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Haematological Indices of *Plasmodium falciparum* Malaria In Immune Adult Population of Port Harcourt, Nigeria

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Abstract

This study examines the hematological changes in adults with acute *Plasmodium falciparum* infection in Port Harcourt, Nigeria reporting to malaria clinics compared with apparently healthy non-parasitemic persons from the same population. A total of one hundred and one (111) patients comprising of 69 males and 42 females and thirty-five(35) control subjects comprising of 19 males and 16 females were recruited. QBC haematological analysis, QBC malaria parasite specie identification and quantification and thin blood film for differential leucocytes count was used. Persons with patent parasitemia tended to have significantly lower lymphocytes and platelets and higher eosinophils and TWBC than those who were malaria-negative. A trend in reduced platelet count with parasitemia was found to be associated with both *P. falciparum* infections. This study supplements previous literature on the hematological effects of malaria and helps define those alterations for an immune population. Reduced platelet count is identified as a key indicator of malaria in these febrile patients.

1.0 Introduction

The prevalence of malaria in Port Harcourt, Nigeria which is located between latitudes 4° 2/ North and 4° 47/ North and longitudes 6° 55/ East and 7° 08/ East has continued to increase despite all the federal government's 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. The Nigerian coastal zone experiences a tropical climate consisting of rainy season (April to November) and dry season (December to March). High temperatures and humidity as well as marked wet and dry seasons characterize the climate. The mean annual rainfall is estimated at about 2,280mm while the average annual minimum and maximum temperatures are 25.1° C and 30.3° C (Igoni *et al*, 2007). The mean annual temperature for Port Harcourt is 26.7° C (Odjugo, 2010). Temperature affects many parts of the malaria life cycle. The duration of the extrinsic phase depends on temperature and on the species of the parasite the mosquito is carrying (Pounniotis *et al.*, 2004) The extrinsic cycle normally lasts nine or ten days, but sometimes can be as short as five days (Pounniotis *et al.*, 2004). As the temperature decreases, the number of days necessary to complete the extrinsic cycle increases for a given *Plasmodium* species. The extrinsic phase takes the least amount of time when the temperature is 27°C (Roshanravan *et al.*, 2003). The time required for development of the ookinete, the egg of the parasite, in the midgut of the Anopheles mosquito, decreases as temperature increases from 21°C to 27°C. The optimum temperature for mosquitoes is 25-27°C (Roshanravan *et al.*, 2003). Rainfall also affects malaria transmission because it increases relative humidity and modifies temperature, and it also affects where and how much mosquito breeding can take place (Roshanravan *et al.*, 2003). Also the amount of rainfall may be secondary in its effects on malaria to the number of rainy days or the degree of wetness that exists after a rain event. The mean annual temperature, relative humidity and rainfall of Port Harcourt therefore favours both the parasite and the vector.

Malaria is holoendemic in Nigeria with *Plasmodium*

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falciparum as the dominant strain (Lesi and Adenuga, 1996). 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-Sahara 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 associated with malaria infection are well recognized, but specific changes may vary with level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity. This study examined the haematological effects of malaria on immune adults in Port Harcourt. Specifically, the haematological profiles of persons infected with *P. falciparum* were compared with expected normal values. Additionally, correlation analysis was employed to ascertain the relationship between parasite density and haematological parameters. This study also examined the effect of sex and age on the haematological parameters of the malaria infected subjects. Also haematological parameters most predictive of malaria in this population are identified.

2.0 Subjects and Methods

The laboratory study was carried out for a period of four months during which a total of one hundred and one (111) patients comprising of 69 males and 42 females and thirty-five(35) control subjects comprising of 19 males and 16 females were recruited. The inclusion criteria were out-patients to the participating clinic site within the age of 21 - 70 years queried for malaria infection with the presentation of at least one 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 out-patients with pathological conditions outside malaria such as protozoan or helminthic infection, typhoid fever and HIV/AIDS, congenital manifestations such as sickle cell disease, physiological manifestations such as pregnancy and history of allergy

Control subjects were selected from among apparently healthy male and female subjects aged 21 - 70 years living in Port Harcourt also without QBC detectable parasitemia. Such subjects must also not have had recent malaria attack/ treatment or evidence of congenital or any recent pathological or physiological manifestations.

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

A volume of 2.6 ml of the venous blood sample was drawn into monovette tubes containing the anticoagulant potasium ethylenediamine-tetraacetate (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 Dacie and Lewis (2006) on 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 data obtained were grouped into males and females and each sex group further divided into four parasite densities to determine the effect of sex and parasite density on the haematological parameters of the malaria infected subjects. Independent-sample t-test was used to compare the haematological parameters of the malaria infected groups with their corresponding sex specific groups. Again the sex groups were divided into age groups to determine the effect of age on the investigated parameters. The mean and standard deviation (SD) and correlation of the haematological parameters were carried out using Excel Statistical Analysis Package. The correlation was performed at 99% confidence level of significance. The significance of the correlation was tested to determine the probability (P) of chance occurrence.

Result obtained for the different sexes were compared to determine the influence of sex on the parameters. The type and degree of association of sex, age and parasitemia with haematological parameters was assessed using the results of correlation analysis.

Each sex group was further divided on the basis of age groups 21-30, 31-40, 41-50, 51-60 and 61-70 years. Each age group was then divided into different parasitemia (slight infection (+1)), moderate infection (+2)), heavy infection (+3)) and very heavy infection (+4)). The control was similarly grouped according to sexes and each sex grouped according to same age groups as in the malaria infected subjects. The reason for the groupings was to determine the effect each of the factors; sex, age and parasitemia had on the haematological parameters of the malaria infected subjects by comparing the values with the corresponding control groups.

3.0 Results

A total of one hundred and one (111) patients comprising of 69 males and 42 females and thirty-five (35) control subjects comprising of 19 males and 16 females were enrolled in this study. The mean values of selected haematological parameters for the malaria infected subjects and control were determined for this population, stratified by sex (Tables 1 and 2).

Haematological parameters of *P. falciparum* malaria infected subjects were compared with those of the sex-specific control group using independent-sample *t*-tests. There were observable variations in the Mean±SD of haematological parameters of the infected male subjects and the control males on one hand and females infected subjects and the control females on the other hand as shown in Tables 1 and 2. The mean values of eosinophil count (8.39 ± 2.54%), and TWBC count (8.39 ± 2.35 X 10^9/L) were significantly higher ($P<0.05$) in the infected males than the corresponding values of eosinophil count (3.55 ± 0.94%), and TWBC count (6.25 ± 1.30 X 10^9/L) in the controls. However, Platelet count (152.99 ± 59.60 X 10^9/L) and Lymphocyte count (25.03 ± 9.12%) in the infected males were significantly lower ($P<0.05$) than their corresponding values of 245.65 ± 51.82 X 10^9/L and 34.60 ± 4.59% in the control. Similarly, the mean values of eosinophil count (8.10 ± 1.85%), and TWBC count (8.53 ± 2.42 X 10^9/L) were significantly higher ($P<0.05$) in the infected females than the corresponding values of eosinophil count (3.75 ± 1.00%), and TWBC count (6.67 ± 0.87X 10^9/L) in the controls. However, Platelet count (162.89 ± 67.19X 10^9/L) and lymphocyte count (25.03 ± 9.12%) %) in the infected females were significantly lower ($P<0.05$) than their corresponding values of 244.13 ± 48.42 X 10^9/L and 34.81 ± 5.05 in the control.

Mean parasitemias for *P. falciparum* ranged from 5.00 ± 2.86 parasites / µl to 1196.67 ± 83.86 parasites / µl in males and 3.14 ± 2.27parasites/µl to 1442.50 ± 374.73parasites/ µl in females (Tables 3 and 4).

Table 1: Mean Value ± SD of Haematological Parameters in Male Malaria Positive and Malaria Negative (Control) Subjects

Haematological Parameters	Male infected subjects	Male control subjects	P-value
[1]PCV (%)	41.74 ± 4.59	42.44 ± 2.88	P>0.05
[2]Hb (g/dl)	13.52 ± 1.49	14.21 ± 1.90	P>0.05
[3]MCHC	32.43 ± 1.42	33.60 ± 1.13	P>0.05
[4]TWBC x 10^9/L	8.39 ± 2.35	6.25 ± 1.30	P<0.05
[5]Plt x 10^9/L	152.99 ± 59.60	245.65 ± 51,82	P<0.05
Neutrophils (%)	58.00 ± 8.75	54.90 ± 4.19	P>0.05
Lymphocytes (%)	25.03 ± 9.12	34.60 ± 4.59	P<0.05
Monocytes (%)	8.72 ± 2.93	6.45 ± 1.36	P>0.05
Eosinophils (%)	8.39 ± 2.54	3.55 ± 0.94	P<0.05
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	P>0.05

[1]Packed Cell Volume, [2]Haemoglobin Concentration, [3]Mean Cell Haemoglobin Concentration, [4]Total White Blood Cell Count, [5]Platelet Count

Table 2: Mean Value ± SD of Haematological Parameters in Female Malaria Positive and Malaria Negative (Control) Subjects

Haematological Parameters	Female infected subjects	Female control subjects	P-value
[1]PCV (%)	37.79 ± 2.77	38.01 ± 3.21	P>0.05
[2]Hb (g/dl)	12.26 ± 0.98	12.66 ± 1.38	P>0.05
[3]MCHC	32.79 ± 1.12	33.28 ± 1.54	P>0.05
[4]TWBC x 10^9/L	8.53 ± 2.42	6.67 ± 0.87	P<0.05
[5]Plt x 10^9/L	162.89 ± 67.19	244.13 ± 48.42	P<0.05
Neutrophils (%)	59.40 ± 6.53	55.00 ± 4.10	P>0.05
Lymphocytes (%)	25.03 ± .12	34.81 ± 5.05	P<0.05
Monocytes (%)	8.43 ± 2.28	7.00 ± 1.59	P>0.05
Eosinophils (%)	8.10 ± 1.85	3.75 ± 1.00	P<0.05
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	P>0.05

[1]Packed Cell Volume, [2]Haemoglobin Concentration, [3]Mean Cell Haemoglobin Concentration, [4]Total White Blood Cell Count, [5]Platelet Count

Table 3: Mean ± SD of Haematological Parameters of Males of different Parasitemia

Parameters	*Parasitemia			
	+1	+2	+3	+4
[1]N (%)	53.93 ± 10.41	58.59 ± 5.19	58.77 ± 9.78	65.00 ± 5.29
[2]L (%)	35.71 ± 10.00	23.95 ± 4.57	21.80 ± 7.54	15.33 ± 4.51
[3]M (%)	6.29 ± 1.82	8.73 ± 2.10	9.77 ± 3.35	9.67 ± 1.53
[4]E (%)	4.79 ± 1.53	8.73 ± 1.67	9.67 ± 1.94	10.00 ± 0.00
[5]B (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
[6]TWBC x 10^9/L	6.71 ± 2.06	8.03 ± 1.90	9.21 ± 2.35	10.80 ± 1.66
[7]Hb (g/dl)	14.24 ± 0.96	13.64 ± 1.35	13.12 ± 1.65	13.33 ± 2.10
[8]PVC (%)	43.34 ± 3.69	42.12 ± 4.48	40.83 ± 4.80	40.67 ± 7.02
[9]MCHC	32.94 ± 1.50	32.45 ± 1.53	32.14 ± 1.32	32.83 ± 0.47
[10]Plt x 10^9/L	237.86 ± 54.68	174.55 ± 57.23	133.39 ± 33.35	128.33 ± 21.94
[11]PD (Parasite/µl)	5.00 ± 2.86	49.68 ± 22.27	389.40 ± 274.85	1196.67 ± 83.86

*Parasitemia: +1 (scanty infection), +2 (moderate infection), +3 (heavy infection), +4 (very heavy infection).
[1]Neutrophils, [2]Lymphocytes, [3]Monocytes, [4]Eosinophils, [5]Basophils, [6]Total White Blood Cell Count, [7]Haemoglobin Concentration, [8]Packed Cell Volume, [9]Mean Cell Haeglobin Concentration, [10]Platelet Count, [11]Parasite Density

Table 4: Mean ± SD of Haematological Parameters of Females of different Parasitemia

Parameters	Parasitemia			
	+1	+2	+3	+4
[1]N (%)	57.71 ± 7.43	60.15 ± 4.24	59.33 ± 8.00	60.25 ± 5.32
[2]L (%)	30.57 ± 7.66	24.92 ± 7.44	22.78 ± 8.40	20.25 ± 4.50
[3]M (%)	5.71 ± 1.98	8.62 ± 1.19	8.94 ± 2.39	10.25 ± 1.26
[4]E (%)	5.86 ± 1.21	7.77 ± 1.30	8.83 ± 1.76	9.75 ± 0.50
[5]B (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
[6]TWBC x 10^9/L	7.37 ± 1.19	7.65 ± 1.96	9.25 ± 2.62	10.20 ± 3.05
[7]Hb (g/dl)	12.97 ± 0.54	12.35 ± 1.30	12.12 ± 0.73	11.35 ± 0.62
[8]PVC (%)	39.43 ± 1.40	37.69 ± 3.20	36.94 ± 2.55	34.75 ± 1.50
[9]MCHC	32.90 ± 0.64	32.72 ± 1.33	32.84 ± 1.22	32.63 ± 0.78
[10]Plt x 10^9/L	279.57 ± 60.65	159.31 ± 32.35	130.41 ± 39.10	116.50 ± 21.49
[11]PD (Parasite/µl)	3.14 ± 2.27	44.46 ± 19.79	407.56 ± 333.07	1442.50 ± 374.73

*Parasitemia: +1 (scanty infection), +2 (moderate infection), +3 (heavy infection), +4 (very heavy infection).
[1]Neutrophils, [2]Lymphocytes, [3]Monocytes, [4]Eosinophils, [5]Basophils, [6]Total White Blood Cell Count, [7]Haemoglobin Concentration, [8]Packed Cell Volume, [9]Mean Cell Haeglobin Concentration, [10]Platelet Count, [11]Parasite Density

Table 5: Mean ± SD of Haematological Parameters of Male Age group of different Ages

Parameters	Age (Years)				
	21-30	31-40	41-50	51-60	61-70
[1]N (%)	55.43 ± 8.03	60.81 ± 5.06	58.60 ± 8.21	55.65 ± 12.74	61.14 ± 3.02
[2]L (%)	26.57 ± 9.28	20.56 ± 4.84	26.13 ± 8.46	28.06 ± 12.35	22.43 ± 5.22
[3]M (%)	9.29 ± 3.00	9.00 ± 2.03	8.53 ± 2.64	8.18 ± 4.02	8.71 ± 2.50
[4]E (%)	8.71 ± 1.98	9.63 ± 1.67	7.40 ± 2.35	8.12 ± 3.33	7.71 ± 2.75
[5]B (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
[6]TWBC x 10^9/L	8.56±2.49	9.16 ± 2.46	8.08 ± 1.95	8.20 ± 2.42	7.44 ± 2.58
[7]Hb (g/dl)	13.59 ± 1.98	13.56 ± 1.11	13.81 ± 1.40	13.41 ± 1.74	12.96 ± 0.50
[8]PVC (%)	42.36 ± 5.67	41.36 ± 3.90	43.13 ± 4.48	41.40 ± 4.91	39.24 ± 2.55
[9]MCHC	32.06 ± 1.23	32.86 ± 1.39	32.07 ± 1.16	32.38 ± 1.62	33.10 ± 1.68
[10]Plt x 10^9/L	133.57 ± 38.86	151.81 ± 34.43	191.13 ± 86.05	164.22 ± 69.60	205.29 ± 51.24
[11]PD (Parasite/µl)	317.14 ± 298.66	265.56 ± 377.50	148.13 ± 265.06	262.65 ± 367.54	151.29 ± 281.16

[1]Neutrophils, [2]Lymphocytes, [3]Monocytes, [4]Eosinophils, [5]Basophils, [6]Total White Blood Cell Count, [7]Haemoglobin Concentration, [8]Packed Cell Volume, [9]Mean Cell Haeglobin Concentration, [10]Platelet Count, [11]Parasite Density

The variations of haematological parameters with changes in parasitemia are also shown in Tables 3 and 4. The value of neutrophil count in males increased progressively from 53.93 ± 10.41% in scanty infection (1+) to 65.00 ± 5.29% in very heavy infection (4+). The same parameter ranged from 57.71 ± 7.43% in scanty infection to 60.25 ± 5.32% in very heavily infected female subjects. In each of the cases, there was increase in neutrophil count with increase in parasitemia. Other haematological parameters with similar trend in the malaria infected male and female subjects include monocyte count, eosinophil count and total WBC count. However, lymphocyte count, Hb, PCV and platelet count decreased gradually across the quartile of parasite density in both infected sexes.

Platelet count was the only parameter for any group that showed a trend across quartiles of parasite densities. The mean platelet count was significantly lower in men and women with higher parasitemia for *P. falciparum* (Tables 3 and 4).

Correlation coefficient was used to identify relationships between haematological parameters and parasite densities. Eosinophil count and total

Table 6: Mean ± SD of Haematological Parameters of Female Age group of different Ages

Parameters	Age (Years)				
	21-30	31-40	41-50	51-60	61-70
[1]N (%)	58.71 ± 5.62	60.36 ± 6.81	59.22 ± 9.31	59.00 ± 2.35	None
[2]L (%)	26.14 ± 7.72	22.07 ± 8.06	24.67 ± 9.50	26.40 ± 6.73	None
[3]M (%)	8.57 ± 1.70	8.93 ± 2.59	7.56 ± 2.07	8.20 ± 3.27	None
[4]E (%)	7.86 ± 1.41	8.64 ± 2.21	8.56 ± 1.51	6.40 ± 1.67	None
[5]B (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	None
[6]TWBC x 10^9/L	9.36 ± 2.68	8.15 ± 2.79	8.46 ± 1.69	7.42 ± 0.98	None
[7]Hb (g/dl)	12.19 ± 1.14	12.17 ± 1.11	12.31 ± 0.75	12.58 ± 0.63	None
[8]PVC (%)	36.57 ± 3.03	38.21 ± 3.02	37.22 ± 2.17	37.60 ± 2.19	None
[9]MCHC	33.31 ± 0.68	31.84 ± 1.17	33.08 ± 0.80	33.46 ± 0.83	None
[10]Plt x 10^9/L	169.79 ± 81.15	151.31 ± 61.87	164.00 ± 72.83	174.00 ± 32.07	None
[11]PD (Parasite/µl)	330.43 ± 443.18	317.71 ± 372.72	488.00 ± 722.47	48.00 ± 69.48	None

[1]Neutrophils, [2]Lymphocytes, [3]Monocytes, [4]Eosinophils, [5]Basophils, [6]Total White Blood Cell Count, [7]Haemoglobin Concentration, [8]Packed Cell Volume, [9]Mean Cell Haeglobin Concentration, [10]Platelet Count, [11]Parasite Density

WBC count had strong positive correlation with parasite density among male and female infected subjects. Conversely lymphocyte count and platelet count correlated negatively with parasite density in both sexes.

The age group with the highest parasite density in the male infected subjects is 21-30 years with a mean value of 317.14 ± 298.66parasites/µl while the least mean value of 148.13 ± 265.06parasites/µl was observed in males in the age group 41-50 years as shown in Table 5. Conversely, in the female infected subjects, the highest parasite density fell within age group 41-50 years with a mean value of 488.00 ± 722.47parasites/ µl and the least mean value of 48.00 ± 69.48parasites/µl fell within51-60 age group as shown in Table 6.

4.0 Discussion

The decreases in platelets counts observed in this study agree with other studies on malaria infection on immune adult populations (Sharma *et al*., 1992, Murty *et al*., 2000). Although low platelet counts have been consistently found for *P. falciparum* (Essien, 2006, Wickramasinghe and Abdalla, 2000), a substantial proportion of cases in this study did not have very low platelet counts except those with hyperparasitaemia because most of them were fully immuned against malaria infection. *Plasmodium falciparum* cases with platelet levels below this count have been documented in populations of semi-immune (Guerin *et al*., 2002, Erhart *et al*., 2004) and non-immune patients (Lesi and Adenuga, 1996, Richards *et al*., 1998, Guerin *et al*., 2002). The trend of decreasing platelet count with increasing levels of parasitemia observed in this study has been previously noted for *P. falciparum* (Sharma *et al*., 1992, Murty *et al*., 2000). Decreased platelet production has been ruled out (Erhart *et al*., 2004). Looareesuwan *et al*., (1992) and Abbro *et al*., (2009) have reported thrombocytopenia in malaria infection to occur as a result of peripheral destruction and consumption of platelets by disseminated intravascular coagulation (DIC). During malaria infection, there are several factors that activate platelets, among which are formation of immune complexes and damage to endothelial cells. Surface contact of platelet membrane to parasitized RBCs is another stimulator (Erhart *et al*., 2004). Intravascular lysis of the activated platelets may also occur. Immune complexes generated by malarial antigen lead to sequestration of the injured platelets by macrophages in the spleen. Oh *et al*., (2001) and Oseni, *et al*., (2006) have similarly reported thrombocytopenia and platelet dysfunction resulting in hyperaggregation as important changes seen in malaria infection. Trends between increasing parasite density with a decrease in the level of hematologic parameters other than platelets count, lymphocyte

count and eosinophil count were not observed in this study.

Both the male and female malaria infected subjects studied had significantly lower lymphocyte counts ($P<0.05$) than their controls. Kern *et al.,* (2000) have attributed this decrease to P. *falciparium*-induced apoptosis. It has been speculated that redistribution of lymphocytes into body compartments and apoptosis of T cells occur in parallel during malaria attacks. The finding of this work agrees with the findings of Price *et al*., (2001) in their study of the haematological effects of acute malaria. Mean lymphocyte counts in the male and female malaria infected subjects showed a consistent decrease with increasing parasite density. This was evident in the strong negative correlation between parasite density and mean lymphocyte count ($P<0.01$) in both sexes.

The mean eosinophil count for the male and female malaria infected subjects respectively were higher than their corresponding control mean values ($P<0.05$). There were no cases of eosinophilia among the controls. Interestingly, eosinophilia was observed more on hyperparasitaemic cases.

The observed elevation in eosinophil count might have been stimulated either directly by the parasites or by cytokines or other mediators produced during the malaria attack. Eosinophils may play a role in protection against malaria by induction of parasite killing as reported by Singh, *et al*., (2009), but they may also contribute to pathology by release of granule proteins such as eosinophil cationic protein (ECP) as reported by Kurtzhals, *et al*., (1997). Another stimulus of eosinophils, according to Kurtzhal *et al*., (1998) could be immunological responses to the infections in which there is a positive association between levels of TNF and serum soluble interleukin 2 receptor (sIL-2R) and both ECP and eosinophil peroxidase (EPX) in the malaria patients, indicating that inflammatory reactions or T cell activation may play a role in eosinophil induction during acute illness. This finding suggests that sequestration and destruction of eosinophil which have often accounted for low eosinophil count in malaria patients did not occur in the studied subjects. This result agrees with the findings of Jandl, (1996) and Ladhani *et al*., (2002) who had independently reported general eosinophilia in malaria patients. It does not however agree with the findings of Layla *et al.,* (2002) who reported only mild eosinophilia in malaria infected subjects.

Mean eosinophil counts in the male and female malaria infected subjects showed a consistent significant increase with increasing parasite density. The counts correlated positively with parasite density ($P<0.01$) in both sexes. This clearly demonstrates that the level of parasitemia is the dominant factor that influences the eosinophil count in malaria infection. This is consistent with earlier works by Jandl, (1996) and Ladhani *et al*., (2002).

The mean total WBC count for the male and female malaria infected subjects respectively were higher than their corresponding control mean values ($P<0.05$). While not all studies have found an increase in leukocytes during malaria infection (Rojanasthien *et al*., 1992), this alteration is certainly not unique for *P. falciparum* (Richards *et al*., 1998).

Anemia was not found in this population even though anemia has been frequently associated with malaria. In regions of sub-Saharan Africa with stable, high malaria transmission, severe anemia is common among children under 5 years or pregnant women infected with *P. falciparum* (Guerin *et al*., 2002). Studies among non-immune or semi-immune populations outside Africa have also found statistically significant levels of mild anemia in falciparum malaria patients (Rojanasthien *et al*., 1992, Das *et al*., 1999). Two possible causes of this anemia are increased hemolysis or a decreased rate of erythrocyte production (Phillips and Pasvol, 1992). Despite the extensive documentation of anemia in malaria, anemia was not observed in this study. This discrepancy may be related to the multifactorial etiology of anemia. Furthermore, some observers have suggested that malaria- related anemia is more severe in areas of intense malaria transmission and in younger children rather than in older children or adults (Menendez *et al*., 2000).

5.0 Conclusion

In this malaria-endemic area, an acutely febrile patient with low platelet count, a reduced lymphocyte count and a reduced eosinophil count,

irrespective of malaria QBC or smear report, should always be thoroughly re-evaluated for malaria. This study used very sensitive QBC procedures, which should minimize misclassification of malaria status. Daily quality control checks of the automated cell counters maintained the accuracy and precision of the hematologic measurements.

Although these basic hematologic changes in association with malaria are not new to the subject, our data add more detailed information to the limited body of knowledge. The observations of reduced platelet, lymphocyte and eosinophil counts are not as serious as those reported in non-immune and semi-immune individuals from Nigeria and other parts of sub-Sahara Africa. This study implies that malaria must always be a key differential diagnosis in acutely febrile patients with reduced platelet, lymphocyte and eosinophil count from this endemic area. Greater exploration of the strong, inverse relationship between platelet levels and malaria infection may afford means to improve diagnosis and alleviate the clinical severity of or accelerate recovery from this disease.

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