 x $10^9$/L was significantly lower than the mean value of 168 ± 48 x $10^9$/L in the control group. The normal platelet count of 150 – 450 x $10^9$/L has been reported in Caucasians. [18] In Nigeria, a normal range of 100 – 300 x $10^9$/L was established by Essien *et al* among healthy subjects in Ibadan. In contrast, a normal range among controls used in this study is 120 – 216 x $10^9$/L, which is higher in the lower range and lower in the upper range.

Thrombocytopenia occurs when platelets are lost from the circulation faster than they can be replaced by the bone marrow. A person is termed thrombocytopenic when the circulating platelets fall below the lower limit of 150 x $10^9$/L [19]. A marked thrombocytopenia occurs when the platelet count is between 40 – 60 x $10^9$/L. Below 40 x $10^9$/L, a patient is regarded as severely thrombocytopenic. In this study, a marked degree of thrombocytopenia, that is, platelet count of less than 60 x $10^9$, was found in 30 patients representing only 5% of the study population. All of them had a *Plasmodium falciparum* 2+ infection of malaria suggesting that malaria could be responsible for the reduced platelet count.

Some workers have suggested mechanism by which malaria causes a reduction in platelet count. The role of immune factors and the destruction or sequestration of platelets seems to be prominent among others [1]. In severe forms, platelets and erythrocyte sequestration are frequent [4] and thrombocytopenia is present [1,4,5,19]. However no studies have shown that thrombocytopenia at the initial stage of acute malaria could be a marker of severity.

Results obtained in this study have proved that the effect of *Plasmodium falciparum* parasite on platelet count is quite independent of the degree of infection. There was no statistically significant difference between the mean value of 99 x $10^9$/L in a Pf 2+ infection and a mean value of 121 x $10^9$/L obtained in a 1+ infection. A mean haematocrit value of .35 L/L was observed in this study. This occurred independent of the degree of parasitaemia.

This prospective cross-sectional study was aimed at assessing the effect of *Plasmodium falciparum* malaria on circulating platelets among hospital patients in Calabar, Nigeria. Malaria is highly endemic in Nigeria and is one of the major causes of ill-health and death [10]. In this study, the highest prevalence rate of 68.8% was observed in children (1-12 years). Adults accounted for 37.7% while adolescents (13-17 years) recorded 22.2%. The prevalence rate among the pregnant women was found to be 33.3%. Many factors have been known to influence the transmission of malaria. In Nigeria, the pattern of malaria is that of intense transmission and remarkable stability. Malaria transmission is intense all year round in the

forest belt of which Calabar belongs but more intense in the rainy season. In the dry Savannah transmission is also relatively low during the dry seasons [10].

This study was carried out between the months of January and April, when the transmission is expected to be relatively lower. This may possibly explain the reason why a heavy infection with *Plasmodium falciparum* was not encountered. Malaria is caused by a parasite, the plasmodium – that lives in and feeds on red blood cells. This parasite is transmitted to humans by Anopheles mosquito and those in Nigeria are very efficient vectors [10]. Also the environment in many parts of Nigeria, including Calabar favours malaria transmission, that is, temperature of $20^0 – 28^oC$, mean monthly rainfall of greater than 10cm, relative humidity greater than 60% and topography with altitudes of less than 2000 meters above sea level [10,11].

The severity and clinical presentation of *Plasmodium falciparum* malaria has also been reported to depend on the age of patients, intensity of transmission and development of immunity [12]. In this study, the prevalence of malaria infection is highest in children and this is in consonance with several other reports for endemic areas [2,3,11,12].

The next group of people so vulnerable to the effect of malaria is the pregnant women as shown in this study, which is also in support of other published reports [11,13]. A unique feature of *Plasmodium falciparum* is its ability to sequenter in deep capillary beds during the asexual stages of parasite replication, thereby avoiding host immune surveillance and splenic clearance. Infected erythrocytes adhere to a variety of ligands on vascular endothelium [14]. It is this feature that is thought to result in *Plasmodium falciparum* being responsible for most of the severe disease and almost all of the mortality associated with malaria worldwide. Until recently, the mechanism through which placental parasite sequestration occurs has been unclear, however, studies in Malawi and Kenya have identified strain of parasite which are found with increased frequency in pregnant women and which may be selected by their ability to adhere to chondroitin sulphate A in the syncytiotrophoblast [15,16]. Since malaria in pregnancy is a potentially life threatening condition, women from malaria endemic areas should be given intermittent preventive treatment.

A seroepidemiologic study conducted in West Africa has revealed that most children (> 2 years) and adults develop antibodies to apical membrane antigen – 1 (AMA - 1) [17]. Age – dependent changes in the serum antibody levels to this antigen were found among children age 2-9 years in their study in Guinea-Bissau. In this present study, the levels of antibody were not tested for, but the comparison of the results between groups indicates that children are more vulnerable to the effect of *Plasmodium falciparum* malaria. There were no significant changes in the age group (13-17 years). This may probably account for the low prevalence

rate of 22.2% among those of the age group 13-17 years as observed in this study.

**Conclusion**

The effect of *Plasmodium falciparum* malaria on platelet count is more pronounced in children (1-12 years) and pregnant women. The effect is moderate on adults aged 18 years and above. No significant effect was noticed among adolescents (13-17 years). The reduction in platelet count is independent of the degree of parasitaemia. Thrombocytopenia might be one of the useful indicators of *Plasmodium falciparum* malaria in children and pregnant women.

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