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Experiential Relationship between Malaria Parasite Density and Some Haematological Parameters in Malaria Infected Female subjects in Port Harcourt, Nigeria

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Abstract
This study examined the experiential relationship between the parasite density and haematological parameters in female patients with *Plasmodium falciparum* infection in Port Harcourt, Nigeria reporting to malaria clinics. A total of ninety (90) patients were recruited. QBC haematological analysis, QBC malaria parasite specie identification and quantification and thin blood film for differential leucocytes count was used. The mean values of the haematological parameters in respective quartile of parasite density were analyzed using Microsoft Excel Statistical Package. Regression analysis was used to model the experiential relationship between parasite density and haematological parameters. All regression relationships were tested and the relationship with the highest coefficient of determination (R^2) was accepted as the valid relationship. The relationships tested included linear, polynomial, exponential, logarithmic and power relationships. The X-axis of the regression graphs stand for the parasite density while the Y-axis stands for the respective haematological parameters. Neutrophil count had a polynomial relationship with the parasite density and is related to the parasite density by a polynomial regression equation model: $y_{nf} = -1E\text{-}05x^2 + 0.0206x + 52.253$ with a very high coefficient of determination (R^2) of 0.9784. The relationship between lymphocyte count, monocyte count and eosinophil count and parasite density in the studied group was logarithmic ($y_{lf} = -1.992\ln(x) + 35.844$, $y_{mf} = 0.3858\ln(x) + 7.3623$ and $y_{ef} = 0.8454\ln(x) + 4.9136$) in the same order. Their respective coefficients of determination were 0.9738, 0.4065 and 0.9328. The best fitting regression equation model for WBC, Hb concentration, PCV and MCHC and parasite density was a linear regression equation models: $y_{WBCf} = 1.5726x + 8048$, $y_{Hbf} = -0.0007x + 12.136$, $y_{PCVf} = -0.0018x + 38.146$ and $y_{MCHCf} = -0.0005x + 31.96$ in the same order. Their respective coefficients of determination were 0.8747, 0.9617, 0.9924 and 0.9551. A power relationship was most suitable between platelet count and parasite density with a very high coefficient of determination of 0.9955 in the studied group and expressed by equation model: $y_{Pltf} = 302292x^{-0.14\,3}$ These regression models developed could be very useful in areas where there may not be functional microscopes or competent microscopists and in medical emergencies.

List of Abbreviations

y_{nf}	neutrophil count in malaria infected females
y_{lf-}	lymphocyte count in malaria infected females
y_{mf-}	monocyte count in malaria infected females
y_{ef}	eosinophil count in malaria infected females
y_{WBCf}	total white cell count in malaria infected females
y_{Hbm}	haemoglobin concentration in malaria infected females
y_{PCVf}	packed cell volume in malaria infected females
y_{MCHCf}	mean cell haemoglobin concentration in malaria infected females
y_{Pltf}	platelet count in malaria infected females

Keywords: mathematical, parasite density, malaria, *Plasmodium,* neutrophil, eosinophil, lymphocyte, haemoglobin, haematocrit, platelets.

INTRODUCTION

Malaria is widespread in tropical and subtropical regions because of the significant amounts of rainfall and consistent high temperatures and high humidity, along with stagnant waters which provide mosquitoes the environment needed for continuous breeding (Prothero and Mansell, 1999). Malaria is caused by invasion of red blood cells with protozoan parasites of the genus *Plasmodium*. The bite of female anopheles mosquito that carries the plasmodium leads to the presence of the parasite in the red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma and death. The four *Plasmodium* species that infect humans are *P. falciparum*, *P. vivax P. ovale* and *P. malariae*. Occasional infections with monkey malaria parasite, such as *P. knowlesi,* also occur (Roberts *et al*., 2002).

Malaria infection is a major public health problem in tropical areas, and it is estimated that the disease is responsible for 1 to 3 million deaths and 300-500 million infections annually worldwide (Desowitz, 1993, Philips & Nicky, 2010). Malaria is holoendemic in Nigeria with *Plasmodium falciparum* as the dominant strain (Lesi &

Adenuga, 1996).

Malaria parasitemia has been reported to have effects on some haematological parameters in many parts of the world (Perrin *et al*., 1982., Abdalla, 1988., Rojanasthien, Surakamolleart, Boonpucknavig and Isarangkura, 1992., Sharma, R.Das, B.Das and P.Das, 1992., Lesi &Adenuga, 1996., Layla *et al*., 2002 and Oseni, Togun, Olowu and Okoli, 2006, Eze, Ezeiruaku and Ukaji, 2012). The vast majority of morbidity and mortality from malaria is caused by infection with *P. falciparum*, mostly among children under the age of 5 years living in sub-Saharan Africa (Guerin *et al*., 2002). The infection with *P. falciparum*, which causes the most severe infections and nearly all malaria-related deaths, has been well documented in areas of high endemicity in Africa (Day and Marsh, 1991).

Haematologic changes, which are the most common complications, play a major role in fatal complications. They include anaemia, cytoadherence of infected red cells, leukocytic changes followed by the induction of cytokines, thrombocytopenia and coagulopathy, particularly disseminated intravascular coagulation (Price *et al.,* 2001).

Malaria parasite identification request is usually accompanied with full blood count for proper diagnosis, treatment and management of malaria infection. There are several methods employed in malaria parasite identification and quantification. Some of these methods include; serological techniques, molecular techniques using polymerase chain reaction (PCR), quantitative buffy coat (QBC) technique and microscopic method using peripheral blood thick and thin film. These techniques are all with some limitations particularly in a resource limited environment like Nigeria.

Several rapid diagnostic tests have been developed which detect malaria parasite antigens in lysed blood using monoclonal antibodies. However, there are currently no known rapid diagnostic tests which can differentiate *P. vivax*, *P. ovale*, or *P. malariae*. These tests are not quantitative and will not provide any information concerning levels of parasitemia. Antigen persistence is also a problem. PCR testing and QBC method offer a rapid, sensitive, and less subjective methods to determine the presence and species of *Plasmodium*. Unfortunately, these malaria tests are expensive and not routinely used in Nigeria.

The gold standard used for malaria parasite diagnosis in Nigeria is the peripheral blood thick and thin film method. This is because it is most economical. It is also reliable, sensitive and specific but the reliability, sensitivity and specificity of this method depend on the efficiency of the microscopist. Unfortunately in many laboratories in Nigeria, particularly in the rural areas, this manpower is lacking. On the contrary, a good number of laboratory scientist and technicians in Nigeria can easily assess haematological parameters.

Many malaria cases particularly children under 5 years come as medical emergency and as such little time is needed to diagnose, treat and save their lives. To reduce malaria-related deaths in future particularly in rural Africa, strategies on quick diagnosis and treatment should be adopted. Eze, Ezeiruaku and Ukaji established the experiential relationship between some haematological parameters and the parasite density of the malaria infected male subjects in Port Harcourt in 2012. Because of the sex differences in some haematological parameters, this study examined the experiential relationships between some haematological parameters and the parasite density of the malaria infected female subjects in Port Harcourt, Nigeria. These relationships can be used to, given any of the haematological parameter, predict the malaria parasitemia within the shortest time even in the absence of a functional microscope or a competent microscopist.

SUBJECTS AND METHODS

2.1 Study Area

The study was carried out in Port Harcourt located between latitudes 4^{o} $2^{/}$ North and 4^{o} $47^{/}$ North and longitudes 6^{o} $55^{/}$ East and 7^{o} $08^{/}$ East. Port Harcourt has continued to record a high incidence of malaria infection despite the federal government efforts to roll back malaria in Nigeria. The reason is that Port Harcourt is a typical coastal zone located in the Niger Delta of Nigeria. High temperatures and humidity as well as marked wet and dry seasons characterize the climate. The mean annual rainfall is estimated at about 2,405mm while the mean monthly temperature varies between 24^{o} C and 32^{o} C throughout the year (Gobo, 1988). The mean annual temperature for Port Harcourt is 26^{o} C (Gobo, 1988). The mean annual temperature, relative humidity and rainfall of Port Harcourt favour the development of both the parasite as well as the vector.

2.2 Study Population

The laboratory study was carried out for three months during which a total of ninety (90) female malaria patients were recruited. The inclusion criteria were female out-patients to the participating clinic site within the age of 1-60 years queried for malaria infection with the presentation of at least two of the following: an oral temperature of 38°C, headache, or a history of fever within the past 72 hours and who must not have commenced any treatments for malaria. Exclusion criteria were female out-patients with pathological conditions outside malaria such as protozoan or helminthes infection, typhoid fever and HIV/AIDS, congenital manifestations such as sickle cell disease, physiological manifestations such as pregnancy and history of allergy. All enrolled patients

were interviewed for information on current symptoms and previous malaria episodes and treatments.

2.3 Blood Sample Collection and Processing

A volume of 2.6 ml of the venous blood sample was drawn from each patient into monovette tubes containing the anticoagulant potasium ethylenediamine-tetra acetic acid (EDTA) for QBC haematological analysis, QBC malaria parasite specie identification and quantification and thin blood film for differential leucocytes count. QBC Autoread Plus provided a diagnostic haematology profile of the following quantitative values from a single tube of blood; packed cell volume (haematocrit), haemoglobin concentration, mean corpuscular haemoglobin concentration, platelet count, white blood cell count, granulocyte count (% and number) and lymphocyte-monocyte count (% and number). Daily quality assurance checks were performed and recorded.

For QBC malaria parasite detection analysis, the centrifuged tube was examined under a fluorescence microscope in the region between the light red blood cells and granulocytes and lymphocytes/monocytes, where the parasites are most abundant. Examination of the centrifuged blood under a fluorescence microscope readily permits the detection of malaria parasite in the infected cells and plasma. Since the parasites contain DNA which takes up the acridine orange stain, they appear as bright specks of light in the dark background of non-fluorescing red cells.

For QBC malaria parasite species identification, at magnification of 600X, all parasites in the red blood cells were easily visualized and their morphologies identified. Species identifications were made based upon the size and shape of the various stages of the parasite and the presence of stippling (i.e. bright red dots) and fimbriation (i.e. ragged ends). Plasmodium parasites are always intracellular, and they demonstrate, if stained correctly, blue cytoplasm with a red chromatin dot. The parasite densities were obtained by multiplying the average number of parasites in 10 QBC fields by a factor of 10.5 (QBC operator's manual, 2006)

For a reliable differential leucocytes count, a thin blood film was prepared by a standard manual technique as described by Bain *et al*., (2008) on a clean grease-free glass slides, allowed to air-dry and fixed in alcohol (methanol) for 2 minutes and then stained with Field's stain. The differential leucocyte count was carried out by the longitudinal technique. The mean values of the data obtained for haematological parameters were grouped into four based on their degree of parasitemia. The experiential relationships between the degree of parasitemia and haematological parameters were assessed using regression equation models.

RESULTS

Heavy infection (3+) represented the highest percentage (42.22%) in the studied group while scanty infection (1+) represented the least percentage (12.22%). The percentage of infected subjects manifesting moderate infection (3+) and very heavy infection (4+) was 23.33 and 22.22% respectively (Table 1).

Table 1: Distribution of the Infected Subjects according to Levels of Parasitemia

Level of Parasitemia	Total	%
1+	11	12.22
2+	21	23.33
3+	38	42.22
4+	20	22.23
Total	**90**	**100**

The variations of haematological parameters with changes in parasitemia are shown in Table 2. The value of neutrophil count in females increased gradually from 57.55±6.14 % in scanty infection to 60.75±4.93 % in very heavy infection. In each of the cases, there was increase in neutrophil count with increase in parasitemia. Other haematological parameters in the studied population include Hb concentration which decreased from 12.65 ± 0.6 g/dl in scantly infected subjects to 10.45±2.13g/dl in very heavily infected subjects.

Table.2: Mean ±SD of Haematological Parameters of Females of Different Parasitemia

Parameters	1+	2+	3+	4+
N (%)	57.55 ± 6.14	59.38 ± 4.41	59.71 ± 8.68	60.75 ± 4.93
L (%)	28.73 ± 6.68	23.81 ± 6.31	21.66 ±7.97	18.55 ± 4.50
M (%)	6.45 ± 2.07	9.57 ± 2.75	9.34 ± 2.77	10.50 ± 1.99
E (%)	6.18 ± 1.25	8.05 ± 1.32	9.11 ± 1.93	10.45 ± 1.43
B (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
WBC /mm^3	7236.36 ± 946.86	7557.14 ± 2042.69	8981.58 ± 2488.66	10270.00 ± 2680.75
HB (g/dl)	12.65 ± 0.61	12.06 ± 1.49	11.63 ± 1.37	10.45 ± 1.50
PCV (%)	39.13 ± 1.93	37.10 ± 4.02	36.07 ± 4.16	33.07 ± 4.12
MCHC	32.37 ± 1.38	32.50 ± 1.76	32.27 ± 1.52	31.49 ± 1.07
PLT /mm^3	272000.00 ± 51828.56	164952.38 ± 41430.03	132050.00 ± 41129.84	106150.00 ± 21111.92
PD (Parasite/µl)	3.64 ± 2.50	46.52 ± 20.36	377.37 ± 305.71	1447.70 ± 319.07

Neutrophil count among the malaria infected female subjects had polynomial relationship with the parasite density (Figure 1).

$$y_{nf} = -1E\text{-}05x^2 + 0.0206x + 52.253 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq. } 1.1$$

The coefficient of determination (R^2) was very high ($R^2 = 0.9784$).

The relationship between lymphocyte count and parasite density was logarithmic (Figure 2) as expressed in equation 2.1

$$y_{lf} = -1.992\ln(x) + 35.844 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq. } 2.1$$

The coefficient of determination was also very high ($R^2 = 0.9738$).

Monocyte and eosinophil counts also had logarithmic relationships with parasite density (Figure 2). These relationships are defined by the equations 2.2 and 2.3 for monocyte and eosinophil counts respectively.

$$y_{mf} = 0.3858\ln(x) + 7.3623 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq. } 2.2$$

$$y_{ef} = 0.8454\ln(x) + 4.9136 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eq. } 2.3$$

The coefficient of determination of monocyte was 0.4065 while that of eosinophil was 0.9328.

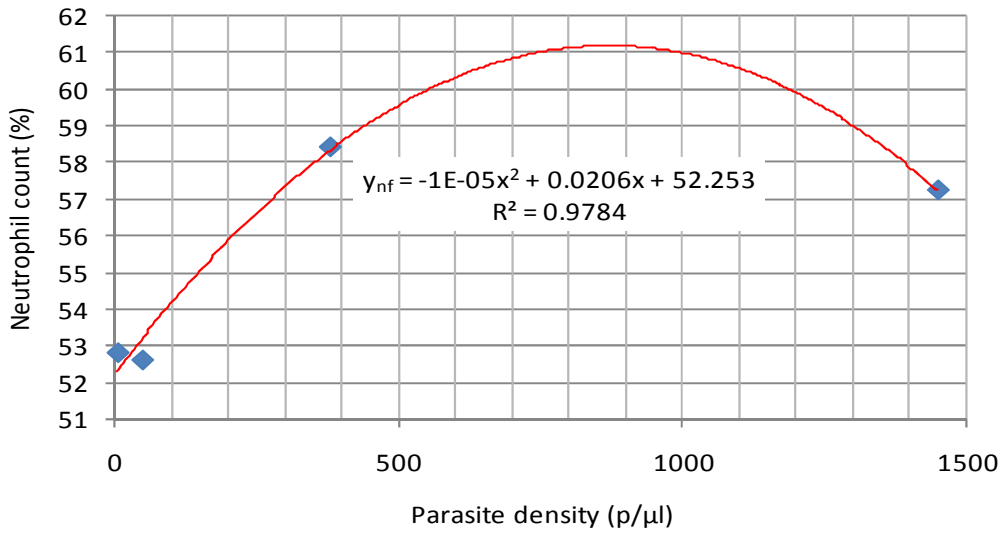

Figure 1: Polynomial Relationship between Neutrophil Count and Parasite Density (Female)

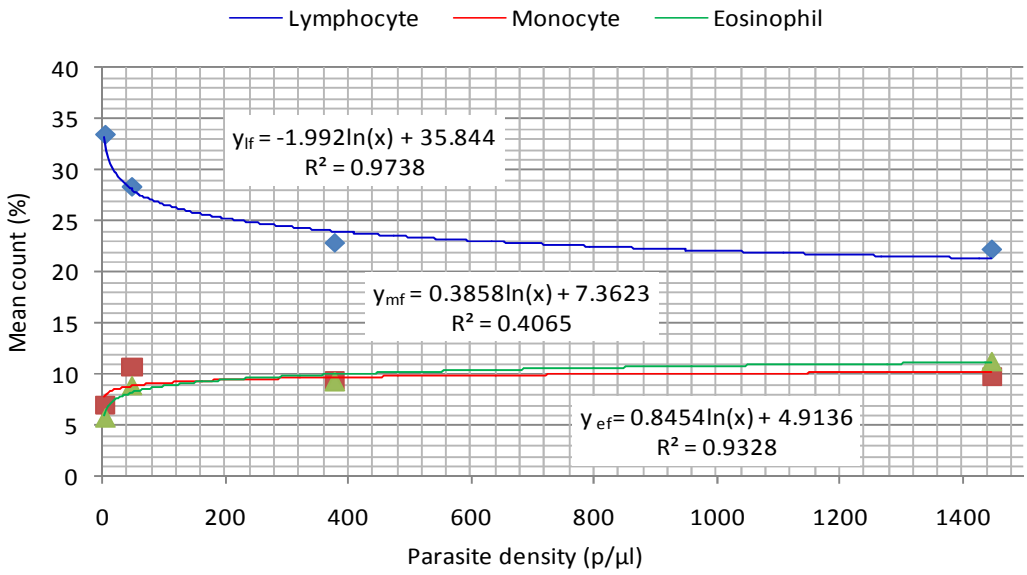

Figure 2: Logarithmic Relationship between Lymphocyte, Monocyte and Eosinophil Counts and Parasite Density (Female)

WBC had a positive linear correlation with parasite density (Figure 3) and a high value of coefficient of determination (R^2 = 0.8747). Equation 3.1 expresses this relationship.

$$y_{WBCf} = 1.5726x + 8048.1 \quad \text{.....eq. 3.1}$$

Hb and PCV each had a negative linear relationship with parasite density (Figures 4 and 5). The relationships are expressed in equations 4.1 and 5.1 respectively.

$$y_{Hbf} = -0.0007x + 12.136 \quad \text{.....eq. 4.1}$$

$$y_{PCVf} = -0.0018x + 38.146 \quad \text{.....eq. 5.1}$$

Their respective coefficients of determination were 0.9617 and 0.9924.

The regression analysis result of MCHC showed that it has a negative linear relationship with parasite density (equation 6.1) as shown in Figure 6

$$y_{MCHCf} = -0.0005x + 31.96 \quad \text{.....eq. 6.1}$$

The coefficient of determination was 0.9551.

Platelet count in malaria infected subjects exhibited a power relationship with parasite density (Figure 7). The relating equation (eq. 7.1) is given as:

$$y_{Pltf} = 302292x^{-0.143} \quad \text{.....eq. 7.1}$$

The coefficient of determination was 0.9955

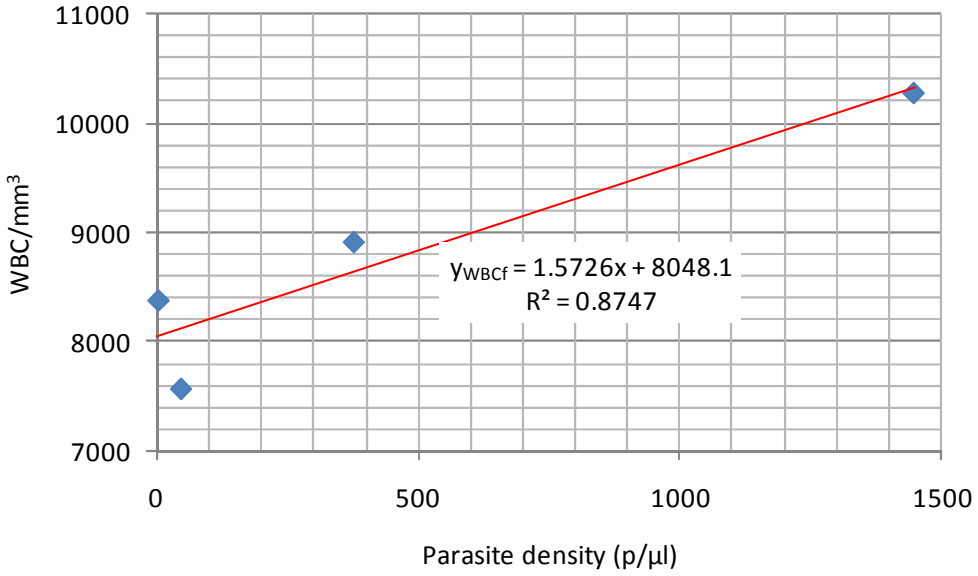

Figure 3: Linear Relationship between WBC Count and Parasite Density (Female)

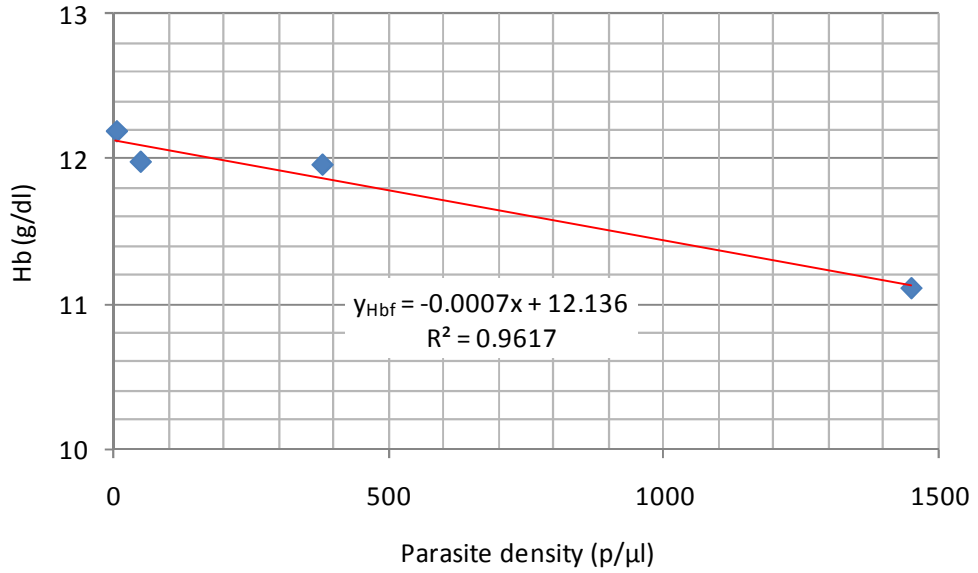

Figure 4: Linear Relationship between Hb Concentration and Parasite Density (Female)

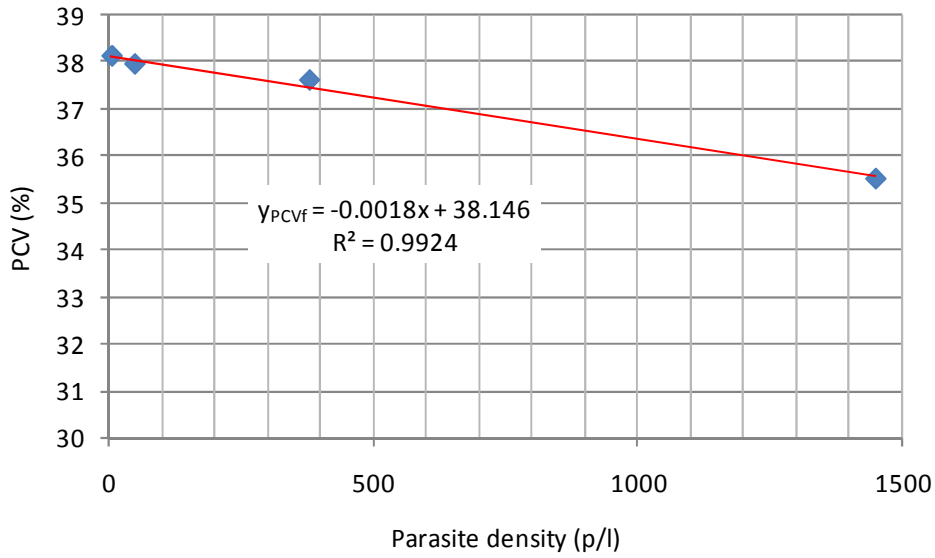

Figure 5: Linear Relationship between PCV and Parasite Density (Female)

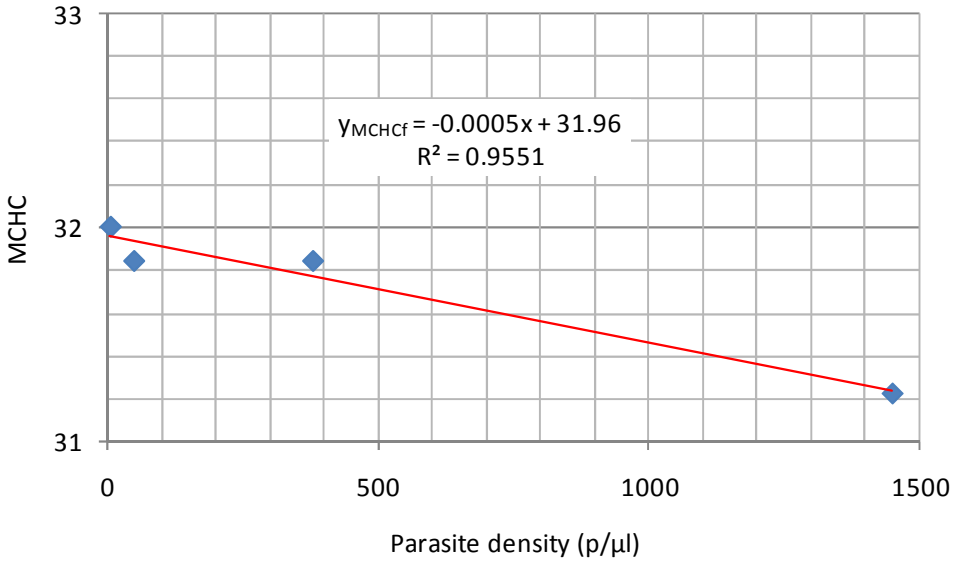

Figure 6: Linear Relationship between MCHC and Parasite Density (Female)

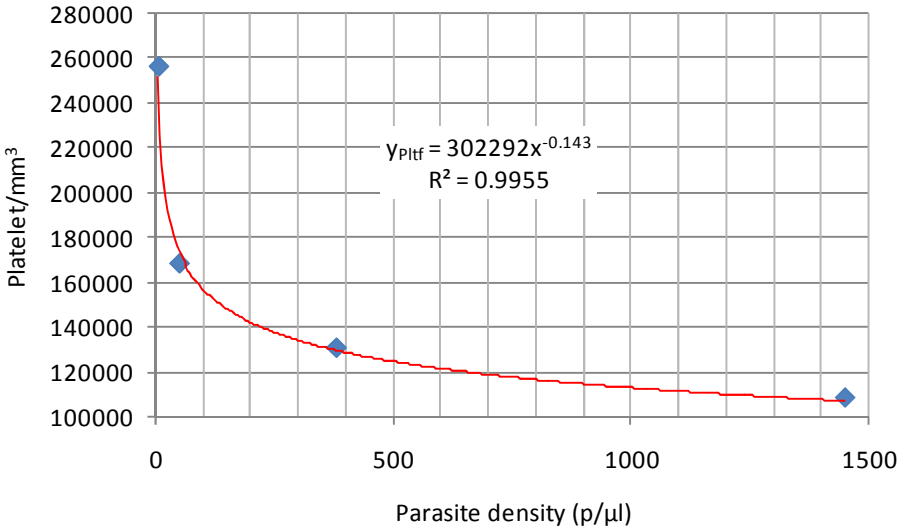

Figure 7: Power Relationship between Platelet Count and Parasite Density (Female)

DISCUSSION

The mean neutrophil counts in most of the infected cases studied were within the normal range of 40-75%. This finding is similar to the works of Layla *et al.,* (2002) who reported that neutrophil count was within the normal range in most malaria patients studied in Saudi Arabia.

Neutrophil count in the studied group had polynomial relationship with the parasite density. As the neutrophil increases, the parasitemia will tend to decrease in double, triple and quadruple manner.

The inverse relationship between lymphocyte count and parasite density from this study suggests an increased apoptosis at higher parasitemia. The best predictive regression models for lymphocyte count and parasite density was logarithmic. This logarithmic relationship means that each doubling of lymphocyte count will cause the same amount of decrease in parasitemia .

The experiential relationship between the parasite density in the studied group and their mean monocyte counts were expressed by logarithmic regression model. This means that each doubling of monocyte count will cause the same amount of increase in parasitemia.

Mean eosinophil counts in the studied group showed a consistent significant increase with increasing parasite density. This agrees with earlier works by Abdallah, (1988) and Jandl, (1996). The experiential relationship between the parasite density and mean eosinophil counts in the studied group were expressed by logarithmic regression model. This means that each doubling of eosinophil count will cause the same amount of increase in parasitemia.

Only 9.9% of the infected females showed leucocytosis. No specific diagnostic indications are given by the white blood cell count during malaria attack and the few cases of leukocytosis may reflect the presence of concomitant bacterial infections. This is in agreement with previous work done by Layla *et al.,* (2002) but Price *et al*., (2001) reported instead, a decrease in WBC count among Thai malaria patients. The experiential relationship between the parasite density and the mean WBC count was expressed by a linear regression model in the studied groupa. This means that as the parasite density is increasing that there is a corresponding increase in total white cell count.

Mean Hb concentration and PCV in the studied group showed a consistent decrease across the quartiles of parasite density. This observation agrees with the report of Oseni *et al*., (2006) that there is a steady fall in haemoglobin and PCV levels during a malaria infection. Anaemia was recorded mostly in the hyperparasitemic cases. The best fitting regression equation models for both Hb concentration and PCV and parasite density in the studied group is linear regression model. This means that as the parasite density is increasing that there is a corresponding decrease in haemoglobin concentration and PCV.

There was no significant variation between the mean values of MCHC in the studied group from the normal range of 32-36. This suggests probably that the Hb content of the circulating non-infected erythrocytes remained intact. The empirical relationship between the parasite density and the mean MCHC was expressed by a linear regression model in the studied group.

The trend of decreasing platelet count with increasing levels of parsitemia observed in this study has been previously noted for *P. falciparum* (Perrin *et al*., 1982, Rojanasthien, *et al*., 1992, Eze, Ezeiruaku & Ukaji, 2012). The best fitting regression equation models for the relationship between platelet count and parasite

density in the studied group is power regression model. The value of the power relationship is less than 1(0.143), it therefore means that a unit increment in the parasite density will lower the platelet count by about a square of 0.143

CONCLUSION

Malaria parasitemia has been shown to have effects on some haematological parameters from this study while some haematological parameters are more predictive of malaria infection than others. Eosinophilia, thrombocytopenia and lymphopenia were identified as the key haematological indicators of malaria infection in the studied population. Eosinophilia can be used to predict the intensity of malaria infection. Thrombocytopenia is strongly associated with malaria infection and malaria parasite density. Lymphopenia is however more indicative of hyperparasitemia. Decrease in Hb and PCV in children is strongly suggestive of malaria infection. Neutrophil, monocyte and basophil counts do not have any significant relation with malaria parasitemia and are therefore not reliable indices for predicting malaria infection.

The results of the regression analyses have shown that different relationships exist among different haematological parameters across quartiles of parasite density in the studied group. The best fitting regression equation models have been developed from this study for estimating the values of haematological parameters from parasite densities and vice versa. This could be very useful in areas where there may not be functional microscopes or competent microscopists and in medical emergencies.

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