MPRI-JMBP | INTERNATIONAL JOURNAL OF MEDICAL, BIOLOGICAL AND PHAR. SCI.
VOL. 13 NO.3 MAR-2021 ISSN: 1999-5650

AGRANULOCYTIC RESPONSES TO PARASITAEMIA OF *PLASMODIUM FALCIPARUM* SPECIES IN CHILDREN (6-59 MONTHS) ATTENDING BULUMKUTU COMPREHENSIVE HEALTH CENTRE, MAIDUGURI, BORNO STATE – NIGERIA

YAHAYA, INUWA[1], MOHAMMED ISA TADA[2], YUSUF SHETTIMA[3], MUSA ALI MUHAMAD[4]

[1]Department of Biological Sciences, Faculty of Science, University of Maiduguri, Maiduguri Nigeria. (Department of Biological Sciences, Faculty of Science, Federal University of Kashere, Gombe State, Nigeria).
[2]Department of Biological Sciences, Faculty of Science, University of Maiduguri, Maiduguri Nigeria. [3]Department of Biological Sciences, Faculty of Science, University of Maiduguri, Maiduguri Nigeria. [4]Department of Biological Sciences, Faculty of Science, University of Maiduguri, Borno State, Nigeria.

Abstract

This study was conducted to assess the influence of *Plasmodium falciparum* parasitaemia on some selected hemalogical parameters in children (6-59months), at Bulumkutu Health Centre, Maiduguri, Bono State, between November 2015 to February 2016. A total of 210 children were enrolled in the study which consisted of 88 (41.90%) patients with positive *P. falciparum* malaria and 122 (58.10%) negative malaria. Hematological parameters were analyzed using sysmex haematology 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 agranulocytes

KEYWORDS:
Responses, Falciparum Species, Agranulocytic, Comprehensive, Health Centre, Parasitaemia

(lymphocytes, and monocytes) were also found to be positively correlated with mean parasite densities among the malaria positive children (6-59 months) (r^2 = 0.521, p = 0.005), (r^2 = 0.520, p = 0.005) as well as among females infected subjects (r^2 = 0.539, p = 0.005), (r^2 = 0.607, p = 0.005) and male infected 'lymphocytes (r2 0.623, p 0.005), but a non significant and negative correlation was observed between parasite densities and monocytes of male positive children (r^2 = 0.410, p = 0.006) respectively.

INTRODUCTION

Plasmodium faciparum is a unicellular protozoan parasite of human, and the deadliest species of Plasmodium that cause malaria in humans. Rich *et al.,* (2009). It is transmitted through the bite of a female *Anopheles* mosquito. It is responsible for roughly 50% of all malaria cases.

Robert and Janovy, (2005) It causes the disease's most dangerous form called *falciparum* malaria Perkins *et al.,* (2011). It is therefore regarded as the deadliest parasite in human s, causing 435,000 deaths in 2007 World Malaria Report (2011).

The potential mechanisms explaining the decrease in circulating lymphocyte depletion are activated by *Plasmodium* infection (El-hassan *et.al.,* 1994; Langborne and Simon-Haarhaus, 1991). The decrease in lymphocyte counts associated with malaria parasitaemia may be due to reflecting redistribution of lumphocytes sequestration in the spleen (Wickramasinghe*et.al.,* 2000). Similar findings were shown in a study conducted in Burkina Faso in which (Sanou *et.al.,* 2012) demonstrated that individuals in the Fulani ethic group, who are naturally less frequently infected by malaria parasites and with low parasitaemia compared to the individuals in the mossi ethic group, which had higher absolute lymphocyte count than Fulani ethic group in both malaria transmission seasons. But, Abdalla and Pasvol, 2004 that the lymphocyte count remain normal during an acute malaria infection.

The potential mechanisms explaining the decrease in circulating lymphocyte depletion are activated by *Plasmodium* infection (El-hassan

et.al., 1994; Langborne and Simon-Haarhaus, 1991). The decrease in lymphocyte counts associated with malaria parasitaemia may be due to reflecting redistribution of lumphocytes sequestration in the spleen (Wickramasinghe *et.al.,* 2000).

The incidence of Disseminated Intravascular Coagulation (DIC) is reported to be 4-12% (Murthy *et.al.,* 2000). Monocyte make up of about 1-9% of the white blood cells, (WHO 1996). It usually occur in patients with *Plasmodium falciparum* infection and hyperparasitaemia. In severe complicated malaria, there is activation of coagulation cascade by the release of various procoagulants. Procoagulants are derived from the various sources such as lysis of patients, red blood cells, release of tissues factors from monocytes and damaged endothelial cell, cytokines and micro-circulatory stasis. *Plasmodium falciparum* infection was associated with increased plasma levels of plasmogen activator inhibitor, factor VIII R:Ag and antithrombin III. The monocyte procoagulants activity was also found to be high in *Plasmodium falciparum* infection (Murthy *et.al.,* 2000), (Pavithran, 2007). Monocyte was positively associated with parasitaemia and negatively with age. The high monocyte count had been reported in patient with an uncomplicated malaria (Abdalla *et.al.,* 1988). Abdalla and Pasvol, 2004 reported monoctosis as one of the most consistent observations reported from prior studies done on hematological studies that characterized malaria.

In innate immunity, monocyte macrophages are the main immune effectors for controlling malaria blood stage infection via phagocytiv activity (Seghides *et.al.,* 2003). Activation through pattern recognition receptors (PRRs) present on monocytes, dendritic cells and neutrophils indece release pro inflammatory cytokines and chemokines the development of acquired immunity (Kolli *et.al.,* 2013). During acute infection monocytes produce high level of IL-IB,OL-112 and TNF-α (Fell and Smith, 1998), where as malaria pigment, hemozoin (Hz), production by monocytes contributing to dendritic cell maturation (Jaramillo *et.al.,* 2004). In humans, an impaired function on the maturation of monocytes and DCs due to malaria has been

indicated by the reduced numbers of blood DCs (Pichyangkul *et.al.,* 2004) inducing pregnan women (Diallo *et.al.,* 2008).

STUDY AREA

Maiduguri Lies on latitude 11° 40'N and longitude 13° 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°C respectively. The month of March and April are the hottest periods of the year with temperatures ranging between 30°C and 40°C. 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 Umaru Shehu Ultra Modern Hospital Bulumkutu, 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.

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 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. (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° 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).

RESULT

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

INTERNATIONAL JOURNAL – MBP
VOL. 13 NO.3 MAR-2021 ISSN: 1999-5650

The agranulocyte (Lymphocytes, Monocytes) were found to be positively correlated with mean parasite densities among the malaria positive children 6-59 months (r^2 = 0521, P = 0.005), (r^2 = 0.520, P = 0.005) and as well as among lymphocyte of male positive children (r^2 = 0.6231 P = 0.005) and females infected subjects (r^2 = 0539, P = 0.005), (r^2 = 0.607, P = 0.005) as indicated in figure 1, 2, 3, 4, 5, and 6 respectively. However, a non significant and negative correlation was observed between parasite density and monocyte of male positive children (r^2 = 0.410) as shown in figure 3.

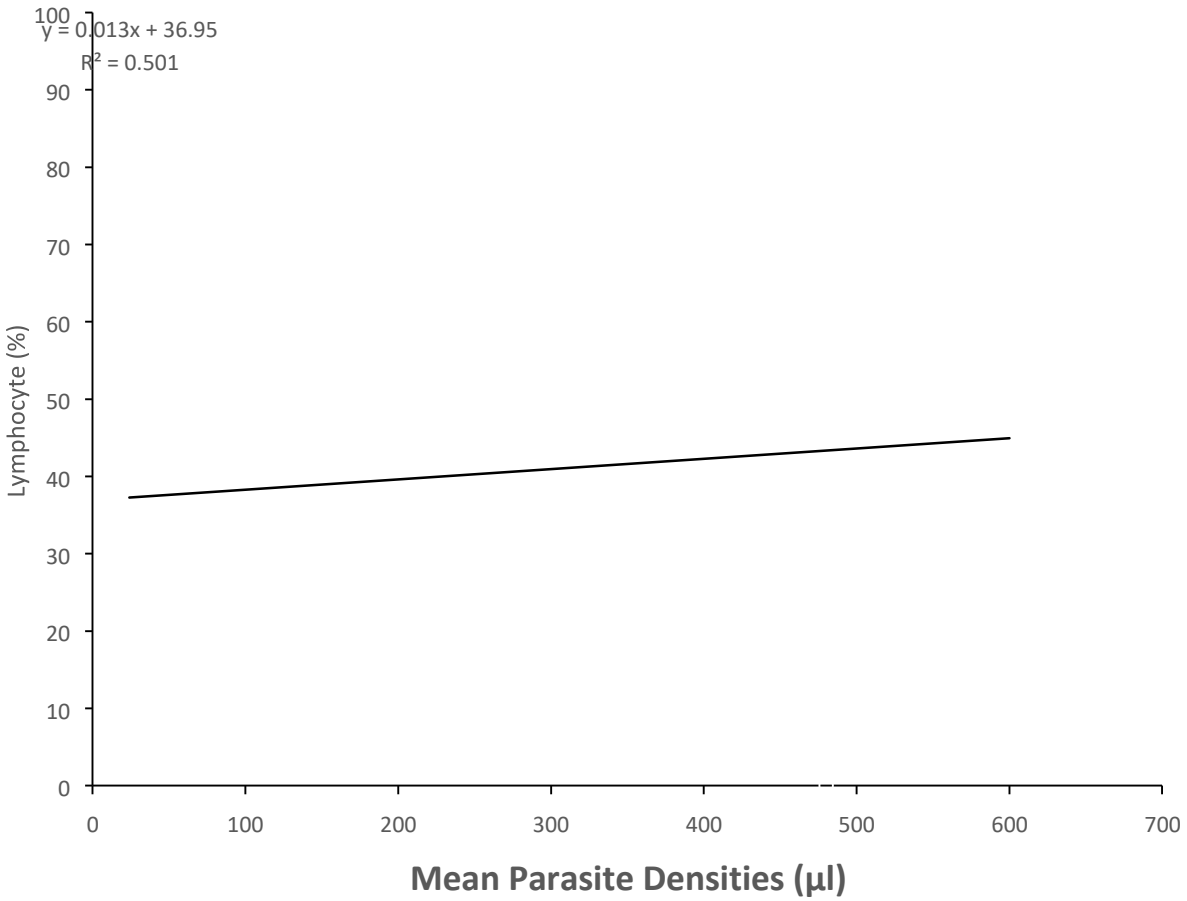

Figure 1: The influence of mean parasite densities (*P. falciparum*) on Lymphocyte in children (6-59 months) infected with malaria.

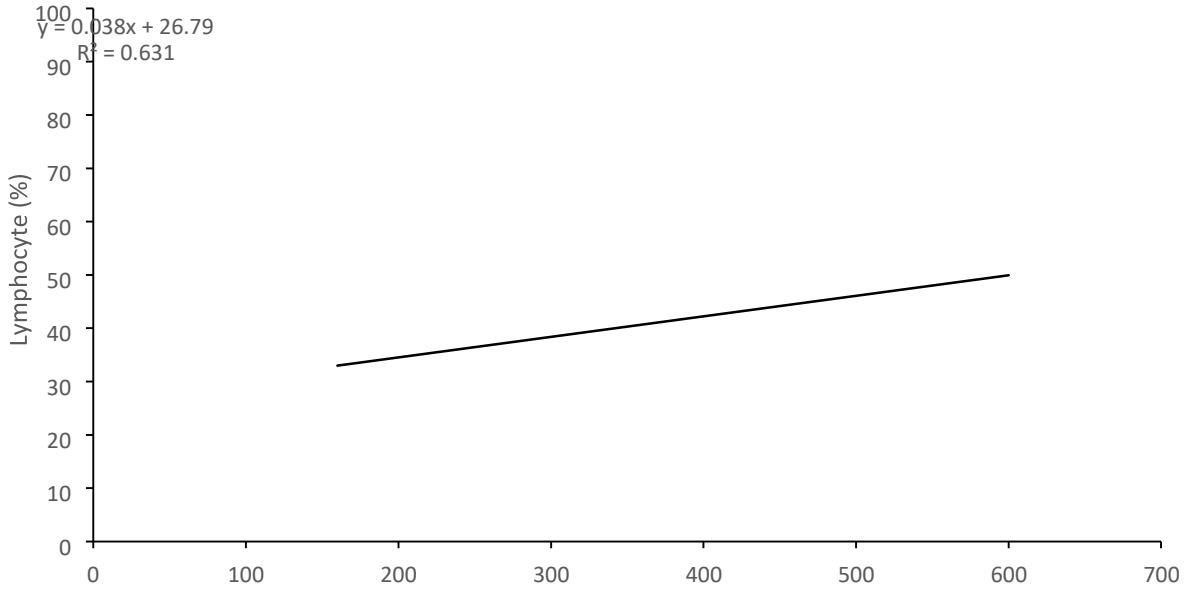

Figure 2: The influence of mean parasite densities (*P. falciparum*) on Lymphocyte in male children (6 -59 months) infected with malaria.

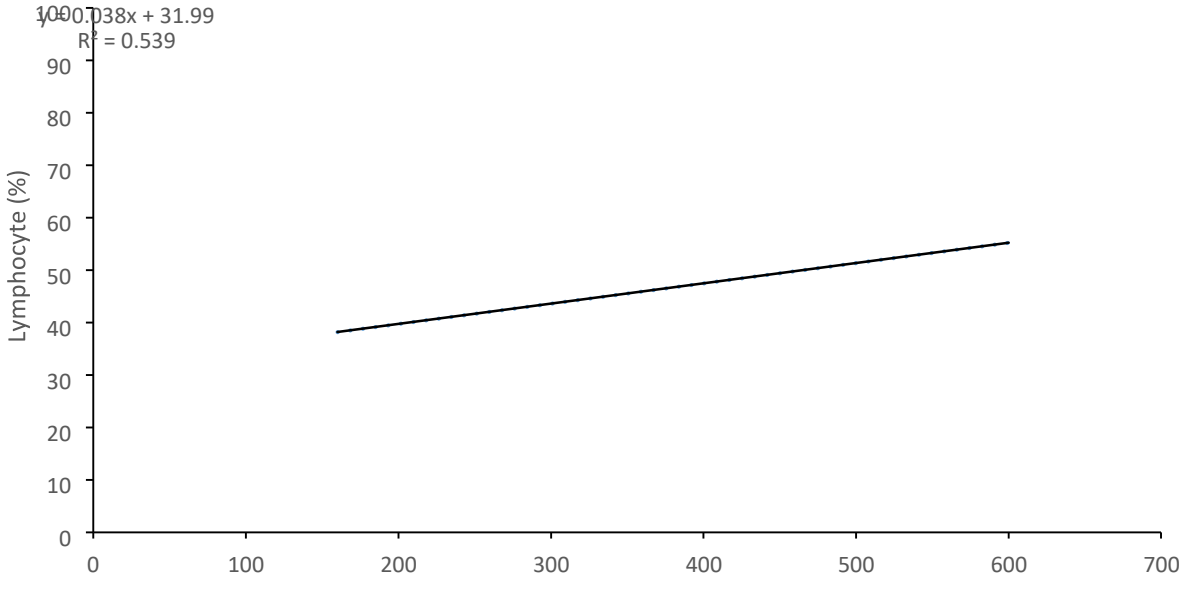

Figure 3: The influence of mean parasite densities (*P. falciparum*) on Lymphocyte in female children (6 -59 months) infected with malaria.

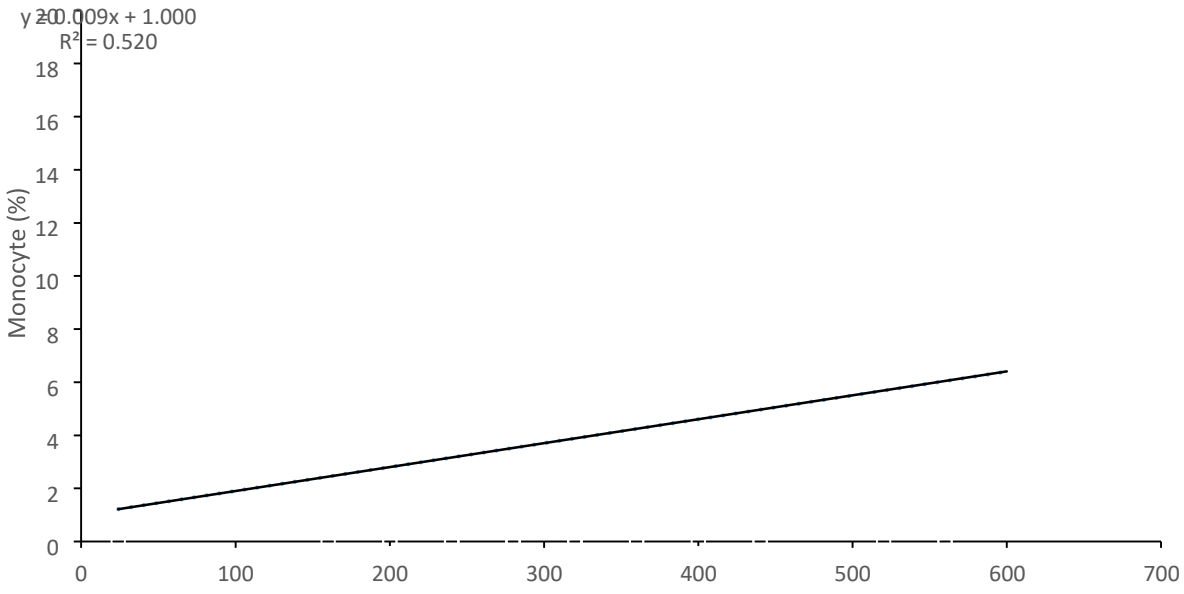

Figure 4: The influence of mean parasite densities (*P. falciparum*) on Monocyte in children (6-59 months) infected with malaria.

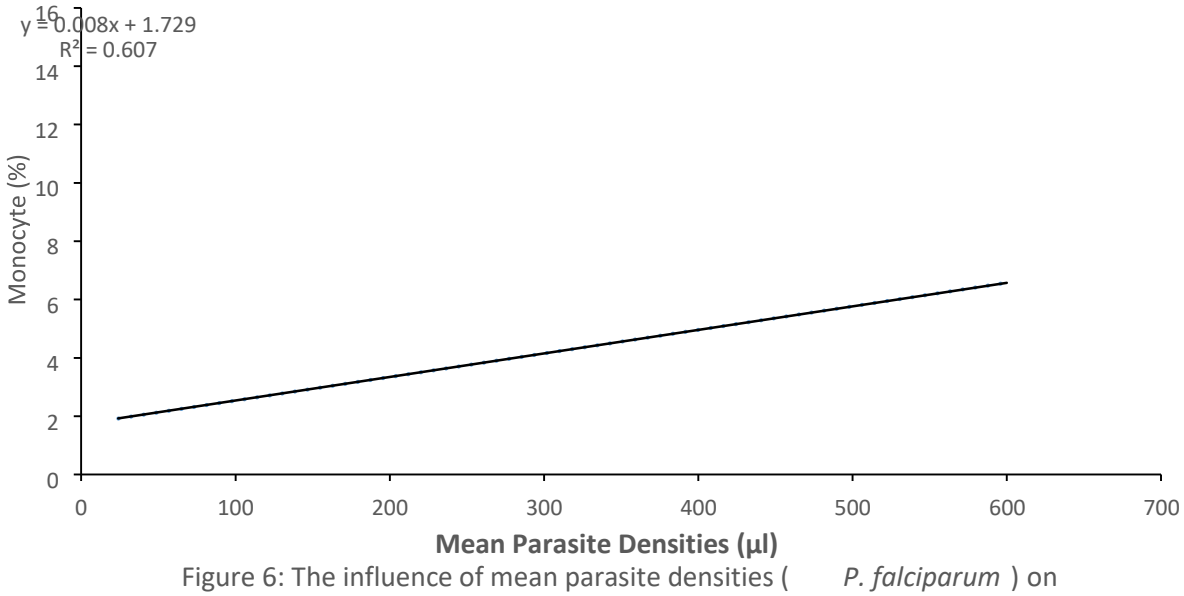

Figure 6: The influence of mean parasite densities (*P. falciparum*) on Monocyte in female children (6 -59 months) infected with malaria.

DISCUSSION

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).

The study showed parasite densities influenced some hematological parameters in positive malaria in children (6-59months). A case study of Umaru Shehu Ultra Modern Hospital Bulumkutu, Maiduguri Borno. 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.

In the present study lymphocyte was positively correlated with parasitaemia as indicated in fig. 1 , 2 and 3 in positive subject, (r^2 = 0.501, P = 00.05) males (r^2 = 0.631, P = 0.005) and females (r^2 = 0.539, P = 00.05) respectively. This finding contradicted a previous study of Erhart, (2004) who reported that the relative lymphocyte was found to be negatively associated with malaria parasitaemia. This study also indicated that there is a positive association between parasite density and monocyte among malarial infected subjects ((r^2 = 0.520, P = 0.005) and females (r^2 = 0.607, P = 0.005) as indicated in figure 4, 5 and 6 respectively. This finding agrees

with previous report stated that monocyte was positively associated with parasitaemia Murthy *et.al.,* (2010) Pavithran, (2007). However, the observation of decreased monocyte counts among males (r^2 = 0.410, P = 0.006) in this study was contrast with previous studies (Maina *et al.,* 2010), (Abdalla, 1988).

REFERENCE

Abdalla S.H (1988) Peripheral blood and bone marrow lencocytes in Gambia children with malaria: numerical changes and evaluation of phagocytosis. Ann trop predator. 1988; 8: 250- 258.

Abdalla S.H., Pasvol G. (2004) Malaria: A Hematological Perspective *Imperal College Press; London, UK:* 2004.

Borno State Information, Federal Republic of Nigeria, National Bureau of Statistics; accessed 28 September, 2015.

Cheesbrough, M, (1999). District Laboratory practice in tropical countries. Cambridge University Press. Volume 1:244-251.

Diallo M, Alderbert D, Moreau J.C., (2008). "Decreases of lymphoid dendrifits cells in blood from malaria infected pregnant women *Int. J parasitol* 2008; 28:1557-65.

Elhassan I.M.., Hviid L, Satti G, Akerstrom B, Jakobsen P.H., Jensen J.B., Theander T.G., (1994). Evidence of endothelial inflammation, T cell activation and T cell reallocation in uncomplicated *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene*. 1994; 51:372-379.

Fell A.H., Smith., (1998). Immunity to Asexual Blood Stages of *Plasmodium*: is Resistance to Acute Malaria adaptive or Innate? *Parasitol Today* 1998; 14: 364-9.

Jaramillo M, Plante I, Ouellet N., (2004). Hemozoin-Inducible Preinfalmmatory Events in *vivo*: Potential Role in Malaria Infection. *J Immunol* 2004; 172:3101-10.

Kolli D, Velayutham T.S., Casola A, (2013). "Host-Viral interactions: role of pattern I recognition receptors (PRRs) in human pneumo virus infections. *Patogens* 2013;2:2.

Murthy, G.L., Sahey, R.K., & Srinivasan, V.R., (2000) clinical profile of *falciparum* malaria in a tertiary care hospital. *Journal of Indian Medical Association* 98: 160-169.

Ojukwu, J.U., (2002). Patter and outcome of Paediatric malaria admissions in Abakaliki, Nigeria Ebonyi *Medical Journal j* (1), 1720.

Pavithran, K, (2007). Hematological changes in Malaria. Clinical pharmacology 1:1-3.

Perkins, D. J. Were, T.; Davenport, D. C.; Kempaiah, P; Hittner, J. B.; J. B.; Ong'Echa, J. M.

(2011). "Severe malarial anemia: Innate immunity and pathogenesis" International Journal of Biological Sciences. **7** (9) 1427-1442

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.

Rich, S. M.; Leendertz, F. H.; Xu, G.; Lebreton, M.; Djoko, C. F.; Aminake, M. N., Takang, E. E.; Diffo, J. L. D. Pike, B. L; Rosenthal, B. M.; Fomently, Boesch, C., Ayala F. J.; Wolfe, N.,

D. (2009) "The Origin of malignant malaria" Proceeding of the National Academy of Sciences. **106** (35): 14902-14907.

Roberts & Janovy 2005.

Sanou G.S., Tiendrebeogo R.W., Ouedraoso A.L., Diara A, Ouedraogo A. Yaro J.B., Ouedrago E, Verra F, Behr C, Traore Y, Sirima S.B., Nebie I, (2012). "Hematological parameter natural regulatory C44+ CD25+ FOXP3 T cells and gamma delta T Cells among two sympatric ethnic groups having different susceptibility to malaria in Burkina Faso. BMC Res Notes 5:76.

Serghides L; Smith T.G., Patel S.N., Kain K.C., (2003)." CD36 and Malaria: Friends or foes? *Trends Parasitol* 2003; 50:143-5.

Wickramasinghe SN, Abdalla SH. Bailliere'sClinHematol.Vol. 13.Harcourt Pub Ltd; 2000.Blood and bone marrow changes in malaria; pp. 277–299. [PubMed]

World Health Organization, (1996). World Health Director, General's report. Fighting Disease, Fostering Development: Geneva: WHO; (1996)

INTERNATIONAL JOURNAL – MBP
VOL. 13 NO.3 MAR-2021 ISSN: 1999-5650

World Health Organization, (2008) "WHO Guidelines for the Treatment of Malaria. Geneva,
Switzerland": Technical document, WHO/HTM/MAL/2006.1108,
World Health Organization, (2008). *World Malaria Report* W.H.O – Global Malaria Programme, Geneva.
World Malaria Report 2018, WHO Retrieved 2nd December, 2018.