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Influence of *Plasmodiumfalciparum*Parasitaemia on Thrombocytes among children (6-59 Months). A Case study of Bulumkutu Comprehensive Health Centre Maiduguri, Borno State – Nigeria

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Abstract
This study was conducted to assess the influence of Plasmodium falciparumparasitaemia on Platelate (children) (6-59), at Bulumkutu Comprehensive Health Centre Maiduguri, Borno State, between August to December. A total of 210 children were enrolled in the study which consisted of 88 (41.9%) patients with positive P. falciparum malaria and 122 (58.10%) negative malaria. Hematological parameters were analyzed using sysmexhaematology auto-analyser (2011), while the Giemsa stained slides thick and thin blood films were prepared from the stock solution, and tested for Plasmodium falciparum malaria and count of malaria parasite density. The result shows that a mean platelate counts were significantly lower compared to malaria negative individual. A negative and significant correlation was observed between the parasite densities platelate (throbocyte) as index of thrombocyteopenia of subjects. (r2 = 0.760, p = 0.001) and also in male subjects (0.716, p = 0.001) while platelate count female subject (r^2 = 0.810, p = 0.001) respectively.

Keywords: *Malaria, Mosquito, Plasmodium, Parasite.*

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I. Introduction

Malaria is among the major public health concern in Sub-Sahara Africa Francis *et al*., (2014). As such it's one of the major causes of mortality and morbidity especially in children Okoroiwu, *et al.,* (2014). Majority of malaria infection occurs in Sub-Sahara Africa as against any other area in the world. Malaria endemicity vary from country to country Okoroiwu, *et al.,* (2014), and Approximately 15 nations accounts for 80% and 78% of cases and deaths emanating from malaria infection on global scale, respectively (Aju-Amehe*t al.,* 2016). Malaria is endemic in Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda and as such approximately half of global malaria case occurs in these regions Fact Sheet (2011).

Malaria is caused by plasmodium species (a protozoan). Four major species of plasmodium exist in Africa including *ovale, vivax, falciparuum, malariae*Bassey and Izah (2017). Among the species, *P. falciparum*is the major cause of malaria in West Africa Idowu*et al.,* (2009). Malaria is transmitted by Mosquito, an iniquitous dipteran fly. Mosquito has the tendency to transmit malaria and host of other diseases Ndiok*et al.,* (2016) including filariasis, yellow fever, dengue fever, encephalitis, chikungunya, yellow and dengue fevers, lymphatic filariasis especially in tropical countries. Different species of mosquito belong to varying genera (viz: *Aedes, Culex, Anopheles and Mansonia*etc) exist and they can transmit different diseases in both humans and animals.

Specifically the genus *Anopheles* is a major vector that transmits malaria parasite. Several species of the genus exist, but the common species include *Anopheles gambiae, A. funestus, A. arabiensis*and *A.*

melasNmadu et al (2015). But in Nigeria, *Anopheles gambiae*is among the frequently encountered species. The parasite is transmitted through bite of an infected female anopheles mosquito (Okoroiwu, *et al.,* 2014). Anopheles mosquito that carries the parasite desposites the sporozoites on the host, which then invades the liver and then the red blood cells, thereby predisposing the individual to intermittent shivering, pyrexia, sweating and spleen enlargement. Francis*et al.*,(2014). This could lead to anaemia in infected patients.

Blood plays a very vital function in the human body. Typically, blood consists of cellular constituent including erythrocytes, leukocytes and thrombocytes that are essential for the various functions in body physiology Okoroiwu, *et al.,* (2014) in disease condition, the cells and roles of the cellular components are altered in both production and function. Blood differentials including neutrophils, eosinophils, lymphocytes, basophils and monocytes provide clues to the kind of infection especially the status of the white blood cells. The effects of malaria often manifest in haematological system Surve*et al.,* (2017) Studies have shown that parasitaemia has the tendency to cause anaemia where it has degenerative impact and suppresses bone marrow Obeagu*et al.,* (2017). Variations in haematological parameters may be influenced by any disease condition such as malaria where they can cause complications probably due to their role in the pathogenesis of malaria Kotepui*et al*., (2017).

Thrombocytopenia or platelet dysfunction is the two most important changes in malarial infection. Thrombocytopenia is seen in 40%-90% of patients infected with *P. falciparum.*maximum thrombocytopenia occurs on the fifth or sixth day of infection, and gradually return to normal within 5-7 days after *parasitaemia* ceased. (FMH and NSP for RBC in Nigeria, (2001), (Ovuakporaye, (2011), Kumar, (2006) found thrombocytopeniaa common occurance in children infected with *Plasmodium falciparum*Parasitaemia. Adedapo*et al.,*(2007) also find thrombocytopenia as one of the haemolytic challenges associated with malaria infection among children. The mechanism of thrombocytopenia in malaria is due to decreased thromobopoiesis despite normal or increased megakaryocytes in bone marrow Abro*et al.,* (2008), peripheral destruction induced by *Plasmodium falciparum* in which immune complexes generated by malarial antigens lead to sequestration of the injured platelets by macrophages in the spleen and consumption by disseminated intravascular coagulation Pavithran, (2007).

Two types of platelets dysfunction are usually seen in malarial initially there is a platelet hyperactivity which is followed by platelet hypoactivity. Platelet hyperactivity result from various aggregating agents like immune complexes, surface contracts of platelet membrane to malaria red blood cells and damage to endothelial cells. The injured platelets undergo lysisintravascularity. The release of platelets contents can activate coagulation cascade and contribute to disseminated intravascular coagulation. Transient platelets hypoactivity is seen following this phase and return to normal in 1 - 2 weeks Pavithran, (2007).

The incidence of disseminated intravascular coagulation (DIC) is reported to be 4-13% Murthy *et al.,* (2000). It usually occurs in patients with *P. falciparum* infection and *hyperration* of coagulation cascade by the release of various sources such as lysis of platelets and red blood cells, *Cytokinsmicro-birculatorystasis*. *P. falciparum* infection was associated with increased plasma levels of *plasmogen* activator inhibitor, factor VIII R: Ag and reduced levels of protein C, protein S, and *antithrombin* III.

II. Study Area

Maiduguri Lies on latitude 11^0 40'N and longitude 13^0 5'E. The state occupies the greater part of the Chad basin and is in the North eastern part of Nigeria, the state share borders with the republic of Niger to the North, Chad to the North east and Cameroon to the East. Within Nigeria, the state shares boundaries with Adamawa state to the south, Gombe state to the west and Yobe state to the North West.

Maiduguri is the Capital of Borno State. It is located in the Sahel Savannah region of northeast Nigeria. The climate of Maiduguri is favorable, with a mean annual rainfall and temperature of about 650 mm and 32^0C respectively. The month of March and April are the hottest periods of the year with temperatures ranging between 30^0C and 40^0C. It is usually cold and dry during the harmattan, November to January being the coldest months. (Borno State Ministry of Information. 2015)

Ethical Clearance

Ethical permission was obtained from the Ethical Committee of the University of Maiduguri Teaching Hospital, to carried out the blood analysis using sysmex*haemotology*auto-analyzer of Immunology laboratory and it was also be obtained from Primary health Care Department, Maiduguri Metropolitan Council. Subject and head of Bulumkutu Comprehensive Health Centre Maiduguri, Borno Statewas educated on the collection of the blood samples and significance of the study.

Inclusion Criteria

All consecutively recruited children aged between 6-59 months visiting the pediatric outpatient department of the Bulumkutu Comprehensive Health Centre, Maiduguri, Borno State with history of febrile

illness and whose parents and guidance consented to their inclusion in this study will be eligible to participate as subjects for this study.

Exclusion Criteria

All children less than 6 months and greater than 59 months and whose parent did not give inform consent were excluded from participating in this study.

Preparation and Examination of Blood Films

Blood samples were obtained from patients by trained laboratory staff on duty. Thick and thin blood films were made by spreading a drop of blood on a clean, grease-free, labelled slide and then allowed to dry. The dried blood films were then stained with 10% Giemsa stain solution and washed after 10 min using clean water. The stained films were allowed to dry and on addition of a drop of immersion oil, each slide was examined under ×100 objective lens for malaria parasites. The examination was conducted according to Cheesbrough (1999), while the densities of positive slides were estimated by the methods described by WHO, (2008)

Thick Blood Film

The drop of well mixed whole blood was placed on clean grease – free slide. Using a glass spreader, it was spread to the size of a small coin. The thickness was made in such a way that the hands of a wrist watch can be seen through the film. It was allowed to air dry free from dust And flies and labeled with patient identity. Cheesbough, (1999)

Thin Blood Film

A drop of blood was placed at the centre near one end of a clean grease free slide. A glass spreader was placed on the slide and drawn back to touch the drop of the blood. When the blood spreads to the edges of the spreader, the spreader was moved forward at an angle of 45^0 without interruption to obtain the thin blood film. It was allowed air dry to free from dust and flies and labeled with patient identify.

Determination of parasite density

The thick film slide was stained for 30 to 45 minutes with 3% Giemsa for the assessment of parasite density. The samples were examined using objectives of a research microscope (x100) asexual parasites were counted alongside with 200 leukocytes. In an even that parasite count was <10 parasites/200 leukocytes; count was continued per 500 leucocytes. The parasite density was expressed as the number of asexual parasites per ml of blood by assuming a mean normal leukocyte count of 8000/µl of blood Gilles and Warrell, (1993) and modified by WHO, (2008). Parasitaemia (per µl) = number of parasites x 8000 / number of leucocytes (200/500).

Blood Analysis

The collected samples was transferred to the laboratory for the estimation of blood parameters such as white blood cells packed cell volume, lymphocytes, monocytes, neutrophil, eosinophil, platelets and by using sysmex hematology Autoanalyser, (2011). The result will be recorded alongside findings of each subject's data.

Statistical Analysis

Data collected were subjected to descriptive statistic using the statistical package for social science SPSS version 20.0 Armand and Jon peck, (2011) and analysis software statistics version 8.0 (Microsoft, 2013) measure of central tendencies (standard deviation percentages) were determined.

III. Results

Results presented in table 1 showed the characteristics of the base line of enrollment in the study population. A total number of 210 children were enrolled for the study52 (24.76%) were male tested negative, 64(30.48%) tested positive and 36 (17.14%) were female tested negative and 58(27.62%) were female tested positive. Mean S.D to estimate variability in the data set was observed, consequently the age of the subject were highly disperse between 6-59 months from the mean SD of 42.0± 55.55 tested positive and 31.0±18.96 tested negative

Table 1: Characteristics Baseline of Enrolment of the participant in Bulumkutu Health Centre, Maiduguri

Variables	Tested positive	Tested negative	Total
No enroll age (month)	88	122	210
Mean	42.00	31.00	73.00
S.D	55.55	18.96	74.51
Range	6-59	6-59	6-59
Gender			
Male	52.0(24.76%)	64.0(30.48%)	116
Female	36.0(17.14%)	58.0(27.62%)	94

Platelate was also negatively correlated with parasite densities among malaria infected subjects age between 5-59 months, as well among males and females subjects with ($r^2 = 0.760$, P = 0.001) ($r^2 = 0.680$, P = 0.005) and ($r^2 = 0.810$, P = 0.001) as shown in figure 1, 2 and 3 respectively.

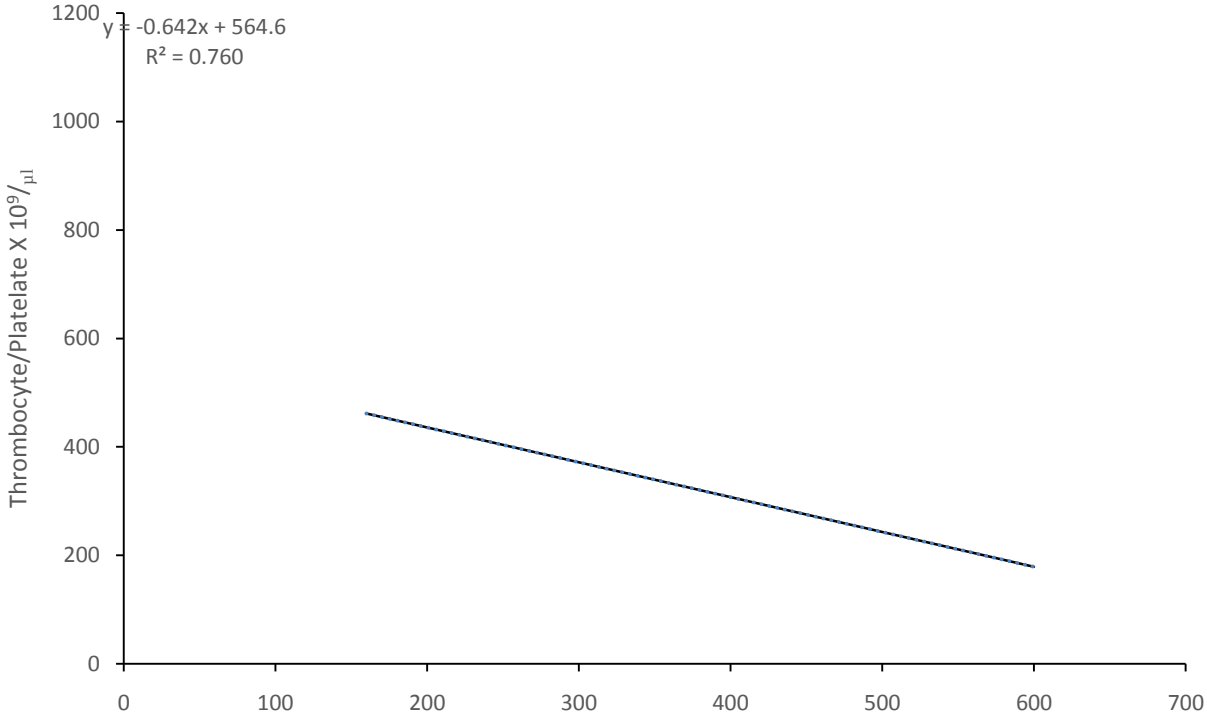

Figure 1: The influence of mean parasite densities (*P. falciparum*) on platelate in children (6-59 months) infected with malaria.

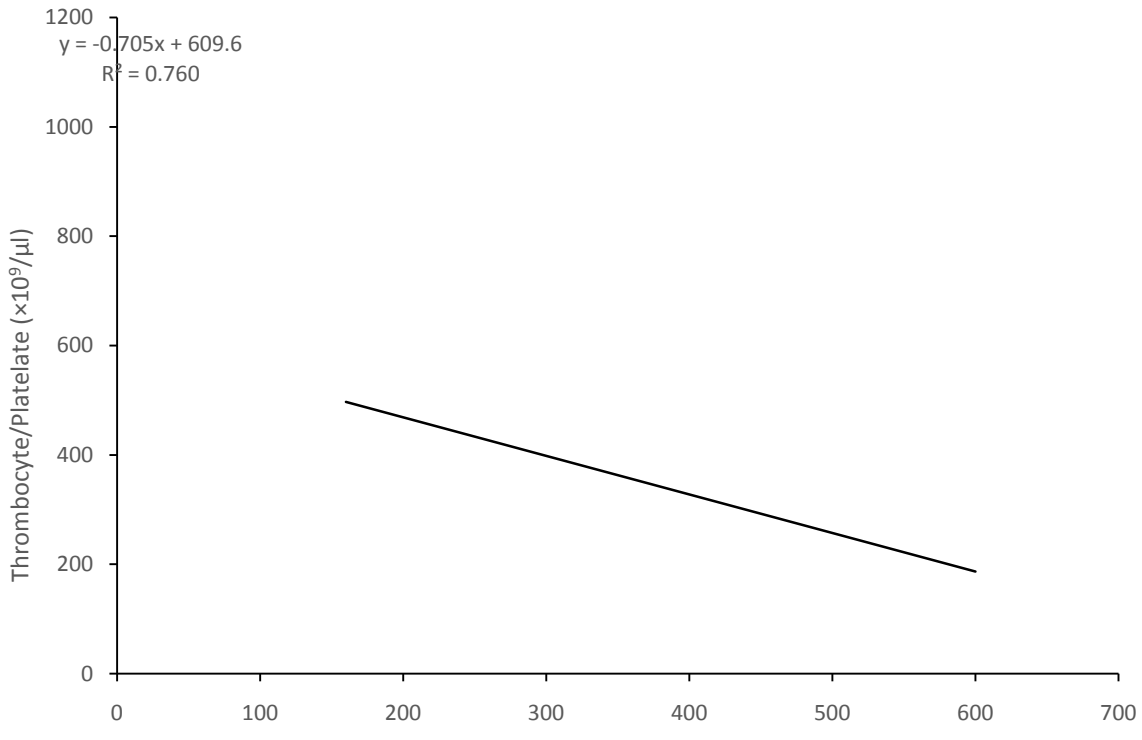

Figure 2: The influence of mean parasite densities (*P. falciparum*) on Platelate in male children (6-59 months) infected with malaria.

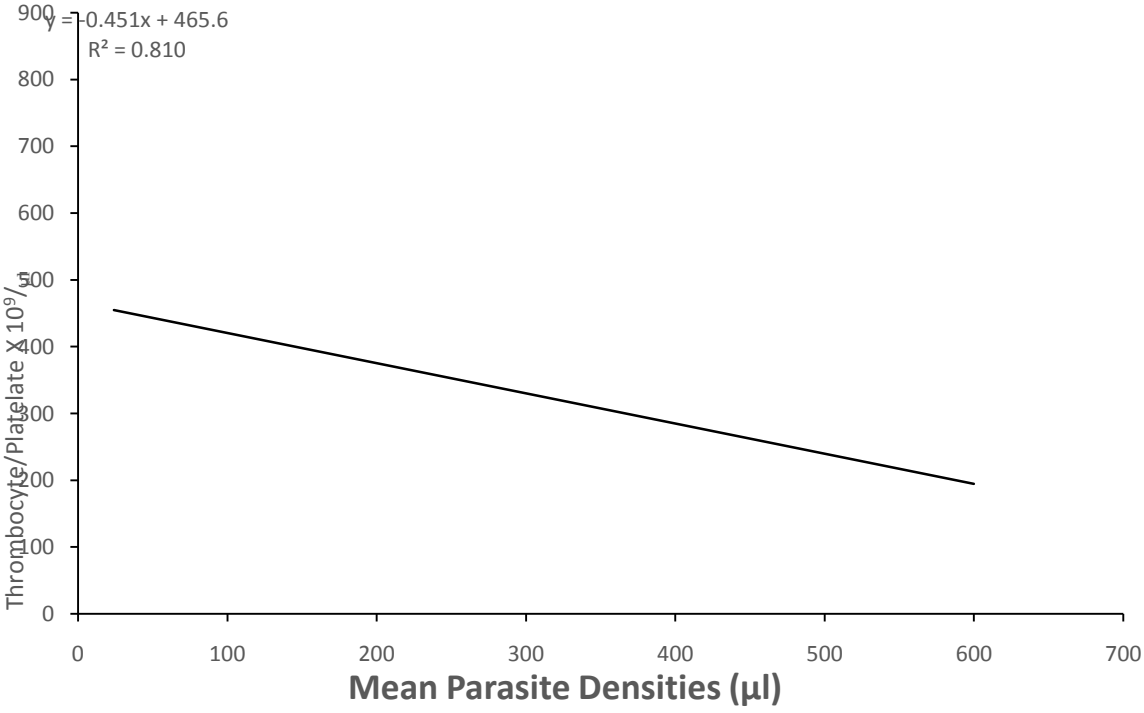

Figure 3: The influence of mean parasite densities (*P. falciparum*) on Platelate in female children (6-59 months) infected with malaria

IV. Discussion:

The study showed parasite densities influenced some hematological parameters in positive malaria in children (6-59months), a case study of Bulumkutu Comprehensive Health Centre Maiduguri, Borno State. During this study it was observed that 210 (41.90%) children aged between 6-59 months visited the pediatric outpatient department were positive for *Plasmodium falciparum* malaria. This finding is concurrent with previous reports from Nigeria by (FMOH, 2005) that obtained 40% annual prevalence rate found in Nigeria. This finding is also concurrent with previous report by Ojukwu, (2002) 50% in North East, North Central, North West and South South regions of Nigeria respectively. But, this study contradicted other finding by Ojukwu, (2002) who in a similar research, in South Eastern part of Nigeria report 17% prevalence rate.

There was a relatively higher prevalence of infection 52 (59.09%) among males than females 36 (40.91%) of female subject (p>0.05%). However reports indicated higher prevalence in males than females WHO, (2005; 2006) with no evidence on higher prevalence to gender susceptibility to malaria infection is not influenced by gender Giles and warell, (1993). The higher prevalence rate among male could just be by chance.

The mechanism of anaemia in parasitized patient is either due to the haemolysis of parasitized red cells, exacerbated removal of parasitized red blood cells bone marrow suppression and decreased enythroprotein level Pavithran, (2007).

The result of the present study showed that platelet (thrombocyte) negatively correlated with parasite densities among malarial positive children with (r^2 = 0.760, P=0.001), males (r^2 0.617, P=0.005) and females (r^2 =0.810, P=0.001) respectively. Similarly with increased age there was a faster parasite clearance, indicated by figure 1, 2 and 3 respectively. This finding is comparable with Memoni*et al.,* (2006), thrombocytopenia is strongly associated with malaria infection.

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