Granunolocytic responses to Parasitaemia of *Plasmodium Falciparum* Species in children (6-59 months) attending bulumktu 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 eosinophil and neutrophil in children (6-59months), at BulumkutuComprehensive Health Centre, Maiduguri, Bono State, between August to December 2018. 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 sysmexhaematology auto-analyzer (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. This study indicated that there was a positive correlation between parasite densities and granulocytes (neutrophil and eosinophil) of the malaria-infected subjects, as well as malaria infected males and females subjects respectively. (r^2 = 0.63 5, p = 0.005), (r^2 = 0.5 10, p = 0.005), (r^2 = 0.602, p = 0.005), (r^2 = 0.504, p 0.005), and (r^2 = 0.890, p = 0.001), (r^2 = 0.623, p = 0.005).

Key words: *Granunolocytic, parasitaemia, plasmodium falciparum, children, Maiduguri*

I. Introduction

The most major parasite species accounting for about 98% of malaria cases in the country is *Plasmodium falciparum*. *P. malariae* usually occurs is the main vector of malaria in Nigeria, but species *Anopheles funestus* 0.5% and *Anopheles arabiansis* 2.0% are also commonly encountered. *Anopheles malariae* is found in coastal areas. (NATP, 2011). In brutal cases of surviving, children can be left with seizures, speech disorders, or partial paralysis. persistent boats of fever drain a child's capacity to learn (Breman, 2001; WHO, 2005).

In Nigeria, Malaria is endemic all over the country with up to 90% of the population living in areas with constant malaria and a National Prevalence of 2.0%. Malaria single-handedly accounts for 60% of outpatient visits in Hospitals and 15-31% of admission (Federal Ministry of Health, National Malaria Control Programme, 2005).

WHO (2005) and WHO (2006) had reported a higher commonness of malaria in males than females. Gills and Warell (1993), reported that there is no scientific evidence of higher prevalence being related to gender as susceptibility to malaria infection is not influenced by gender, but the higher prevalence rate among male could just be by chance.

Haemalologic changes which are the most common ramifications in Malaria play a major role in the fatal complications. They include anemia, cytoadherence of infected red blood cells, leucocyte changes, thrombocytopenia, and coagulopathy (Pavithran, 2007). Changes in leucocyte proliferation and function are seen with severe *Plasmodium* infection. Leucocyte proliferation is associated with the release of cytokines that are involved in cytoadherence, thrombocytopenia, disseminated intravascular coagulation hypoglacemia, and lactic acidosis (Pavithran, 2007).

II. Literature review

Granulocytic Changes in *Plasmodium* Infections

Eosinophils may contributeto the protection against malaria (*Plasmodium falciparum*) by induction of parasite killing Walter, et al, (1987) but they may also contribute to pathology by the release of granule proteins such as eosinoiphil cationic protein (EC) as eosinophil protein x leasinophil-derived neurotoxin (EPX) (Duracket al., 1981). Acute malaria in children is associated with decreased numbers of easinophils in peripheral blood, it also induces eosinophil production. Eosinophils might be stimulated either directly by the parasites or other mediators produced during the malaria attack (Walters et al., 1987)

The mechanism of neutropenia in malaria has been postulated to involve increased margination and sequestration of neutrophils (Dole and Wolff, 1973) as a result of the increased expression of cell adhesion molecules (ICAM-I and VCAM-I) that occurs in malaria (Clark, et. al., 2006).

Patients suffering from malaria present with several hematological changes of which anemia is a major complication especially in those infected with *P. falciparum*. (Weatherayet al., 1982). Other changes include *easinophil* concentration on *neutrophils* and lymphocyte, thrombocytes, and neutrophilic leukocytosis (Pukrittayakarneet al., 1989). *Falciparum* malaria also causes accelerated turnover of the coagulation cascade. Ina terrible disease, there is increased fibrinogen consumption, but in many cases, plasma concentrations are either normal or elevated (Pukrittayakarneeet al., 1989).

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 Northeastern part of Nigeria, the state share borders with the Republic of Niger to the North, Chad to the Northeast, and Cameroon to the East. Maiduguri is the capital of Borno State, 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).

III. 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, granulocytes,(nutrophil and eosinophil) 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.

IV. Results

Results presented in the table showed the characteristics of the baseline 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 was highly disperse between 6-59 months from the mean SD of 42.0± 55.55 tested positive and 31.0±18.96 tested negative.

This study indicated that there was a positive association between parasite densities and granulocytes (Neutrophil and Eosinophil) among malarial infected subjects and as well as males and females, infected subjects (r^2 0.635, P = 0.005), (r^2 = 0.510, P = 0.005), (r^2 = 0.602, P = 0.005), (r^2 = 0.504, P = 0.005 and (r^2 = 0.890, P = 0.001), (r^2 0.623, P = 0.005) as indicated in figure 1, 2, 3, 4, 5, and 6 respectively.

Table: Characteristics baseline of enrolment of the participant in Bulumkutu health center, 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

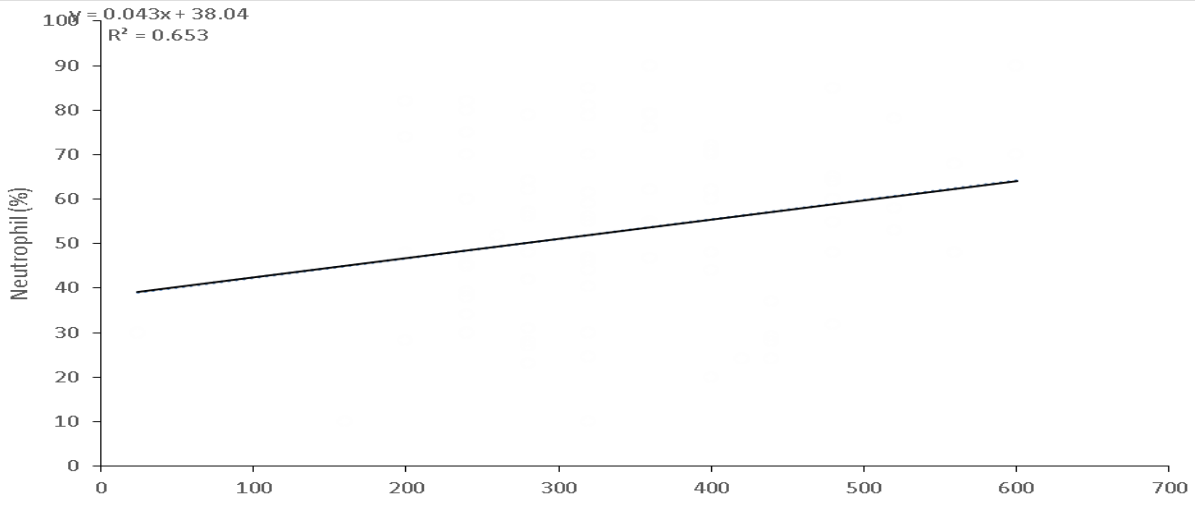

Figure 1: The influence of mean parasite densities (*P. falciparum*) on Neutrophii in children (6-59 months) infected with malaria.

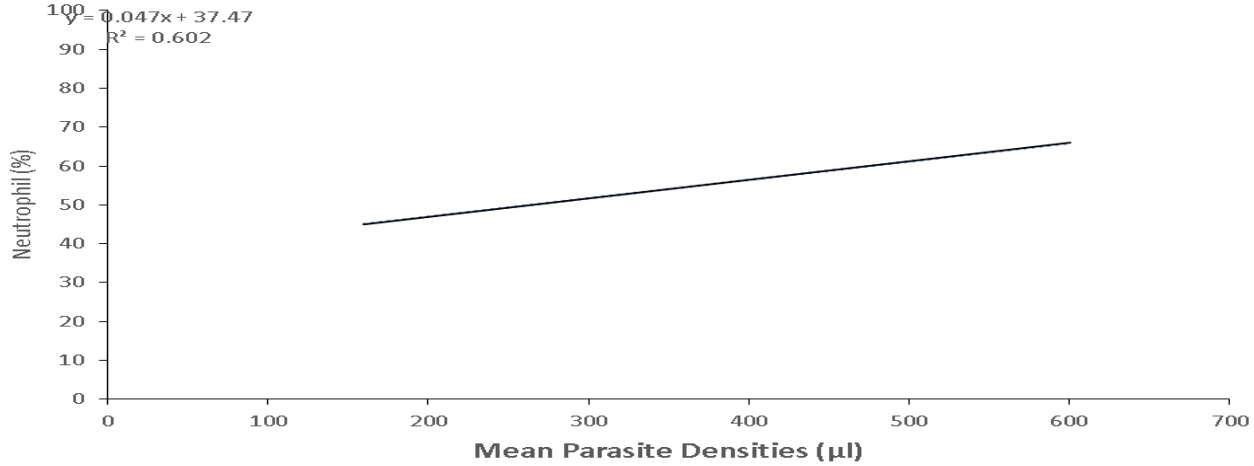

Figure 2: The influence of mean parasite densities (*P. falciparum*) on Neutrophil in male children (6-59 months) infected with malaria.

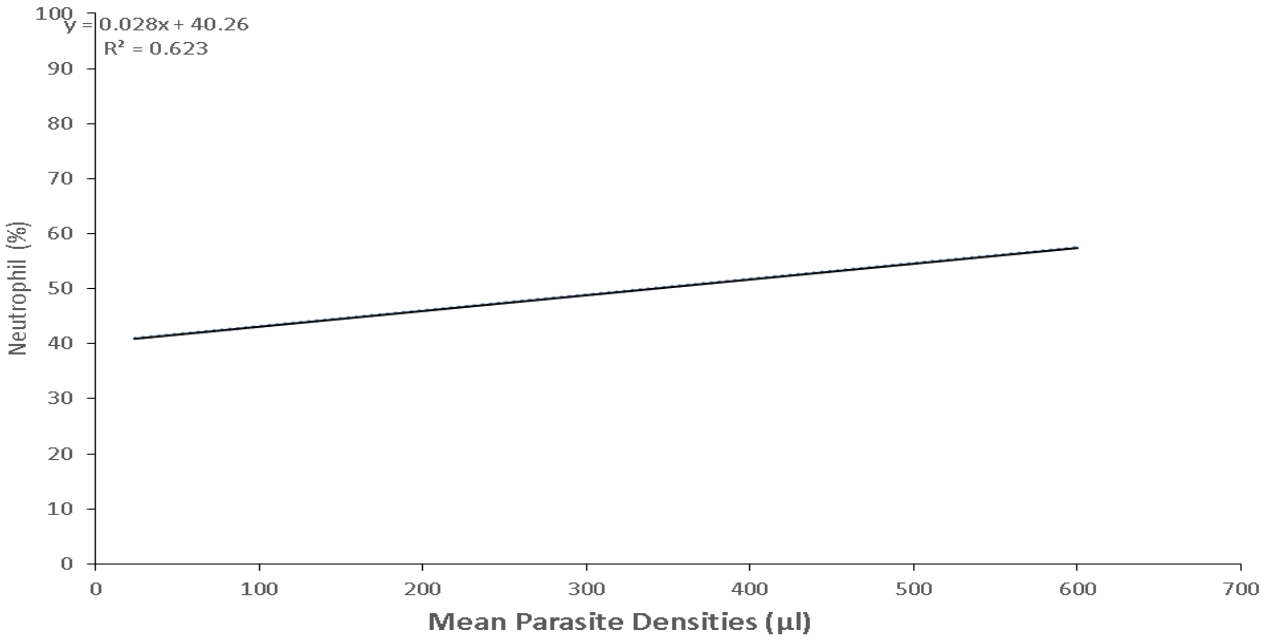

Figure 3: The influence of mean parasite densities (*P. falciparum*) on Neutrophil in female children (6-59 months) infected with malaria.

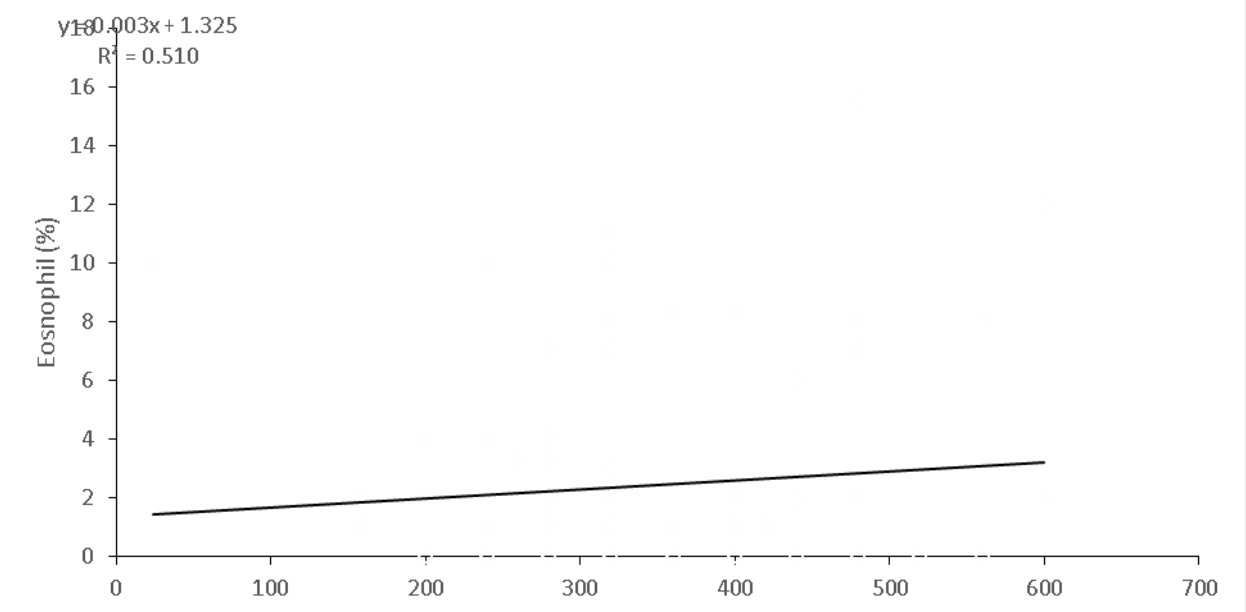

Figure 4: The influence of mean parasite densities (*P. falciparum*) on Eosinophil in children (6-59 months) infected with malaria.

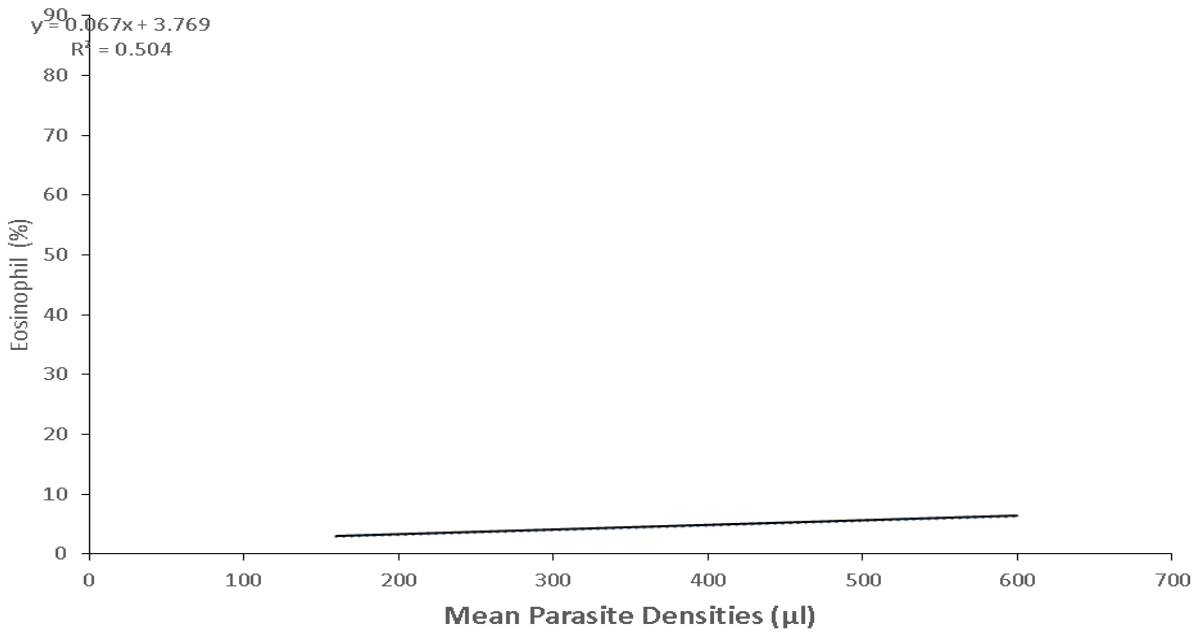

Figure 5: The influence of mean parasite densities (*P. falciparum*) on Eosinophil in male children (6-59 months) infected with malaria.

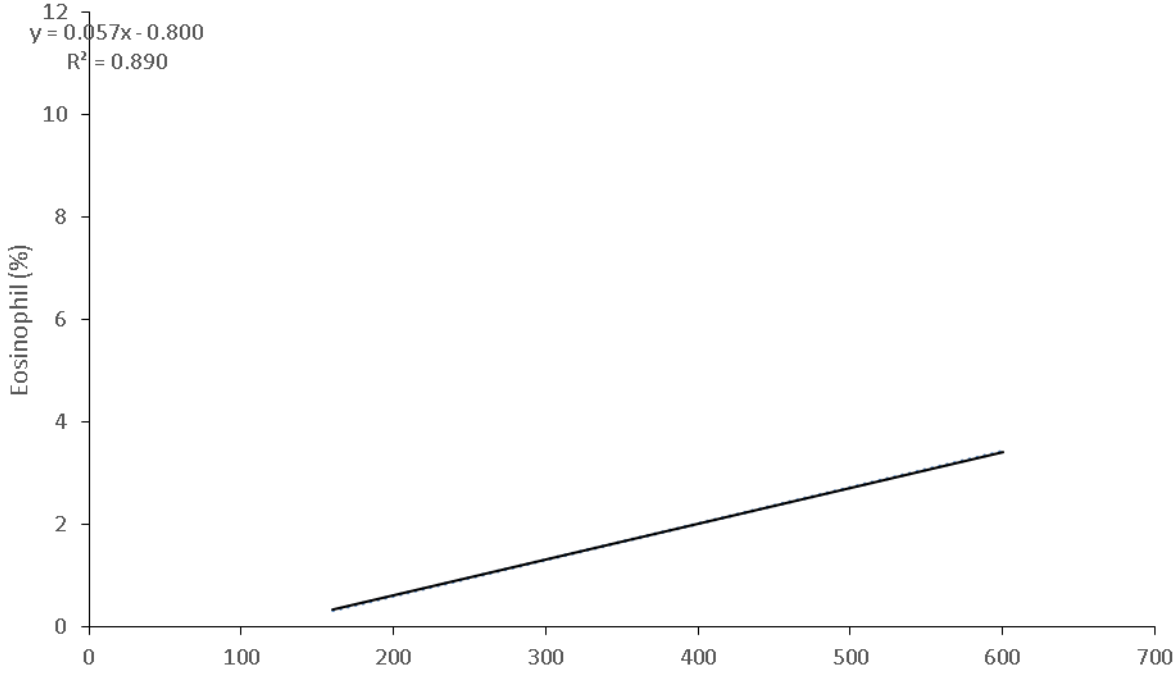

Figure 6: The influence of mean parasite densities (*P. falciparum*) on Eosinophil in female children (6-59 months) infected with malaria.

V. 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 anemia cytoadherence of infected red cells, leucocytes change coagulopathy, particularly intravascular coagulated (Pavithran, 2007), others are lymphocytosis, leucopenia leucocytosis, neutrophilia and monocytosis have all been reported Abroet.al., (2008).

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

There was a relatively higher commonness of infection 52 (59.09%) among males than females 36 (40.91%) of a female subject (p>0.05%). However, reports indicated higher commonness 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 wall, (1993). The higher prevalence rate among males could just be by chance.

VI. Conclusion

The result presented on figure 1, 2 and 3 also revealed significant positive correlation exist between parasite density and neutrophil in malarial positive children (r^2 = 0.653, P=0.005) and as well as among males (r^2 = 0.602, P=0.005) and females (r^2 = 0.890, P=0.001). This shows that as parasite density increase neutrophil also increases. This finding is in line with Abdalla, (1988) who reported neutrophilia among malaria cases especially in pediatric patients (Mainaet.al., 2010).

Eosinophilia was however found to be positively correlated with parasite density (r^2=0.510, P=0.005) in the study population, and (r^2=0.504, P=0.005), (r^2=0.623, P=0.005) for males and females subject as indicated in figure 4, 5, and 6 respectively. When parasite density increased, eosinophil correspondingly also increased (eosinophilia) (Decie and Lewis, (2001).

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