Changes in Haematological Parameters in *Plasmodium falciparum* Infected Malaria Patients in an Urban Slum of Lagos, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author UOO designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors NA and OOB performed the statistical analysis and Authors NA, AUH, OOB and SAE managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

The present study was carried to determine the changes in haematological parameters in *P. falciparum* infected patients in Ajeromi Ifelodun area of Lagos, Nigeria. Seventy (70) human subjects comprising of 50 *P. falciparum* malarial infected and 20 non-infected (control) subjects between 10-60 years were selected for this study. RDT test and microscopy were carried out to ascertain the presence of *P. falciparum.* They were grouped based on age criteria and level of parasitaemia. This work was carried out in the Department of Biochemistry, Nigeria Institute for Medical Research Laboratory, Yaba, Lagos, Nigeria between August 2016 and January 2017. Blood samples were collected for the determination of *P. falciparum*, level of parasitaemia and haematological parameters. Haematological parameters were determined using a Coulter A-T Pierce haematology

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analyzer (Beckman Coulter, Inc. Fullerton, CA, USA), *P. falciparum* was determined by rapid diagnostic test (RDT) and Microscopy. There was a significant increase in the mean level of total white blood cells (WBC) and red blood cells distribution width (RDW), and a significant decrease in the mean level of haematocrit (HCT), haemoglobin (HGB), red blood cells (RBC), and platelets (PLT) in the malaria infected patients than in the controls (p<0.05). There was also a higher malaria parasite density among malaria infected patients for ages above 20 and a lower malaria parasite density for ages below 20 in this study. The findings of this study show that infection with *P. falciparum* produces changes in haematological parameters in those infected and tested positive for malaria. The most commonly affected parameters are haemoglobin, haematocrit, white blood cells and platelet count.

1. INTRODUCTION

Malaria remains a major cause of mortality in the tropical world, with as many as 500 million cases annually [1]. According to WHO report, almost 90% of all malaria deaths in the world occur in Africa. An estimated one million people in Africa die from malaria each year. In Nigeria, close to 96 million people are exposed to malaria, out of which 64 million get infected, and almost 300,000 deaths, reported annually [2]. Malaria poses a lot of challenges despite enormous government, foreign and donor partner efforts. The climatic condition in the tropic encourages the reproduction of the vector and the development of the parasite in the vector and host's body. Certain epidemiological factors make malaria endemic in the tropics and they include; climatic conditions (relative humidity, altitudes, rainfall level, mean temperature between 18-29ºC) and socio-economic factors.

The important ways for reducing malaria morbidity and mortality is best achieved through early diagnosis and prompt, effective treatment, which is the base for management of malaria.

Changes in haematological parameters are likely to be influenced by any disease condition including endemic diseases, such as malaria, that can affect health of mankind with various clinical presentations [3]. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. These changes involve the major cell types such as RBCs, leucocytes and thrombocytes [3–5]. Severe malaria is a disorder that affects different parts of the human biological systems, presenting with multiple manifestations, requiring hospitalization, parenteral antimalarial therapy, as well as appropriate management of evolving complications. The malaria plasmodia spend most of their complex life cycle within the cell or intracellularly, primarily within their host–cell erythrocyte [6]. Because of this parasites–red cells association, there are numerous consequences to the host's blood extending far beyond the direct effect of parasitized red blood cells, including severe anaemia, coagulation disturbances, leukocyte numerical or functional changes and spleen involvement [6–7].

Hematological alterations associated with malaria infection may vary depending on the following factors: level of malaria endemicity, background haemoglobinopathy, demographic factors, nutritional status and malaria immunity. The pathophysiological processes causing the hematological changes in malaria are complex, multiple, and incompletely understood [8].

The haematological manifestations that have been reported with malaria include anaemia, thrombocytopenia, splenomegaly [9]. There have also been reports of leucopoenia and leucocytosis [10]. In Nigeria, there have been conflicting reports on the changes in haematological parameters based on the available work. This small study was carried out in a malaria endemic area in Lagos, Nigeria and aims to determine the changes in haematological parameters in *P. falciparum* infected patients in Ajeromi ifelodun, a semi urban area in Lagos, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Laboratory and Period

This research was conducted in the Nigerian Institute of Medical Research Laboratory, Department of Biochemistry, Yaba, and the study site was in Ajegunle with Geo-coordinates,

Latitude: 6 36' 22" and Longitude: 3 16' 57''.The patients prior consent was sought before blood samples were collected from them at Ajeromi General Hospital, Ajegunle, Lagos between August 2016 and January, 2017.

2.2 Subject's Selection

Seventy (70) human subjects comprising of 50 *P. falciparum* malarial infected and 20 non-infected (control) subjects between 10-60 years were selected for the study. The place where this study was conducted is an urban slum area with high episode of malaria infection. Selection and pre-qualification was done by simple random sampling of males and females who presented for malaria parasite test at Ajeromi General Hospital Laboratory, Lagos State, Nigeria, with a history of fever (temperature > 37°C), headache and malaise within a period of 2- 8 days and who were subsequently confirmed to be *Plasmodium falciparum* malaria positive by RDT Kits (rapid diagnostics test) and later microscopic examination of Giemsa Stained thin and thick blood slide.

2.3 Patient's Selection Criteria

Based on the following selection criteria, 50 patients found to be qualified for participation in the study were selected. The ages of patients ranged from 10-60 years. All the patients diagnosed with febrile condition suggesting the presence of malaria parasites, referred to the laboratory for investigation, were recruited. All patients who are not diagnosed with febrile conditions, suggestive of malaria parasites and were not referred to the laboratory were excluded. Similarly, patients on self-medication with any antimalarial drug prior to presentation were also excluded from the study. Twenty (20) subjects in apparent good health and malaria parasite negative were included as control individuals. Consent was sought and obtained from the subjects and all the malaria positive patients were subsequently treated after blood samples were collected from them. The malaria patients and control subjects were sex - and age - matched. Presence or absence of malaria infection was confirmed using Giemsa Stain procedure.

2.4 Collection of Blood Samples

Blood sample collection was carried out through the medical officers of the Ajeromi General Hospital. A 5ml whole blood sample was drawn with 10 ml syringe from subjects by venipunture into Lithium Heparin and EDTA vacutainer tubes to prevent blood clot. Only the samples intended for biochemical assay were centrifuged at 3000 g for 10 minutes at room temperature (about 29-30°C) to obtain the plasma. The plasma was removed from the mixture using a micropipette and placed in a separate labeled container and freeze stored until required for analysis which was done within 48 h and biochemical assay was carried out within 48 h of collection.

2.5 Parasitological Examination

Randomization was performed through the selection of patients with the diagnosis of malaria by thick and thin blood smearing. The presence and density of *P. falciparum* in each blood sample was determined from Giemsa-stained thin and thick blood films. Thick blood film was prepared on the slide from the whole blood collected from each patient. The slide was stained with 3% Giemsa' stain [11] and left for about 45 min, after which the slide was washed with clean water and allowed to dry. The slide was mounted on the light microscope and screened for the presence of malaria parasite. A slide was considered negative when no parasite was found after screening of 200 fields. For those slides that were positive the number of parasites counted per 200 white blood cells was used to calculate parasite density on the basis of 8000 leukocytes per µl of blood. Parasitaemia was calculated using the formula:

$$\frac{No.\,of\;parasite \;\times\; 8000}{Count\;WBC\;\;200}$$

Positive smears were grouped into two:

Low parasitaemia, with parasite density of <1000 asexual forms per ml of blood.
High parasitaemia, with parasite density of > 10,000 asexual forms per ml of blood.

2.5.1 Procedure for staining thick and thin blood film

Thick blood film was made and stained using Giemsa's staining technique for malaria parasite detection and malaria parasite count. However, thin blood film was also made and stained with Giemsa's staining technique for plasmodium species identification. The number of asexual *P. falciparium* and other species per 200 leukocytes were counted and if ten or more parasites were identified, then the number was recorded, a

blood sample was regarded as negative if the examination of thick films failed to show the presence of asexual parasites. The parasite count in relation to the leukocyte count was converted to parasite per micro litre of blood using this mathematical formula;

$$\frac{Number\ of\ parasites}{Number\ of\ leukocytes} \times Total\ WBC\ count$$
$$=\ Parasite\ per\ micro\ litre\ of\ blood.$$

The procedure was described by Monica Cheesbrough, 2005, 3% of stock Giemsa stain was diluted in buffered water immediately before use. Thick blood film was made on clean grease free glass slide, allowed to air-dry and stained with prepared Giemsa stain for 30mintues. Stained slide was rinsed in clean water and allowed to air dry before examining under microscope using X100 objective lens. Chromatin of malaria parasite stained dark red and cytoplasm stained blue with Giemsa's stain. The diagnosis of malaria was made with certainty on identification of malaria parasite together with other symptoms associated with malaria infection. The presence of malaria parasite, identification of the species of human parasites and relative malaria parasite count in each blood sample was determined from Giemsa stained thick films and thin blood film. Malaria Parasitaemia was confirmed by microscopic examination using X100 objective lens (oil immersion lens).Thin blood film was made on clean grease free glass slide and stained using Giemsa's staining technique; the procedure was described by Monica Cheesbrough, 2005; the film was allowed to air dry and fixed with methanol, allowed to dry and stained using Giemsa stain for 30minutes. The slide was diluted with buffered distilled water and allowed to stain for 10minutes. Slide was rinsed with water; back of the slide was cleaned with damped cotton wool in methylated spirit. The slide was allowed to air dry and examined under microscope using X100 objective lens.

2.5.2 Estimating and grading of parasitaemia

Malaria parasitaemia was graded as + = 1 – 10 parasites per 100 thick film field, ++ = 11 – 100 parasites per 100 thick film field, +++ = 1 – 10 parasites per single thick film field, ++++ = more than 10 parasites per single thick film field after staining for 30 min. Identification of the species of human parasites in the blood films was carried out according to WHO method. A slide was scored as negative when 100 high power fields had been examined for about 30 minutes without

seeing any parasites. The amount of relative parasite count in positive smears was done using a simple code from one to four crosses (+ - ++++) [12].

2.6 Diagnosis of Malaria Parasite Using a Rapid Diagnostic Kit Test

Malaria *Plasmodium falciparum* was screened using commercially prepared malaria rapid test kit (Acon). The test device is a rapid chromatographic immunoassay for the qualitative detection of circulation of *Plasmodium falciparum* in whole blood.

2.6.1 Procedure

The procedure was as described by the manufacturer of the kit (Acon Laboratories, Inc.). 20 µl of whole blood was pipette into clean labeled test tube, 120 µl of buffer solution was added and waited for 1minute, contents in the test tube were mixed, 140 µl of mixed blood sample and buffer solution was pipette into specimen well on test device and waited for colour line(s) to appear. The result was read after 15 minutes. Interpretation of results: for positive result, two distinct coloured lines appeared: one line was in control region and another line was in test region. For negative result: only one coloured line appeared in the control region, result was invalid if control line fails to appear.

2.7 Full Blood Count (FBC)

A sample of 5 ml of venous blood was collected from each participant into ethylene diamine tetra-acetic acid (EDTA) bottles and promptly analyzed for routine hematological parameters which included white blood cell count (WBC), Hemoglobin level (HB), red blood cell count (RBC), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count (PLT), and mean platelet volume (MPV). The hematological parameters were determined using a Coulter A-T Pierce hematology analyzer (Beckman Coulter, Inc. Fullerton, CA, USA).

2.8 Statistical Analysis

The results were expressed as Mean ± Standard Deviation. Data obtained were analysed using student t-test to compare means. Analysis was performed using computer database software from the statistical package for social sciences

(version 16.0 SPSS). A p-value< 0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

3. RESULTS

The results obtained from this study were presented in Tables 1 and 2. Table 1 shows the haematological parameters of malaria infected patients and the controls. The total WBC, RDW were significantly higher in the malaria infected patients than in the controls (p<0.05). However, Haematocrit (HCT), Haemoglobin (HGB), Red Blood Cell (RBC), and Platelet (PLT) were significantly lower in the malaria patients than in the controls (p<0.05). Table 2 shows the malaria parasite density of different age groups in both the malaria infected patients and controls. There was an increase in malaria parasite density with age.

Table 1. Table showing mean value of haematological parameters in sample and control of malaria *P. falciparum* isolates

Haematological parameters	Sample	Control	P value
WBC(10^9/L)	7.71±4.69	5.76±1.54	<0.05
LYMPH (10^9/L)	2.83±2.25	2.25±0.66	
MID (10^9/L)	0.81±0.59	0.66±0.34	
GRAN (10^9/L)	3.48±2.3	3.02±1.61	
LYMPH (%)	40.13±16.71	40.87±14.82	
MID (%)	11.68±5.38	11.23±5.62	
GRAN (%)	48.2±16.39	47.62±14.65	
HGB (g/dL)	10.15±3.29	12.83±2.25	
RBC X(10^{12}/L)	3.7±1.15	4.61±0.81	
HCT (%)	32.42±11.12	39.63±6.1	
MCV (fl)	87.36±9.28	86.23±9.88	
MCH (pg)	27.13±3.07	28±3.2	
MCHC (g/l)	31.42±2.25	31.48±1.27	
RDW-CV(%)	15.18±2.88	13.1±1.02	
RDW-SD	47.46±7.87	46.41±5.93	
PLT(10^9/L)	169.74±118.94	235.19±73.5	
MPV(fl)	9.69±0.95	9.39±0.76	
PDW (%)	15.73±0.7	15.55±0.32	
PCT (%)	0.16±0.11	0.22±0.07	

Results were presented as Mean ± SD of three determinations.
(WBC)-white blood cell count, (HB)-Haemoglobin level, (RBC)-red blood cell count, (HCT)-haematocrit, (MCV) mean cell volume, (MCH)-mean cell haemoglobin, (MCHC)-mean cell haemoglobin concentration, (RDW)-red blood cell distribution width, (PLT)-platelet count, (MPV)-mean platelet volume, (PDW)-platelet distribution width, (LYMPH)-Lymphocyte, (PCT)-Plateletcrit, (RDW-CV)-red blood cell distribution width coefficient of variation, (RDW-SD)-red blood cell distribution width standard deviation, (GRAN) Granulocytes,(MID)-Mid-range.
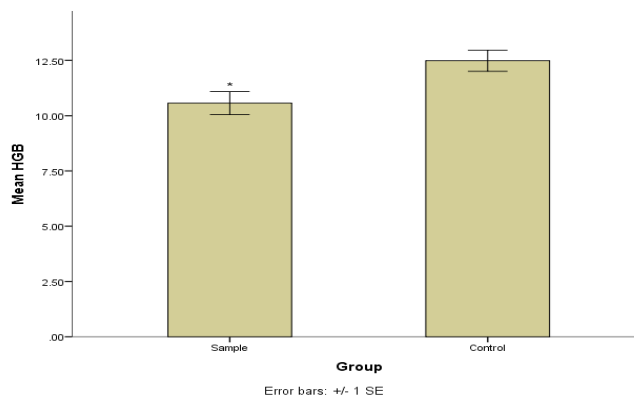

Fig. 1. Mean levels of HGB in *P. falciparum* positive and negative control samples for malaria parasite test
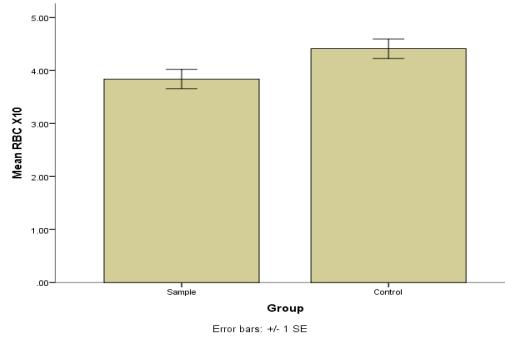
Fig. 2. Mean levels of RBC in *P. falciparum* patients and control subjects
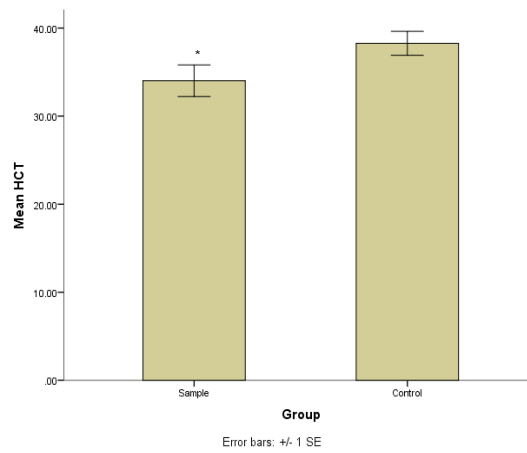

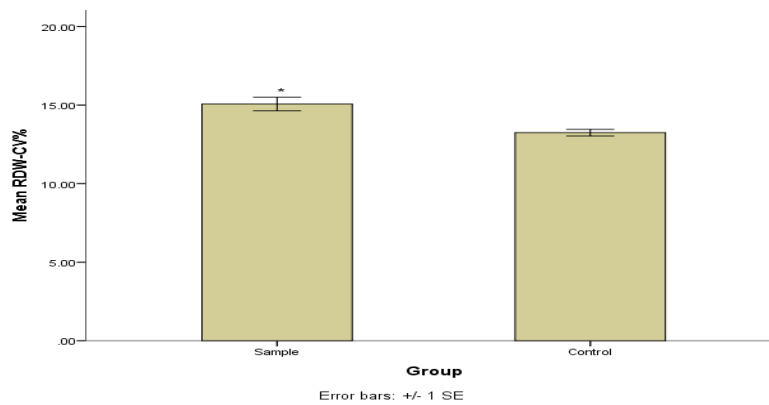**Fig. 3. Mean levels of HCT in *P. falciparum* patients and control subjects**

Fig. 4. Mean levels of RDW-CV% in *P. falciparum* patients and control subjects

Table 2. Age group and malaria parasite density (MPD)

Age (yrs)	MPD (par/µl)	P value
10-19	892.7 ± 45.2	
20-29	35365.5 ± 19561.0	.668
>30	47989.68 ± 15.83	

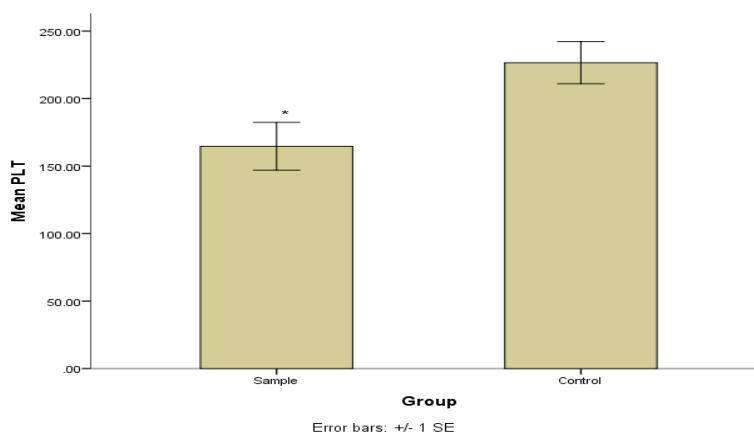*Results were presented as Mean ± SEM of three determinations*

Fig. 5. Mean levels of PLT in *P. falciparum* patients and control subjects

Table 3. Effect of parasite density on haematological parameters in *P. falciparum* positive and control samples using ANOVA table

Hematological parameters	Test	Control	P value
WBC	6.7605 ± .749	5.3423 ± .238	.903
GRAN	2.8300 ± .409	2.5577 ± .248	.026
LYM	3.4733 ± .419	2.300 ± .133	.425
PCV	34.0211 ± 1.799	38.2643 ± 1.359	.709
MCV	88.337 ± 1.567	86.832 ± 1.484	.026
MCH	27.308 ± .462	27.8500 ± .52464	.550
MCHC	31.239 ± .355	226.6071 ± 15.609	.239
PLT	164.658 ± 17.753	31.7143 ± .242	.073
HB	10.568 ± .519	12.4846 ± .479	.098

Results were presented as Mean ± SEM of three determinations.
(WBC)-white blood cell count, (HB)-Haemoglobin level, (PCV)-Packed cell volume, (MCV) mean cell volume, (MCH)-mean cell haemoglobin, (MCHC)-mean cell haemoglobin concentration, (PLT)-platelet count, , (LYM)-Lymphocyte, (GRAN)-Granulocytes

4. DISCUSSION

Malaria is a major health problem in the tropical regions of the world including Nigeria, and poses a great burden on the health sector of countries in this region of the world. In Nigeria, malaria is not just confined to urban slums but is also prevalent in the cities and rural areas. Proper and early diagnosis is critical towards the efficient and effective management of malaria.

The results obtained from this study showed that there was a significant decrease in the mean level of haematocrit (HCT/PCV), haemoglobin (HGB), red blood cells (RBC), and platelets (PLT) in *P. falciparum* infected malaria patients compared to the healthy subjects. There was also an increase in the mean level of WBC and RDW in *P. falciparum* infected malaria patients compared to the healthy subjects. The result obtained from this study showed that the mean value of HCT was lower in the malaria infected patients which is in concordance with the work carried out by [13]. Bhawna et al [14] reported a significantly lower platelet count and also lower HCT/PCV in the patients which also agree with our work and shows parasitaemia and haematological alterations in malaria. The decrease in HCT/PCV levels may be as a result of anaemia which is due to the breakdown of parasitized red cells and the subsequent removal of parasitized and deformed erythrocytes by the spleen. Infected RBCs display a reduced deformability and altered surface characteristics, which usually would lead to them being filtered and cleared by the spleen.

However, the malaria parasite *P. falciparum* has found a way to counter this protective measure. They modify their host cell membrane, which ultimately results to the cytoadherence of RBCs onto the endothelium. Infected and uninfected erythrocytes cluster together, a process called

sequestration and rosetting, and clog up the capillary and postcapillary venules of various organs [15]. In addition, the enhanced destruction of uninfected erythrocytes coupled with a decrease in erythrocyte production all add to malaria related anemia [16]. In accordance with this, RBC counts in patients with *falciparum* malaria were lower than in those patients without malaria.

In this study, there was a significant decrease in the level of HGB and RBC. Akogwu et al [17] reported a decrease mean level of PCV/HGB (anaemia). The decrease in HGB and RBC could be as a result of the breakdown of haemoglobin by the parasite in malaria infected patients and the subsequent removal of parasitized red blood cells from circulation by the reticuloendothelial system. The effect of malaria infection on haemoglobin and red blood cells can lead to a reduction in the amount of oxygen supplied to the body tissues and amount of carbondioxide removed as the number of haemoglobin and red blood cells are reduced. This can result into fatigue, causing muscle pain and convulsions [18].

A significant decrease in PLT was observed in this study (p<0.05). This result agrees with the work of Senthilkumaar and Sarojini [19] which reported that platelet counts and serum potassium levels in malaria infected blood were significantly lower in the patients when compared to the non-infected blood. Previous study has revealed the prevalence of thrombocytopenia in patients with *falciparum* malaria, but patients with severe falciparum malaria had a significantly lower platelet count compared to the non-severe *falciparum* malarial patients [20]. Results from other previous studies showed that thrombocytopenia seems to occur through peripheral destruction [21]. Immune-mediated destruction of circulating platelets may be a cause of thrombocytopenia in malaria infections, especially those caused by *P. falciparum* [22]. The pathogenic mechanisms by which platelets mediate disease severity in patients with *falciparum* malaria remains to be delineated. However, clinical studies have shown that platelets in patients with *P. falciparum* expressed Toll-like receptors (TLRs), which release prepackaged inflammatory mediators [23] such as Nitric oxide (NO), a key mediator of platelet homeostasis. A decreased bioavailability of NO was found in patients with severe malaria, which may contribute to increased platelet activation and consumption [24].

In this study, there was a significant increase in the level of total WBC in the malaria patients when compared to the controls (P<0.05). This probably reflects effective immune response to malaria being a feature in endemic areas like our study area. Sumbele et al [25] also reported an increase in WBC count in malaria patients and this study corroborates with that report. This increase in WBC count is probably a result of an increase in the release of leukocytes at the early stage of the infection, to contend and fight against the infection. In this present study, RDW values were found to be significantly higher in the malaria patients when compared to the controls, and this is in concordance with the work of Koltas et al [26], however this was in contrast to the findings of Lathia et al [27]. Elevated RDW may arise from high parasitemia and increased destruction of RBCs in P. falciparum malaria resulting in RBCs of varying sizes [26].

From this study, the malaria parasite density was higher from age group 20 years and above. This could be as a result of this age group being the most active age group and are more exposed to the vector based on their level of activity. The significance of the relation between age and malaria parasite density in this study cannot be fully ascertained because of the relatively small and uneven number of sample size in the various age groups.

5. CONCLUSION

The findings of this study shows that infection with *P. falciparum* produces changes in haematological parameters in those infected and tested positive for malaria. The most commonly affected parameters are haemoglobin, haematocrit, white blood cells and platelet count. Presence of thrombocytopaenia in combination with anaemia in *P. falciparum* positive isolate may be useful as supportive diagnostic criteria for malaria in circumstances where definitive microscopic or Rapid Diagnostic Test (RDT) may be sub-optimal, as may be the case with low parasite density. Therefore, when used in addition to clinical and microscopy parameters, it can significantly improve malaria diagnosis and ideally prompt timely initiation of anti-malarial therapy.

CONSENT

The patients prior consent was sought before blood samples were collected from them at

Ajeromi General Hospital, Ajegunle, Lagos between August 2016 and January, 2017.

ETHICAL APPROVAL

Ethical approval to conduct this study was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research, Lagos. Permission was obtained from the health facility where the study was carried out. All the research participants gave written informed consent, guardian/parental consent and/or accent for participants less than 16 years (children). Participants who declined participation were not denied treatment. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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