Impact of *Plasmodium Falciparum* Parasitaemia on packed cell volume among Children (6-59 months) attending bulumkutu comprehensive health center maiduguri, Borno State – Nigeria

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ABSTRACT

This study was conducted to assess the influence of Plasmodium falciparum parasitaemia on PCV Packed Cell Volume in (children) (6-59), at Bulumkutu Comprehensive Health Center Maiduguri, Borno State, between August to December, 2019. 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 falciparummalaria and count of malaria parasite density. The result shows that a mean parked cell volume. countswere significantly lower compared to malaria negative individual. A negative and significant orre1a1ion was observed between the parasite densities parked cell volumes index of anaemia of subject. (r^2 0.508, p 0.005) and also in male subject.(r^2 0.680, p 0.005) while the parked cell volume and count female subjects (r2 0.537, p 0.005) respectively.

I. Introduction

Malaria is a major public health problem and cause of suffering and premature death in tropical and subtropical countries Cheesbrough, (1998). In many prevalent areas it is becoming increasingly complicated to control because of the resistance of the parasite to antimalarial drugs and the failure of

vector control actions Cheesbrough, (1998). Malarial parasites belong to the genus *Plasmodium*. *Plasmodium* is the only genus belonging to the family Plasmodiidae, order Haemosporida, class Coccidea, phylum Sporozoa (Apicomplexa). The genus contains over 125 species that cause malaria in mammals, reptiles and birds Hommel and Gilles, (2006). There are four species, which include; P. falciparum, P. vivax (wide spread), P. ovale and P.malariae (less widely spread) Hommel and Gilles, (2006).

In Nigeria, there are over 100 million people at threat of malaria every year and it is projected that about 50 % of the adult population experience at least one episode yearly Igbeneghu and Odaibo, (2013).Malaria causes a lot of debilitating effect in adults and the yearly economic loss due to malaria in Nigeria has been put at 132 billion Naira comprising cost of treatment and transport to source of treatment, loss of man-hours, absenteeism fromplaces of work and other indirect cost Igbeneghu and Odaibo, (2013).

Hematological alterations that are thought to characterize malaria may be related to the overt biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite. Entry of P. falciparuminto erythrocytes usually leads to a marked increase in secretion of inflammatory cytokines (TNFα, IL-1, IL-10, and IFNg), endothelial cell activation (due to overexpression of cell adhesion molecules; ICAM-1, VCAM-1), activation of the coagulation cascade (due to platelet consumption and endothelial damage), and sequestration of parasitized RBCs (Haruna et al., 2013). Certain haematological changes which include low platelet count, haemoglobin concentration and hematocrit have been reported to be associated with malaria (Kumar and Shashirekha, 2006). This study was aimed to assess the effects of malaria parasites on some haematological parameters in Sokoto Nigeria, with the objective to determine the PCV, TWBC and PLC among malaria infected patients and apparently healthy non malaria infected students.

Justification of the Study

Information on malaria at local settlements is scanty and the effects of the diseases on the population are not well documented, for this reason, this study is designed to provide baseline data required for health care planning and administration. In Nigeria, malaria is the most common cause of outpatient to hospitals and this is due to the high number of vector presence and potential of malaria parasite (mosquito) in the country especially during raining season, and it is consistently ranked among the three most important cause of death among children in sub-Saharan Africa (CDC, 2004). Since this study is designed to assess the effect of *Plasmodium falciparum* infection on some blood parameters among children (6-59 months). It has become paramount in the assessment of health care studies which have become inevitable due to it devastating nature.

Anaemia is the most common complication in malaria. The incidence of anaemia in malaria was reported to be as high as 74%. Severe anaemia was observed predominantly observed in *P. falciparum* infection which characterized by hyper *parasitaemia* and systematic complication such as disseminated intravascular coagulation Pavithran, (2007); Kwadwo*et al.*,(2003) reported that *Plasmodium falciparum* malaria infection is a contributory factor to the etiology of the anaemia in children in malaria endemic areas of the world. Abro*et al*., (2008) reported incidence of anaemia of up to 64% and are usually normocytic normachromic type in majority of cases. Menendez *et al*., (2007) also reported anaemia as one of the most common complications in malaria especially in younger children and pregnant women

in high transmission areas. The mechanism of anaemia in *Plasmodium falciparum* parasitized patients is either due to haemolysis of parasitized red cells, exacerbated removal of parasitized red blood cells, bone marrow suppression, decreased erythropoietin level or due to ineffective erythropoiesis Pavithran, (2007). *Plasmodium falciparum* malaria is one of the commonest causes of anaemia and correlates with its infection Erhabor *et al.*, (2006).*Plasmodium falciparum* was found to be the causes of malaria among parasitized subjects 210 (100%). This finding is consistent with a previous report that found *Plasmodium falciparum* as the predominant cause of malaria in Nigeria (Erhabor *et al*, 2006).

The pathogenesis of anaemia in malaria is extremely complex and factorial. It is thought to result from a combination of *haemolysis* of parasite red blood cells and accelerated removal of both parasitized and innocently *non parasitized* red blood cells, depressed as well as ineffective *erythropoiesis* with *dyserythropoietic* changes and anaemia of chronic disease. Other factors contributing to anaemia in malaria include decreased red blood cells deformability, *splenic* pooling and/or phagocytosis resulting in increased rate of clearance from circulation Abro*et al.,* (2008).

II. Methodology

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 carry out the blood analysis using sysmex *haemotology* auto-analyzer of Immunology laboratory and it was also obtained from Primary health Care Department, Maiduguri Metropolitan Council to collectthe blood sample from children attending the general out patient department of Bulumkutu Health Center Maiduguri, Borno State. Subject and head of Bulumkutu Health Center, Maiduguri, Borno State were 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.

Collection of Venous Blood by venipuncture

(I) Label collection tubes and pre-cleaned slides preferably (frosted end) with the patient's name, date and time of collection

(II) Clean the slide with alcohol and allow it to dry

(III) Collect the venous blood in a vacuum tube containing anti-coagulant (preferably EDTA)

(IV) Prepare at least two thick smears and two thin smears as soon as possible after collection (CDC, 2004).

Preparation and Examination of Blood Films

The examination was conducted according to Cheesbrough (1999) in the following procedures: thick film is completely dry a drop of immersion oil was applied onto the film. Oil was spread to cover an area of 10mm in diameter. The preliminary scanning of the blood film was done with x10 and x40 objective to select area of good staining and correct thickness, the slides was viewed using x100 objective for presence of parasitized cells. (up to 100 high power microscope field was examined). 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, labeled 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 a 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. (Cheesbrough, 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 to 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 were transferred to the laboratory for the estimation of blood parameters such as, packed cell volume, by using sysmex hematology Autoanalyser, (2011).The Standard Operating Procedure for Sysmex Hematology Autanalyser (2011).

(I) Switch on the machine and wait until, the visual Display unit (VDU) is in the "ready" mode, this takes about five (5) minutes (SOP, 2011).

(II) Place the sample on a mixer to mix for about five (5) minutes.

(III) Open the well–mixed sample of blood in EDTA and prime it with probe.

(IV) Using the sysmexautoanalyser aspirate the sample to analyze automatically.

(V) Remove from the auto-analyser as bit sound is made.

(VI) The result of blood parameters such as White Blood Cells, Red Blood Cells, Platelets, Packed Cell Volume, lymphocytes counts, neutrophlyles, Haemoglobin and percentage are recorded.

(VII) Any blood sample where haemoglobin concentration of less than 8.0d/dl considered prick value to be reported to the clinic immediately.viii.

(VIII) The result are recorded in result sheets after quality officer is certified. `

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. Differences were considered significant when $P<0.01$ or 0.05.

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 Bulunkutu 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

Packed Cell Volume was found to be negatively correlated with parasite densities among malaria infected subject age 6-59 months (r^2 = 0.508, P = 0.005) and as well as among males infected subjects (r^2 = 0.680, P = 0.005) and females infected subjects (r^2 = 0.537, P = 0.005) as shown in figure 1, 2 and 3 respectively

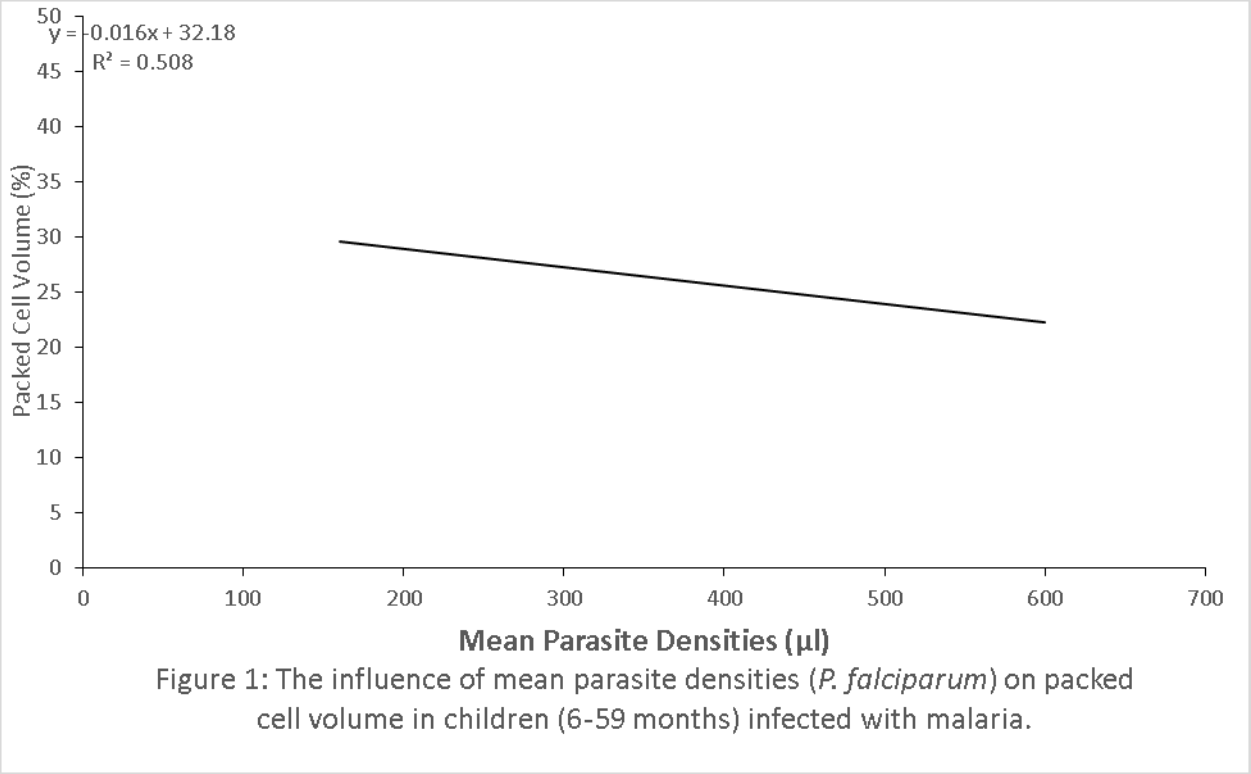

Figure 1: The influence of mean parasite densities (*P. falciparum*) on packed cell volume in children (6-59 months) infected with malaria.

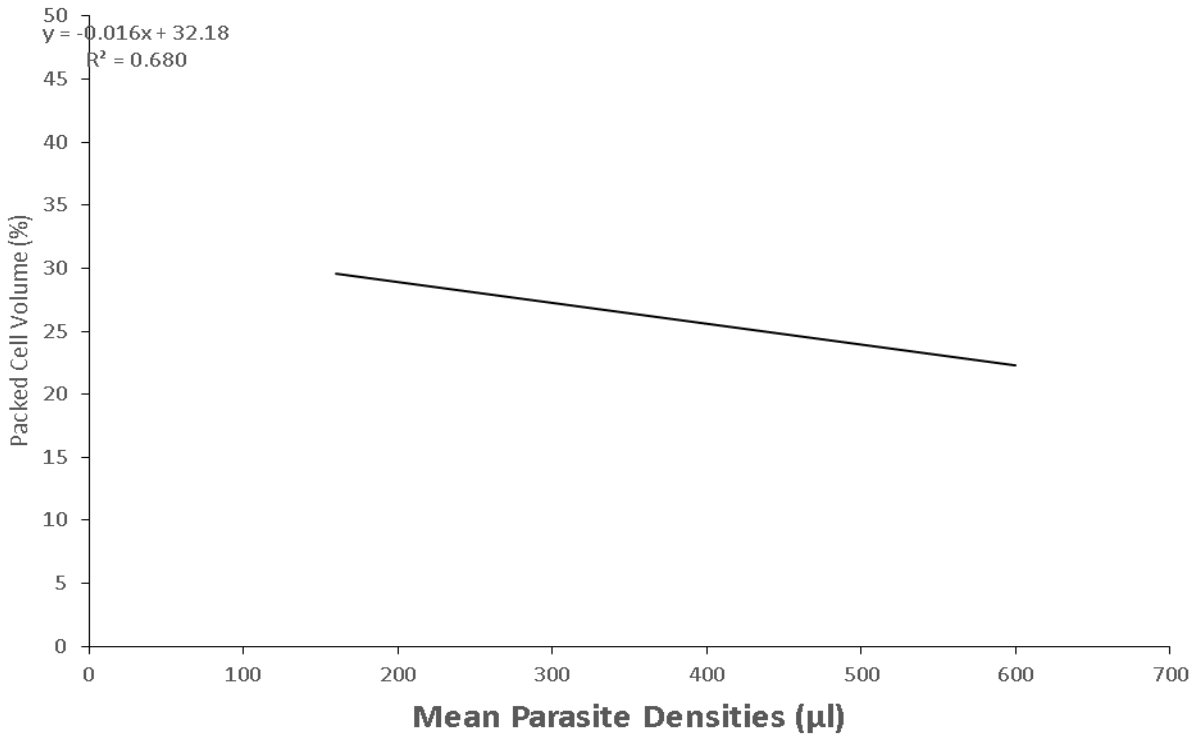

Figure 2: The influence of mean parasite densities (*P. falciparum*) on Packed Cell Volume in male children (6-59 months) infected with malaria.

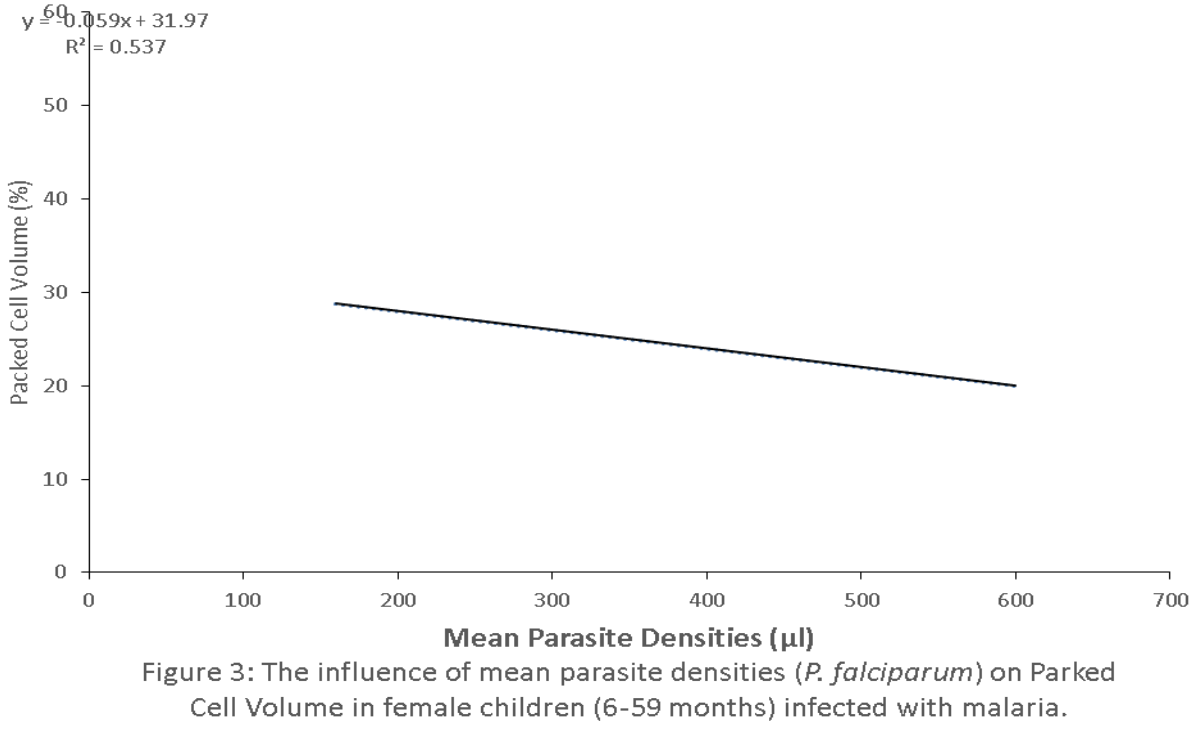

Figure 3: The influence of mean parasite densities (*P. falciparum*) on Parked Cell Volume in female children (6-59 months) infected with malaria.

IV. Discussion

Hematological changes are the most common complications that play a major role in those fatal complications. They include lysis of the red blood cell, leading to anaemiacytoadherence of infected red cells, leucocytes changes coagulopathy, particularly intravascular coagulated (Pavithran, 2007), others are lymphocytosis, leucopenia leucocytosis, neutrophilia and monocytosis have all been reported Abro*et.al.,* (2008).

The study showed parasite densities influenced some hematological parameters in positive malaria in children (6-59months), attending Bulumkutu Comprehensive Health Center 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; WHO, 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).

This study linked *hematological* abnormalities as a hallmark for assessing malaria infection. The abnormalities previously reported include changes in packed cell volume (anemia/pcv<33%), platelets, leucocytes, differential leucocytes counts and disseminated intravascular coagulation (DIC) Reyburn*et al.,* (2007).

V. Conclusion

The presence study indicated that there was a correlation between parasite densities and PCV among children aged 6-59 months with $r^2 = 0.508$, P=0.005) and as well as among males ($r^2 = 0.680$, P= 0.05) and females ($r^2 = 0.537$, P= 0.005) infected subjects as shown in fig. 1, 2 and 3 respectively. This means that as parasite density increases, PCV decreased. This agrees with an earlier investigation by Erhabor*et al.,* (2006) that *Plasmodium falciparum* parasitaemia is one of the commonest causes of anaemia and correlates with the severity of the infection. Furthermore, Kokori*et al.,* (2013) also reported that anaemia has correlated directly with *Plasmodium falciparum* parasitamia with r^2 = (0.9841,P= 0.001).

References:

1. Borno State Information, Federal Republic of Nigeria, National Bureau of Statistics; accessed 28 September, 2015.
2. Center for Diseases Control and Prevention (2004). Biology of malaria, assessed online at www.CDC.gov-malerial biology-index.htm.
3. Cheesbrough, M, (1999). District Laboratory practice in tropical countries. Cambridge University Press.Volume 1:244-251.
4. FMH (2005a) national treatment guideline federal ministry of health publication of the FMH Nigeria P. 44.
5. Gilles H.M, warrell D.A (1993). In Bruce- chwatt's essential malariology, 3rd Ed. Edward Arnold pp. 19- 24.
6. Haruna, M. Sharif, K. Larry, F.S. Abdul, S. and Christine N. (2013). How Reliable Are Hematological Parameters in Predicting Uncomplicated Plasmodium falciparum Malaria in an Endemic Region?Hindawi Publishing Corporation, *ISRN Tropical Medicine*, Volume 2013, Article ID 673798, 9 pages, http://dx.doi.org/10.1155/2013/673798
7. Hommel, M. and Gilles, H.M. (2006).Topley and Wilson's microbiology and microbial infections: Malaria. 4th ed. Edward Amold (Publishers) Ltd.
8. Igbeneghu, C. and Odaibo,A.B.(2013**).**Impact of Acute Malaria on Some Haematological Parameters in A Semi-Urban Community in Southwestern Nigeria.ActaParasitologicaGlobalis 4 (1)**.**
9. Kumar A.S (2006), thrombocytopenia an indicial for of acute malaria. *Indian pattol microbial* 2006; 49(4): 505-508
10. Kwadwo A, Koramt Seth Owusn – Agyci, DavidJ, fryauf T, Abraham Hogson and Francis K, Nkrumah (2003). Seasonal profile of malaria infection anaemia and
11. Menendez C, Fleming A.F, and Alonso Pl: Malaria related anaemia. *Parasitol. Today* 2000; 16: 469-476
12. Ojukwu, J.U., (2002). Patter and outcome of Paediatric malaria admissions in Abakaliki, Nigeria *Medical Journal* (1), 1720.
13. Pavithran, K, (2007). Haematological changes in Malaria. Clinical pharmacology 1:1-3.
14. Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, Drakeley C, Whitty C.J (2007). Rapid diagnostic tests compared with malaria microslopy for guiding outpatient treatment of febrile illness in Tanzania randomized trial BMJ. 2007; 334: 403.Doi 10.1136/bmj. 39073. 496829. AE
15. World Health Organization (2006) Indoor Residual Spraying: Use of Indoor Residual Spraying for Scaling Up Global Malaria Control and Elimination.
16. World Health Organization (2003) "Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated *falciparum* Malaria" Geneva,
17. World Health Organization (2008) "WHO Guidelines for the Treatment of Malaria. Geneva, Switzerland": Technical document, WHO/HTM/MAL/2006.1108