Effect of Anti-malaria Drugs on Some Blood Cell Lines Parameters in Adult Individuals Infected with Acute Uncomplicated *Plasmodium falciparum* Malaria

ESAN AYODELE. J

Department of Haematology and Blood Transfusion Science, Federal Medical Centre, Ido-Ekiti Ekiti State

*Corresponding author: ayodelejacob4u@gmail.com

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Abstract  Malaria remains a major public health problem in Nigeria where it is endemic, especially in rural populations as is the case elsewhere in Africa, it causes significant human suffering and impacts on social and economic development. The aim of this study is to determine the prevalence and severity of malaria parasitaemia in adult individuals caused by *P. falciparum* and to determine the effects of anti-malaria drugs on some blood cell line parameters. 202 confirmed malaria infected patients were recruited for the study between the ages of 15 – 64 years of both sexes at the general outpatient clinic of the Federal Medical Centre, Ido-Ekiti, Nigeria. 4ml of blood sample was collected twice from the same patient before and after taking anti-malaria drug for blood cell lines analysis. Malaria parasite detection, malaria parasite count and malaria parasite species identification were determined. All the subjects were infected with *Plasmodium falciparum* specie; 129(63.9%) were males and 73(36.1%) were females. In pre anti-malaria drug treatment group, mean ± SD of relative and absolute neutrophil 60.18 ± 9.41 and 4.16 ± 1.69 respectively in age group A were higher compared to other age groups, the difference was statistically significant (p<0.05) except in relative neutrophil. Changes in the white blood cell lines are less dramatic in adult individuals infected with acute uncomplicated malaria; proper malaria control strategies targeted at the less immune age group especially young adult and aging will likely reduce the burden of malaria resulting in greater clinical and haematological benefits.

Keywords: anti-malaria drug, blood cell lines, malaria parasite


1. Introduction

Malaria causes significant human suffering and impacts on social and economic development. There are four main types of parasite that cause human malaria - *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium falciparum*. *Plasmodium falciparum* malaria is the most deadly and is most common in sub-Saharan Africa, accounting in large part for the extremely high malaria-related mortality in this region [1]. There were 216 million cases of malaria, with 81% of these in the World Health Organization (WHO) African Region. An estimated 3.3 billion people were at risk of malaria in 2010 [2]. Malaria remains a major public health problem in Nigeria where it is endemic, especially in rural populations as is the case elsewhere in Africa [3]. The World Malaria Report indicated that Nigeria accounts for a quarter of all malaria cases in the 45 malaria endemic countries in Africa, showing clearly the challenge of malaria in Nigeria [4]. This may be due to the large population; approximately 140 million inhabitants [5] live in areas of stable malaria transmission. More than 90% of the total Nigerian population is at risk of malaria and at least 50% of the population suffers from at least one episode of malaria each year [6,7]. The initiative ‘Roll Back Malaria’ launched in 1998 in partnership with the United Nations Children's Fund (UNICEF), WHO and many other non-governmental agencies seems not to be producing effective results in some malaria endemic communities of Nigeria as malaria problem is still on the increase. With such reported high prevalence, there is a need to determine the extent of infection by *Plasmodium falciparum* in endemic communities of Nigeria as this will help in the proper management of the disease. The number of exposure to mosquito bites by individuals had been confirmed to increase the level of immunity against malaria infection [8] this level of immunity against malaria has also been related to age of the individuals living in malaria endemic areas [9]. Hence, notable reduction in malaria infection duration with increasing age is likely due to gradual acquisition of immunity through re-infection [10,11]. Microscopic examination of stained blood films is the gold standard for routine malaria diagnosis. Parasite density has to be reliably evaluated in order to deal with discriminatory thresholds of parasitemia. Besides, parasite counts are important especially in
Plasmodium falciparum infections, which are always considered as potentially dangerous because Plasmodium falciparum infects erythrocytes of any age with the potential of development of high-grade parasitaemia, due to the rapid multiplication of this parasite, the parasite count can increase up to 20-fold over a period of 48 hours without treatment. In the clinical setting, the level of parasitaemia is useful as one of the criteria in defining “severe P. falciparum malaria” and to monitor the effect of anti-malarial therapy [12]. A clinical malaria was defined as Plasmodium falciparum parasitaemia >2,500 parasites/μl and axillary temperature ≥37.5°C or reported fever over the previous 24 hours. Artemisinin is widely used as an agent to treat malaria; during recent years this compound and several derivatives have emerged as the most potent anti-malaria agents, being especially active against chloroquine-resistant malaria strains [13]. The mode of action of artemisinin on Plasmodium falciparum has been associated with the generation of oxidative stress. Artemisinin-based combination therapies (ACTs) are the best anti-malaria drugs available now. World Health Organization has recommended that artemisinin combination therapies (ACT) be first-line therapy for malaria worldwide [14]. Combinations are effective because the artemisinin component kills the majority of parasites at the start of the treatment while the more slowly eliminated partner drug clears the remaining parasites [15]. Artemisinin enhances efficacy and has the potential of lowering the rate at which resistance emerges and spreads. The anti-malaria activity of artemisinin and its derivatives is extremely rapid and most patients show clinical improvement within 1-3 days after treatment. All artemisinins used today are pro-drugs of the biologically active metabolite di-hydro artemisinin, which is active during the stage when the parasite is located inside red blood cells. When the parasite that causes malaria infects a red blood cell, it consumes haemoglobin within its digestive vacuole, liberating free haeme, an iron-porphyrin complex. According to one theory about the action of artemisinin, the iron of the haeme reduces the peroxide bond in artemisinin, generating high-valent iron-oxo species and resulting in a cascade of reactions that produce reactive oxygen radicals which damage the parasite and lead to its death [16]. Despite prevention and control efforts, malaria still remains a leading cause of morbidity and mortality worldwide. The true burden of malaria is difficult to estimate as many people are treated without treatment. In the clinical setting, the level of parasitaemia in adult individuals caused by P. falciparum and to determine the effects of anti-malaria drugs on some blood cell line parameters.

2. Materials and Methods

2.1. Study Location

The study site was Ido town, the headquarters of ido-osi local government in Ekiti State, Nigeria. The secretariat sited in between Ido town and Usi town. It is very close to other local government districts, (Moba, Ijero, Illejemeje and Ado). The local government comprises rural towns: Aaye, Ido, Usi, Ayetoro, Ilogbo, Osi, Ifaki, Orin, Ora, Igbole and some other smaller villages, inhabited mainly by the Ekitis, but with some non-Ekitis fund living peacefully among the people. People in Ido - Osu cherish farming, education, trading and practicing majorly Christianity religion. According to 1991 Census, the Local government has a total population of 107,000 people with eleven electoral wards in the Local government. The climate is characterized by two main seasons; the rainy and the dry season, the rainy season starts in March to October while the dry season is between November to February. The total rainfall in the area is 450mm giving a mean monthly rainfall of 121mm.There is a sharp fall in rainfall at a period between July and August. Temperature in the region is high throughout the year with a means monthly temperature of 27°C and a range of 3.7°C between the month of highest temperature (February) and the month of lowest (August).

2.2. Subjects Selection

Two hundred and two confirmed malaria infected patients with signs and symptoms of malaria were recruited for the study between the ages of 15 – 64 years of both sexes at the general outpatient clinic of the Federal Medical Centre, Ido-Ekiti, Ekiti State, Nigeria. One hundred and two apparently healthy malaria negative subjects were drowning from hospital staff at Federal Medical Centre, Ido-Ekiti which serves as study control group and comprising both sexes between the ages 15 – 64 years within March 2012 and November 2013. Similar drug treatment was given to the entire participant. The study was conducted with an informed consent of the patients. Ethical approval was obtained from ethical committee of Federal Medical Centre, Ido-Ekiti, Ekiti State.

2.3. Sample Collection

About 4ml of blood sample was collected from each subject on the first day of visiting hospital as baseline sample grouped as pre anti-malaria drug treatment sample, after the patient has been clinically diagnosis for malaria infection and confirmed using malaria rapid kit, another 4ml of blood sample was collected on the second or third day from the same patient after taking anti-malaria drugs grouped as post anti-malaria drug treatment sample. Blood sample collected was dispensed into di-potassium ethylenediaminetetraacetic acid (K2EDTA) vacuettainer bottles used for blood cells parameters analysis using haematology analyzer (sysmex model KX-21N), white
blood cells differential was counted manually using thin blood film stained with Leishman staining technique for proper identification of white blood cells. Malaria parasites were seen for using commercially prepared malaria rapid test kit; also thick and thin blood film was made for microscopic gold standard diagnosis of malaria parasite infection; for malaria parasite detection, malaria parasite count and malaria parasite species identification. Questionnaire was used to obtain the demographic characteristics and other relevant information for the study.

2.4. Methodology

2.4.1. Diagnosis of Malaria Parasite Using a Rapid Diagnostic Kit Test

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

2.4.2. Principle

The malaria *plasmodium falciparum* rapid test device (whole blood) is a qualitative, membrane based immunoassay for the detection of *plasmodium falciparum* antigen in whole blood. The membrane is pre-coated with anti-HRP-II antibody. During testing, the whole blood specimen reacts with the dye conjugate, which has been pre-coated in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with anti-HRP-II antibody on the membrane on the test line. If the specimen contains HRP-II, a coloured line will appear in the test region. The absence of the coloured line in the test region indicates that the specimen does not contain HRP-II. To serve as a procedure control, a coloured line will always appear in the control region indicating that proper volume of specimen has been added and membrane wicking has coloured.

2.4.3. Procedure

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

2.5. Microscopic Diagnosis of Malaria Parasite Using Thick and Thin Stained Blood Film

2.5.1. Thick And Thin Stained Blood Film

Thick blood film was made from EDTA blood sample and stained using Giemsa’s staining technique for malaria parasite detection and malaria parasite count. However, thin blood film was also made from well mixed anticoagulated EDTA blood sample and stained with Leishman staining technique for plasmodium species identification. Changes in parasitized red cells help to identity plasmodium species and to detect mixed infection of malaria parasite. The number of asexual *P.falciparum* 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:

\[
\text{Number of parasites} = \frac{\text{Number of leukocytes} \times \text{Total WBC count}}{\text{Parasite per micro litre of blood}}
\]

2.5.2. Principle of the Staining

Staining usually take place at a neutral pH. pH of blood is 7.4, when buffered at pH 6.8, it brings the pH to neutral pH i.e. pH 7.0. Unlike charges of stain and blood will attract, the basic part of stain methylene blue stained the acidic part of the cell i.e. the nucleus while the acidic part of stain eosin stained the basic part of the stain i.e. cytoplasm.

2.5.3. Procedure for Staining Thick Blood Film

The procedure was described by Monica Cheesbrough, 2005 (20). 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 allow air-drying before examined 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 signs and symptoms of malaria infection in humans are caused by the asexual blood stage of the parasite which includes: fever, headache, joint pains, abdominal upset, nausea, vomiting, diarrhoea, and digestive disorders. 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 Leishman stained thin blood film. Malaria Parasitaemia was confirmed by microscopic examination using X100 objective lens (oil immersion lens). 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 (+ - ++++) [21].

2.5.4. Procedure for Staining Thin Blood Film
Thin blood film was made on clean grease free glass slide and stained using Leishman staining technique; the procedure was described by Monica Cheesbrough, 2005 [20]. Thin blood film was made from well mixed EDTA anticoagulated blood; the film was allowed to air dry and flooded with Leishman stain for 3minutes. 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 dampened cotton wool in methylated spirit. The slide was allowed to air dry and examined under microscope using X100 objective lens.

2.6. Malaria Parasite Count

The determination of the number of circulating parasites is exceedingly important for clinical purposes to monitor the evolution of the disease and the efficacy of therapy. Quantitative parasitaemia count (Parasite density) was determined by counting the number of asexual parasites (trophozoites, schizonts) present in as many microscopic fields (100x) necessary to count 200 leukocytes in each thick blood film and multiplies by the total white blood cells count of each blood sample. Parasitaemia was graded as low (parasite <1000 μ L⁻¹), moderate (>1000-9,999 μ L⁻¹) and high (>10,000 μ L⁻¹) [22].

\[
\text{parasites / ul of blood} = \frac{\text{Number of observed asexual parasites}}{200(\text{Number of leucocytes counted})} \times \text{total WBC count / ul}
\]

2.7. Haematological Parameters Analysed Using Haematology Analyser (Sysmex Automated Haematology Analyser Model KX-21N, Maunfactured By Sysmex Co-Operation Kobe, Japan).

2.7.1. Principle

The aspirated blood sample is measured to a predetermined volume diluted at the specified ratio and then fed into each transducer chamber, which has a minute hole aperture and also contains electrodes through which direct current flows. Blood cells suspended in the diluents sample, pass through the aperture, causing direct current resistance to change between the electrodes, blood cell size is detected by electric pulses. Blood cell count is calculated by counting the pulses and the histogram determined by the pulse sizes.

2.7.2. Procedure

Sysmex machine was inspected (for instrument, reagents, waste bin and printer paper) before switch on the machine from power source. The machine was calibrated before used and control sample was run along each batches of sample analysis. Well mixed EDTA blood sample was used for the analysis of complete blood count, blood sample was aspirated through the sample probe one after another by pressing start switch, sample was analyzed, rinsed and display the result on the LCD screen of the machine also printed the results out. After the analysis, machine was shut down by aspirating cell clean which washed and rinsed the machine before finally shutdown and switch off from the power source.

2.7.3. Statistical Analysis

Results obtained were analyzed using student t-test to compare the means. Analysis was performed using computer database software from the statistical package for social sciences (version 16.0 SPSS). A P-value of < 0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

3. Results

Most of the participants were from rural residence and all of them were infected with *Plasmodium falciparum* species; patients without malaria parasite in their blood were disqualified from the study. Out of the 202 malaria infected patients 129 (63.9%) were males and 73 (36.1%) were females; in control subjects 58 (56.9%) were males and 44 (43.1%) were females as shown in the Figure 1.
Multiple comparisons between age group C and E show that age group C had higher mean± SD of MPC, platelet, MPV, WBC, relative neutrophil, lymphocyte, and monocyte compared to mean± SD in age group D, while age group D had higher mean± SD of PDW, relative neutrophil, lymphocyte, and monocyte compared to mean± SD in age group C; the comparison shows statistical significant difference (P<0.05) in relative neutrophil and lymphocyte. Multiple comparison between age group C and E shows that age group C had higher mean± SD of MPC, platelet, MPV, WBC, relative neutrophil, lymphocyte, and monocyte compared to mean± SD in age group D, while age group D had higher mean± SD of PDW, relative neutrophil, lymphocyte, and monocyte compared to mean± SD in age group C; the comparison shows statistical significant difference (P<0.05) in relative neutrophil and lymphocyte.

Multiple comparisons between age group A and D shows that age group A had higher mean± SD of Platelet, MPV, relative neutrophil, absolute neutrophil, absolute monocyte compared to mean± SD in age group A while age group A had higher mean± SD of Platelet, MPV, relative neutrophil, lymphocyte, and eosinophil compared to mean± SD in age group A while age group A had higher mean± SD of Platelet, MPV, relative neutrophil, absolute neutrophil, absolute monocyte compared to mean± SD in age group A; the comparison shows statistical significant difference (P<0.05) in PDW, MPV, and absolute lymphocyte. Multiple comparison between age group A and D shows that age group A had higher mean± SD of Platelet, MPV, relative neutrophil, and absolute neutrophil compared to mean± SD in age group D; the comparison shows statistical significant difference (P<0.05) in PDW, MPV, relative neutrophil, lymphocyte, and absolute lymphocyte. Multiple comparison between age group A and C shows that age group A had higher mean± SD of PDW, WBC, relative lymphocyte, absolute neutrophil, lymphocyte, and eosinophil compared to mean± SD in age group A; the comparison shows statistical significant difference (P<0.05) in WBC, relative neutrophil, absolute neutrophil, and absolute lymphocyte as compared to mean± SD in age group A respectively. Mean± SD of platelet, relative monocyte, F=5.54, P=0.00; F=3.32; P=0.00 and F=0.16, P=0.96 respectively. Mean± SD of platelets and absolute monocyte 178.42 ± 60.92 and 0.27 ± 0.18 respectively in age group E were higher compared to other age groups, F=1.69, P=0.16; F=6.36 P=0.00; F=2.71, P=0.03 and F=1.64, P=0.17 respectively. Mean± SD of WBC, absolute lymphocyte 6.22 ± 1.61 and 2.85 ± 0.90 in age group C were higher compared to other age groups; there was statistical significant difference (P<0.05) in absolute lymphocyte, F=1.36, P=0.25 and F=2.45, P=0.05 respectively. Mean± SD of PDW and relative lymphocyte 15.15 ± 2.92 and 49.53 ± 4.57 in age group D were higher compared to other age groups, there was statistical significant difference (P<0.05) observed in PDW and relative lymphocyte F=4.71, P=0.00 and F=2.40 P=0.05 respectively. Mean± SD of platelet, relative monocyte, eosinophil, absolute monocyte 187.08 ± 64.62, 0.54 ± 0.72 and 0.15 ± 0.13 in age group E were higher compared to other age groups, F=1.82, P=0.13; F=1.76, P=0.14; F=0.10, P=0.98 and F=1.15, P=0.34 respectively. Multiple comparison between age group A and B shows that age group A had higher mean± SD of MPC, platelet, MPV, WBC, relative neutrophil, absolute neutrophil, lymphocyte, and eosinophil compared to mean± SD in age group B while age group B had higher mean± SD of MPV, WBC, relative neutrophil and eosinophil compared to mean± SD in age group E; comparison show significant difference (P<0.05) in PDW. Multiple comparison between age group C and D shows that age group C had higher mean± SD of MPC, platelet, MPV, WBC, relative neutrophil, lymphocyte, and monocyte, compared to mean± SD in age group D, while age group D had higher mean± SD of PDW, relative neutrophil, lymphocyte, and monocyte compared to mean± SD in age group C; comparison show significant difference (P<0.05) in relative neutrophil and lymphocyte. Multiple comparison between age group C and E shows that age group D had higher mean± SD of platelet, MPV, relative monocyte, eosinophil and absolute monocyte compared to mean± SD in age group C; multiple comparison between age group A and D shows that age group D had higher mean± SD of platelet, MPV, relative neutrophil, lymphocyte, absolute neutrophil and lymphocyte compared to mean± SD in age group D; while age group D had higher mean± SD of PDW, relative neutrophil, lymphocyte, and monocyte compared to mean± SD in age group D; while age group D had higher mean± SD of PDW, relative monocyte, monocyte, eosinophil, absolute lymphocyte and monocyte compared to mean± SD in age group D.
SD in age group A; comparison shows significant difference (P<0.05) in MPV, relative neutrophil and lymphocyte. Multiple comparison between age group A and E shows that age group A had higher mean± SD of MPC, MPV, WBC, relative neutrophil, and absolute neutrophil compared to mean± SD in age group E while age group E had higher mean± SD of platelet, PDW, relative lymphocyte, monocyte, eosinophil, absolute lymphocyte and monocyte, compared to mean± SD in age group A; comparison shows significant difference (P<0.05) only in MPC. Multiple comparison between age group B and C shows that age group C had higher mean± SD of MPC, PDW, WBC, relative neutrophil, lymphocyte, absolute neutrophil and lymphocyte compared to mean± SD in age group B, while age group B had higher mean± SD of platelet; MPV, relative monocyte, eosinophil and absolute monocyte as compared to mean± SD in age group C. Multiple comparison between age group B and D shows that age group B had higher mean± SD of MPC, platelet, MPV, relative neutrophil, eosinophil, and absolute neutrophil compared to mean± SD in age group D, while age group D had higher mean± SD of PDW, WBC, relative lymphocyte, monocyte, absolute lymphocyte and monocyte as compared to mean± SD in age group B. comparison shows significant difference (P<0.05) in MPV, relative lymphocyte and absolute lymphocyte. Multiple comparison between age group B and E shows that age group E had higher MPC, platelet, PDW, WBC, relative lymphocyte, monocyte, eosinophil, absolute neutrophil, lymphocyte and monocyte compared mean± SD in age group B; while age group B had higher mean± SD of MPV and relative neutrophil compared to mean± SD in age group E. Multiple comparison between age group C and D shows that age group C had higher mean± SD of MPC, platelet, MPV, WBC, relative neutrophil, eosinophil, absolute neutrophil and lymphocyte compare to mean± SD in age group D while age group D had higher mean± SD of PDW, relative lymphocyte, monocyte and absolute monocyte; comparison shows significant difference (P<0.05) in only MPV. Multiple comparison between age group C and E shows that age group C had higher mean± SD of MPC, PDW, WBC, relative neutrophil, absolute neutrophil and lymphocyte compared to mean± SD in age group E; while age group E had higher mean± SD of platelet, MPV, relative lymphocyte, monocyte, eosinophil and absolute monocyte compared to mean± SD in age group C; Multiple comparison between age group D and E show that age group E had higher mean± SD of MPC, platelet, MPV, WBC, relative neutrophil, monocyte, eosinophil, absolute neutrophil and monocyte compared to mean± SD in age group D, while age group D had higher mean± SD of PDW, relative lymphocyte, and absolute lymphocyte compared to mean± SD in age group E. The comparison show significant difference (P<0.05) only in MPV as show in Table 2.

Table 1. Mean±SD of Blood Cell Lines Parameters in Pre-Treatment Malaria Infected Subjects

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>MPC µ/l</th>
<th>PLT X10^9/L</th>
<th>PDW fl</th>
<th>MPV fl</th>
<th>WBC X10^9/L</th>
<th>Neu %</th>
<th>Lym %</th>
<th>Mono %</th>
<th>Eosin %</th>
<th>Neu X10^9/L</th>
<th>Lymp X10^9/L</th>
<th>Mono X10^9/L</th>
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</thead>
<tbody>
<tr>
<td>A (n=68)</td>
<td>2606.40</td>
<td>±526.03</td>
<td>175.12</td>
<td>±50.13</td>
<td>12.84 ± 2.25</td>
<td>9.96 ± 0.74</td>
<td>6.72 ± 2.05</td>
<td>60.18 ± 9.41</td>
<td>35.50 ± 9.03</td>
<td>3.69 ± 2.79</td>
<td>0.67 ± 1.03</td>
<td>4.16 ± 1.69</td>
</tr>
<tr>
<td>B (n=59)</td>
<td>2515.7 ± 436.72</td>
<td>171.29 ± 49.67</td>
<td>13.40 ± 2.25</td>
<td>9.96 ± 0.79</td>
<td>6.24 ± 1.70</td>
<td>56.36 ± 4.36</td>
<td>38.86 ± 1.86</td>
<td>12.22 ± 1.26</td>
<td>3.61 ± 1.40</td>
<td>2.35 ± 1.06</td>
<td>0.63 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>C (n=34)</td>
<td>2782.20 ± 286.19</td>
<td>153.12 ± 40.67</td>
<td>14.71 ± 2.30</td>
<td>9.59 ± 0.77</td>
<td>7.17 ± 1.21</td>
<td>59.50 ± 8.85</td>
<td>39.00 ± 8.56</td>
<td>3.58 ± 1.42</td>
<td>9.04 ± 2.16</td>
<td>4.05 ± 0.88</td>
<td>2.81 ± 0.26</td>
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<td>D (n=17)</td>
<td>2693.70 ± 18.43</td>
<td>150.59 ± 44.45</td>
<td>15.09 ± 2.61</td>
<td>9.16 ± 0.79</td>
<td>6.84 ± 0.97</td>
<td>59.88 ± 7.15</td>
<td>45.35 ± 6.45</td>
<td>9.46 ± 1.78</td>
<td>9.94 ± 0.89</td>
<td>3.42 ± 0.73</td>
<td>2.09 ± 0.24</td>
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<td>E (n=24)</td>
<td>2702.40 ± 123.99</td>
<td>178.42 ± 60.92</td>
<td>13.60 ± 2.24</td>
<td>9.75 ± 1.08</td>
<td>6.11 ± 1.08</td>
<td>59.46 ± 10.43</td>
<td>38.58 ± 2.22</td>
<td>11.33 ± 1.55</td>
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<td>F (p-value)</td>
<td>2.68 (0.03)</td>
<td>1.96 (0.10)</td>
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<td>4.32 (0.00)</td>
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<td>1.38 (0.24)</td>
<td>1.93 (0.11)</td>
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<td>39.00 ± 8.56</td>
<td>3.58 ± 1.42</td>
<td>9.04 ± 2.16</td>
<td>4.05 ± 0.88</td>
<td>2.81 ± 0.26</td>
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<tr>
<td>D (n=17)</td>
<td>2693.70 ± 18.43</td>
<td>150.59 ± 44.45</td>
<td>15.09 ± 2.61</td>
<td>9.16 ± 0.79</td>
<td>6.84 ± 0.97</td>
<td>59.88 ± 7.15</td>
<td>45.35 ± 6.45</td>
<td>9.46 ± 1.78</td>
<td>9.94 ± 0.89</td>
<td>3.42 ± 0.73</td>
<td>2.09 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>E (n=24)</td>
<td>2702.40 ± 123.99</td>
<td>178.42 ± 60.92</td>
<td>13.60 ± 2.24</td>
<td>9.75 ± 1.08</td>
<td>6.11 ± 1.08</td>
<td>59.46 ± 10.43</td>
<td>38.58 ± 2.22</td>
<td>11.33 ± 1.55</td>
<td>19.71 ± 1.04</td>
<td>2.63 ± 0.68</td>
<td>0.27 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>F (p-value)</td>
<td>2.68 (0.03)</td>
<td>1.96 (0.10)</td>
<td>5.54 (0.00)</td>
<td>6.48 (0.00)</td>
<td>1.00 (0.10)</td>
<td>4.72 (0.00)</td>
<td>4.32 (0.00)</td>
<td>0.16 (0.06)</td>
<td>1.38 (0.24)</td>
<td>1.93 (0.11)</td>
<td>8.22 (0.66)</td>
<td></td>
</tr>
</tbody>
</table>

N.B: A = (15-24), B = (25-34), C = (35-44), D = (45-54), E = (55-64)
P<0.05 significance, P>0.05 no Significant, F (P-value) = mean ± SD of parameters compared using ANOVA.
Table 2. Mean±SD of Blood Cell Lines Parameters in Post Anti-Malaria Drug Treatment In Malaria Infected Subjects

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>MPC  µ/l</th>
<th>PLT X10^11/L</th>
<th>PDW fl</th>
<th>MPV p/l</th>
<th>WBC X10^9/L</th>
<th>Neu %</th>
<th>Lym %</th>
<th>Mono %</th>
<th>Eosin %</th>
<th>Neu X10^11/L</th>
<th>Lymp X10^11/L</th>
<th>Mono X10^11/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2410.30</td>
<td>186.96</td>
<td>13.12 ± 2.14</td>
<td>9.75 ± 0.55</td>
<td>6.04 ± 2.43</td>
<td>54.96 ± 8.35</td>
<td>43.07 ± 7.90</td>
<td>1.54 ± 1.81</td>
<td>0.44 ± 0.90</td>
<td>5.40 ± 1.63</td>
<td>3.50 ± 0.94</td>
<td>5.09 ± 1.38</td>
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<tr>
<td>(n=68)</td>
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</tr>
<tr>
<td>B</td>
<td>2480.79</td>
<td>187.01</td>
<td>13.47 ± 2.62</td>
<td>9.51 ± 0.69</td>
<td>5.39 ± 1.87</td>
<td>52.56 ± 8.99</td>
<td>45.08 ± 8.13</td>
<td>13.81 ± 1.39</td>
<td>0.77 ± 0.13</td>
<td>2.90 ± 2.07</td>
<td>3.27 ± 1.30</td>
<td>0.12 ± 0.13</td>
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<td>(n=59)</td>
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<tr>
<td>C</td>
<td>2369.50</td>
<td>162.38</td>
<td>14.84 ± 2.49</td>
<td>9.51 ± 0.69</td>
<td>6.22 ± 1.61</td>
<td>52.65 ± 7.92</td>
<td>45.15 ± 7.74</td>
<td>1.71 ± 1.06</td>
<td>0.50 ± 0.98</td>
<td>3.25 ± 0.98</td>
<td>2.85 ± 0.90</td>
<td>0.11 ± 0.73</td>
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<td>(n=34)</td>
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<tr>
<td>D</td>
<td>2172.60</td>
<td>159.12</td>
<td>15.15 ± 2.92</td>
<td>9.85 ± 0.64</td>
<td>5.72 ± 1.05</td>
<td>47.76 ± 5.23</td>
<td>49.53 ± 4.57</td>
<td>2.24 ± 0.97</td>
<td>0.51 ± 0.64</td>
<td>2.74 ± 0.64</td>
<td>2.83 ± 0.64</td>
<td>0.13 ± 0.13</td>
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<tr>
<td>(n=17)</td>
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<td></td>
</tr>
<tr>
<td>E</td>
<td>2393.00</td>
<td>184.51</td>
<td>13.47 ± 2.68</td>
<td>9.51 ± 0.70</td>
<td>6.00 ± 1.44</td>
<td>52.92 ± 9.54</td>
<td>45.17 ± 9.03</td>
<td>1.64 ± 0.72</td>
<td>0.96 ± 0.81</td>
<td>3.14 ± 2.69</td>
<td>0.15 ± 0.13</td>
<td></td>
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<tr>
<td>(n=24)</td>
<td></td>
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</tr>
</tbody>
</table>

N.B: A= (15-24), B = (25-34), C = (35-44), D = (45-54), E = (55-64)

Since control subjects have no malaria infection, MPC was null in all the age groups, however mean± SD of relative lymphocyte, monocyte, eosinophil and absolute monocytes 42.64 ± 4.20, 1.17 ± 1.06, 4.44 ± 0.77, and 0.46 ± 0.01 respectively in age group A were higher compared to other age groups, F=1.64, P=0.17, F=2.40, P=0.06; F=1.32, P=0.27 and F=2.24, P=0.07 respectively. Mean± SD of MPV, and absolute neutrophils 9.55± 0.27 and 0.30 ± 0.06 respectively in age group A were higher compared to other age groups; the difference was significance (p<0.05) in absolute neutrophil F=0.20, P=0.94 and F=2.89, P=0.03 respectively. Mean± SD of platelets, relative neutrophil and absolute lymphocyte 302.58 ± 60.46, 59.47 ± 4.74 and 0.24 ± 0.05 respectively in age group D were higher compared to other age groups; the difference was significance (P<0.05) in relative neutrophil, F=1.34, P=0.26; F=2.50 P=0.05 and F=0.07, P=0.76 respectively. Mean± SD of PDW and WBC 13.07 ± 3.59 and 4.57 ±0.12 respectively in age group E were higher compared to other age groups; F=0.59, P=0.67 and F=1.50, P=0.39 respectively. Multiple comparison between age group A and B shows that age group B had higher mean± SD of platelet, WBC, relative neutrophils and absolute lymphocyte compared to mean± SD in age group A; while age group A had higher mean± SD of MPV, MPV, relative neutrophil, absolute neutrophil and lymphocyte compared to mean± SD in age group B; while age group A had higher mean± SD of platelet, PDW, relative lymphocyte, monocyte, eosinophil and absolute monocyte compared to mean± SD in age group C while age group C had higher mean± SD of MPV, WBC, relative neutrophil, absolute neutrophil and lymphocyte compared to mean± SD in age group A. Multiple comparison between age group A and D shows that; age group A had higher mean± SD of PDW, MPV, relative lymphocyte, monocyte, eosinophil and absolute monocyte compared to mean± SD in age group B while age group D had higher mean± SD of platelet, WBC, relative neutrophil, absolute neutrophil and lymphocyte compared to mean± SD in age group A. The comparison shows significant difference (P<0.05) in relative monocyte, absolute neutrophil and monocyte. Multiple comparison between age group A and E shows that age group A had higher mean± SD of platelet, relative lymphocyte, monocyte, eosinophil, absolute neutrophil, lymphocyte and monocyte compared to mean± SD in age group E while age group E had higher mean± SD of PDW, MPV, WBC, and relative neutrophil compared to mean± SD in age group A. The comparison shows significant difference (P<0.05) in relative monocytes, eosinophil and absolute monocytes. Multiple comparison between age group B and C shows that age group B had higher mean± SD of platelet, relative lymphocyte, monocyte, eosinophil, absolute lymphocyte and monocytes compared to mean± SD in age group C while age group C had higher mean± SD of PDW, MPV, WBC, and relative neutrophil compared to mean± SD in age group D while age group D had higher mean± SD of platelet, MPV, WBC, relative neutrophil, monocyte,
absolute neutrophil, lymphocyte and monocyte compared to mean± SD in age group B. Multiple comparison between age group B and E shows that age group B had higher mean± SD of platelet, relative lymphocyte, monocyte, eosinophil, absolute neutrophil, lymphocytes and monocytes compared to mean± SD in age group E while age group E had higher mean± SD of PDW, MPV, WBC and relative neutrophils as compared to mean± SD of age group B. Comparison shows significant difference (P<0.05) only in absolute monocyte. Multiple comparison between age group C and D, shows that age group C had higher mean± SD of PDW, MPV, relative lymphocyte, eosinophil and absolute neutrophil compared to mean± SD in age group D while age group D had higher mean± SD of platelet, WBC, relative neutrophil, monocytes, absolute lymphocytes and monocytes compared to mean± SD in age group C. Multiple comparison between age group C and E shows that age group C had higher mean± SD of platelet, MPV, relative monocyte, eosinophil, absolute neutrophil, lymphocyte and monocytes compared to mean± SD of PDW, WBC, relative neutrophil and lymphocyte as compared to mean± SD in age group C; comparison shows significant difference (P<0.05) in relative and absolute monocytes. Multiple comparison between age group D ad E shows that age group D had higher mean± SD of platelet, relative neutrophil, monocytes, eosinophil, absolute neutrophils, lymphocytes and monocytes compared to mean± SD in age group E while age group E had higher mean± SD of PDW, MPV, WBC and relative lymphocytes compared to mean± SD in age group D, as shown in Table 3.

### Table 3. Mean±SD of Blood Cell Lines Parameters in Non-Malaria Infected Subjects (Controls)

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>MPC µ/l</th>
<th>PLT X10^12/L</th>
<th>PDW fl</th>
<th>MPV fl</th>
<th>WBC X10^9/L</th>
<th>Neu %</th>
<th>Lym %</th>
<th>Mono %</th>
<th>Eosin %</th>
<th>Neu 10^9/L</th>
<th>Lymp X10^9/L</th>
<th>Mono X10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=36)</td>
<td>-</td>
<td>280.33±42.68</td>
<td>12.20±1.72</td>
<td>9.51±0.29</td>
<td>4.34±0.33</td>
<td>55.78±4.49</td>
<td>42.64±2.20</td>
<td>1.17±0.29</td>
<td>4.44±0.77</td>
<td>0.28±0.05</td>
<td>0.22±0.07</td>
<td>0.46±0.01</td>
</tr>
<tr>
<td>B (n=20)</td>
<td>-</td>
<td>294.25±43.46</td>
<td>11.77±1.60</td>
<td>9.51±0.21</td>
<td>4.33±0.33</td>
<td>56.05±4.48</td>
<td>42.35±2.04</td>
<td>1.05±0.12</td>
<td>0.45±0.04</td>
<td>0.24±0.05</td>
<td>0.04±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>C (n=24)</td>
<td>-</td>
<td>276.55±44.55</td>
<td>12.02±2.68</td>
<td>9.55±0.27</td>
<td>4.37±0.36</td>
<td>58.2±4.56</td>
<td>40.54±1.62</td>
<td>1.00±0.38</td>
<td>0.21±0.04</td>
<td>0.23±0.05</td>
<td>0.04±0.01</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>D (n=19)</td>
<td>-</td>
<td>302.58±60.46</td>
<td>11.72±11.80</td>
<td>9.18±0.29</td>
<td>4.49±0.74</td>
<td>59.47±4.00</td>
<td>40.0±0.42</td>
<td>1.11±0.32</td>
<td>0.29±0.07</td>
<td>0.24±0.08</td>
<td>0.32±0.01</td>
<td></td>
</tr>
<tr>
<td>E (n=30)</td>
<td>-</td>
<td>60.91±13.07</td>
<td>5.59±3.59</td>
<td>9.51±0.15</td>
<td>4.57±0.12</td>
<td>58.6±3.21</td>
<td>41.33±3.21</td>
<td>-</td>
<td>-</td>
<td>0.18±0.10</td>
<td>0.14±0.78</td>
<td>-</td>
</tr>
<tr>
<td>F (p-value)</td>
<td>-</td>
<td>1.34±0.26</td>
<td>0.59±0.67</td>
<td>0.20±0.94</td>
<td>1.05±0.39</td>
<td>2.50±0.05</td>
<td>1.64±0.17</td>
<td>2.40±0.06</td>
<td>1.32±0.27</td>
<td>2.89±0.07</td>
<td>0.76±0.24</td>
<td>2.24±0.07</td>
</tr>
</tbody>
</table>

N.B: A= (15-24), B = (25-34), C = (35-44), D = (45-54), E = (55-64)

P<0.05 significance, P>0.05 no Significant, F (p-value) = mean ± SD of parameters compared using ANOVA.

### 4. Discussion

Prevalence of *P. falciparum* specie observed in this present study was supported by the previous study stated that *P. falciparum* is the predominant specie responsible for malaria infection in Nigeria. This present study agree with the fact that, prevalence of malaria infection decrease with advance in age as a result of gradual acquisition of immunity against malaria infection with increasing age upon repeated infection, the decrease in parasite density with increasing age is consistent with finding from previous studies performed elsewhere and is typical of infections in malaria endemic area [23,24,25]. Unpleasant effect of young age is probably related to a lack of acquired anti-malaria immunity and reduced ability to clear parasites. Acquired immunity requires exposure to several infections and develops over several years. White blood cells (WBC) play a vital role in the body’s immune defence against disease. The number of WBC may be reduced or increased depending on a disease condition or reaction occurring in the body, such reactions could be normal or abnormal. WBCs are indirectly and relatively used in estimating Plasmodium parasitaemia by counting, upon microscopic examination of Giemsa-stained blood smears, the number of parasites against a predetermined number of WBC [26]. Leucocytes count was significant higher in parasitized subjects compared to that of the
controls subjects. In this present study, WBC count in post anti-malaria drug treatment was observed lower in all age groups compared to pre anti-malaria drug treatment this may be due to the effect of anti-malaria drug which is associated with a decline in the total white cell and neutrophil counts, neutropenia is related to increased splenic sequestration. Similar to this present study, Adjuik et al., 2002 reported that there was a general trend of a slight decrease in both total WBCs and neutrophil counts after anti-malaria drug treatment [27]. However, contrary to this present study, previous study has reported increase in WBC count in the treated malaria group following treatment, lends support to the finding that malaria infection (P. falciparum) may contributes to the localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis [26,28]. Similar to this present study, Sumbele et al., 2010 reported that neutrophil counts increased over time in the untreated group while a drop was observed for those treated. Conversely, monocyte count decreased in the treated groups while an increase was observed in the untreated group [29]. Although neutrophil count positively correlated with parasitaemia density following treatment. On the contrary, Ladhani et al., 2002 [18] reported that neutrophil counts were not raised by hyperparasitaemia. The positive association of neutrophil and parasitaemia may have been influenced by intercurrent bacterial infection which was not investigated. Monocytes are important in developing immunity against P. falciparum malaria. The decrease in monocyte count in post anti-malaria drug treatment and an increase in monocyte count in the pre anti-malaria drug treatment group is this study is not unprecedented. Monocytes have been reported to act against the malaria parasite through several mechanisms; phagocytosis of malaria pigment by monocytes/ macrophages, and less frequently by neutrophils, has been observed in peripheral blood cells and bone marrow of patients with malaria [30,31,32]. High WBC count, lymphocyte and granulocyte counts observed in this study during the progress of malaria could be associated with severe or acute malaria [33]. Contrary to this present study, eosinophilia has been reported to occur after initiation of anti-malaria treatment [30]. In this present study, platelet count in pre anti-malaria drug treatment was lower than post anti-malaria drug treatment supporting that platelet counts of less than 150 x 109/ L increase the likelihood of malaria by 12–15 times [34]. Similar to this present study, thrombocytopenia usually disappears with the treatment of malaria and requires no treatment [35]. According to the previous studies, malaria parasite was found to exert a significant reduction in platelet count in parasitized subjects. An inverse relationship was established between parasite density and platelet count [36,37]. The mechanisms leading to thrombocytopenia in malaria is thought to include immune mechanisms, oxidative stress, alterations in splenic functions, and a direct interaction between plasmodium and platelets; that platelets engulf malaria parasites, and in the process they are damaged and thus being removed from circulation [30,38]. Acute malaria is often associate with mild or moderate thrombocytopenia in non immune adult from malaria endemic area and is a sensitive but non specific indicator infection with malaria profound thrombocytopenia associated with hemorrhagic manifestation or a component of dissemination intravascular coagulation [18].

5. Conclusion

Changes in the white blood cell lines are less dramatic in adult individuals infected with acute uncomplicated malaria. Hence, proper malaria control strategies targeted at the less immune age group especially young adult and aging will likely reduce the burden of malaria resulting in greater clinical and haematological benefits. Prompt use of effective anti-malaria therapy will reduce the burden of malaria.

References


[34] Lathia TB, Joshi R. Can hematological parameters discriminate malaria from non malarious acute febrile illness in the tropics? Indian J Med Sci. (2004); 58 (6): 239-244.


