Evaluation of Blood Cells and Platelets in *Plasmodium Falciparum* Malaria Infected Individuals

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Abstract  The aim of this study was to evaluate blood cells and platelets in *Plasmodium falciparum* malaria infected individuals. The study was conducted at Federal Medical Centre Ido-Ekiti, Nigeria. Two hundred and two blood samples were collected twice from each of the subjects; after obtaining informed consent and grouped as pre and post anti-malaria drug treatment samples. Thick blood film was made and stained with Giemsa’s staining technique for malaria parasite detection and malaria parasite count as described by Monica Cheesbrough, blood cell parameters were analysed using Haematology Analyser. Data obtained was analysed using SPSS version 16. The result of this present study showed that, the mean±SD of malaria parasite count, mean platelet volume, total white blood cell count, relative and absolute neutrophil count, relative monocyte count, relative eosinophil count, in pre treatment were significantly (p<0.05) higher compared to post anti-malaria treatment and control. However, the mean±SD of absolute platelet count, relative lymphocyte count in pre treatment were significantly (p<0.05) lower compared to post treatment and control. The prevalence of *Plasmodium falciparum* malaria infection in male was higher compared to female. The study showed that, high parasite density was associated with severe clinical illness and complications which significantly reduced after taking anti-malaria drug. Thrombocytopaenia is one of the haematological abnormalities observed in patients with malaria parasite infection which usually disappears with the treatment of malaria infection, changes in the white blood cells were less dramatic in this study.

Keywords: malaria parasite, blood cells and anti-malaria drug


1. Introduction

The aim of this study was to evaluate blood cells and platelets parameters in *Plasmodium falciparum* malaria infected individuals. Malaria is a serious public health problem in most countries of the tropics. It is a major cause of mortality and morbidity, between 300 and 500 million people suffer acute cases of malaria in 100 developing countries each year, and the majority of the victims are children [1]. In Nigeria about 96 million people are exposed to malaria, and out of these 64 million people get infected and almost 300,000 deaths are being reported annually in the general population, of which over 100,000 deaths are of children [2]. Malaria parasite counts is the most important aspects of reporting malaria infections, it is used to estimate the level of parasitemia present in the blood, assessment of malaria parasites morphological is also critical for accurate interpretation. Assessment of the parasite density provides a useful indicator of severity of infection, particularly in non-immune patients, and the level of parasitaemia (parasite burden or load) correlates generally with clinical features and prognosis. 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. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathology [3,4]. These changes involve the major cell lines (red blood cells, leucocytes and thrombocytes). An understanding of these hematological changes will help in diagnosis and treatment and may also serve to predict and prevent various complications [3,4]. White blood cell differential measures the percentage of each type of white blood cells (WBC) that is in the blood. It reveals if there are any abnormal or immature cells. White blood cells are an important part of body’s defense which buildup the body immune system. Other hematological reactions to malaria infection that have been reported include neutropenia, eosinophilia, neutrophilia and monocytosis [5,6]. Among the various haematological changes in malaria infection; thrombocytopaenia is the most consistent one, which occurs in more than half of the patients [7,8]. In endemic areas, malaria has been reported as the major cause of low platelet counts [9]. This is so characteristic of malaria, that in some places, it is used as an indicator of malaria in patients presenting with fever [8]. Platelet counts of less than 150 x 10⁹/ L increase the likelihood of malaria by
12–15 times \[10\]. Thrombocytopenia was seen in about 85% of the patients with uncomplicated malaria and in all the patients with severe \textit{falciparum} malaria [8]. Faseela reported that, 82.8% patients had thrombocytopenia whereas 17% patients had a normal platelet count. 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 [11,12]. The mechanisms leading to thrombocytopenia in malaria is thought to include immune mechanisms, oxidative stress, alterations in splenic functions, and a direct interaction between \textit{plasmodium} and platelets \[13\]. The precise mechanism behind thrombocytopenia, however, remains unclear. Both the immunological as well as the non immunological destruction of platelets have been implicated \[7,8,13,14,16\].

2. Materials and Methods

2.1. Subjects, Study Design and Sample Collection

This study was conducted at Federal Medical Centre, Ido-Ekiti, Ekiti State Nigeria; between November 2012 and March 2013. Subjects were \textit{Plasmodium falciparum} malaria infected adult individuals; presented with signs and symptoms of malaria infection. This was confirmed using malaria rapid kit test and microscopy detection of malaria parasite. Two hundred and two blood samples were collected twice from each malaria infected individuals; grouped as pre-treatment (at presentation) and post anti-malaria treatment using Artemisinins combination therapy, (artesunate and artemether). One hundred and two blood samples from apparently healthy adult individuals negative to malaria infection was collected for control; both \textit{Plasmodium falciparum} malaria infected subjects and controls were within the age 15-64 years. Patient’s consent was sort for through an informed consent form; also ethical approval was obtained from the hospital. Structured questionnaire was used to obtained demographic characteristic and other relevant information for the study.

2.2. Methodology

Thick blood film was made from EDTA blood sample and stained with Giemsa’s staining technique for malaria parasite detection and malaria parasite count as described by Monica Cheesbrough, (2005). Blood cell parameters which includes absolute platelet count, mean platelet volume, platelet distribution width, total white blood cell count, relative and absolute differential white blood cell count were analysed using haematology analyser (Sysmex Automated Haematology Analyser Model kx-21n, manufactured by Sysmex Co-operation Kobe, Japan)

3. Statistical Analysis

Data obtained were analysed for mean and standard deviation; to compare the mean and standard deviation in each of the groups (pre, post treatment and control group).

Significance test was done by ANOVA and t-test. Level of significance was considered as <0.05.

4. Results

Table 1: showed comparisons of \textit{Plasmodium falciparum} infected blood cell parameters in pre treatment, post anti-malaria treatment and control. The parameters includes malaria parasite count (per μl), absolute platelet count (X10⁹), platelets distribution width (%), mean platelet volume (pL), white blood cell count (X10⁹), relative neutrophil count(%), relative lymphocyte count(%), relative monocyte count(%), relative eosinophil count(%), absolute neutrophil (X10⁹), absolute lymphocyte (X10⁹) and absolute monocyte(X10⁹). The mean±SD of malaria parasite count 2628±415.22 in pre treatment was significantly (p<0.05) higher compared to 2305.20±538.62 in post treatment (F = 1369.00; P= 0.00). The mean ± SD of absolute platelet count 168.62 ± 30.05 in pre treatment was significantly (p<0.05) lower compared to 179.78± 57.41 and 285.65 in post treatment and control respectively. (F=184.38; P=0.00).The mean ± SD platelet distribution width 12.01 ± 1.75 in control was significantly (p<0.05) lower compared to mean ± SD of platelet distribution width 13.60 ± 2.44 and 13.76 ± 2.46 in pre and post treatment respectively. (F=15.50; p = 0.00). The mean ± SD of total white blood cell count 6.69± 1.67 in pre treatment was significantly (p<0.05) higher compared with 5.85 ± 1.96 and 4.42 ± 0.33 in post treatment and control respectively (F = 65.26 p = 0.00). The mean ± SD of relative neutrophil count 57.43 ± 1.67 in pre treatment and 57. 28 ± 4.66 in controls were significantly (p<0.05) higher in each case compared with 52.90 ± 8.56 in post treatment (F = 15.50 p = 0.00). The mean ± SD of relative lymphocyte count 38.27 ± 9.34 in pre treatment was significantly (p<0.05) lower compared to 44.80 ± 7.98 and 41.56 ± 4.44 in post treatment and control respectively. (F = 33.55 p = 0.00). The mean ± SD of absolute monocyte count 3.67 ± 2.18 in pre treatment was significantly (p<0.05) higher compared to 1.81 ± 1.51 and 0.93 ± 1.05 in post treatment and control respectively (F = 101.14; p= 0.00) The mean ± SD of relative eosinophil count 0.96 ± 1.36 in pre treatment was significantly (p<0.05) higher compared to 0.49 ± 0.89 and 0.31 ± 0.67 in post treatment and control respectively (F = 15.53; p<0.00). The mean ± SD of absolute neutrophil count 3.89 ± 1.37 in pre treatment was significantly (p<0.05) higher compared to 3.14 ± 1.31 and 2.54 ± 0.29 in post treatment and control respectively (F = 45.69; p<0.00). The mean ± SD of absolute lymphocyte count 1.84 ± 0.23 in control was significantly (p<0.05) lower compared to 2.49 ± 0.70 and 2.57 ± 0.35 in pre treatment and post treatment respectively (F = 40.32; p< 0.00) The mean ± SD of absolute monocyte count 0.11 ± 0.12 in post treatment was significantly (p<0.05) lower compared to 0.24 ± 0.18 and 0.42 ± 0.47 in pre treatment and control respectively (F = 88.98; p< 0.00). However, in between comparison between pre treatment and post treatment show that pre treatment had higher mean ± SD of malaria parasite count, total white blood cell count, relative neutrophil count, relative monocyte count, relative eosinophil count, absolute neutrophil count and absolute monocyte count compared to mean ± SD in post treatment.
while post treatment had higher mean ± SD of relative lymphocyte compared to mean±SD in pre treatment. However, the mean±SD of absolute platelet count, platelet distribution width (PDW) and absolute lymphocyte were higher in post treatment compared to pre treatment while mean platelet volume (MPV) mean ± SD in pre treatment compared to post treatment, show no statistical significant difference (p>0.05). In between comparison between pre treatment and control show that pre treatment had higher mean ± SD of malaria parasite count (MPC), platelet distribution width (PDW), platelet volume (MPV), white blood cell count (WBC), relative monocyte, relative eosinophil, absolute neutrophil and absolute lymphocyte compared to mean ± SD in control; while control had significantly (p<0.05) higher mean ± SD of absolute platelet, relative lymphocyte and absolute monocyte compared to mean ± SD in pre treatment. The mean ± SD of relative neutrophil in pre treatment was higher when compared to mean ± SD in pre treatment. The mean ± SD of relative neutrophil in pre treatment was higher when compared to mean ± SD of relative neutrophil in control, comparisons show no statistical significant difference (p>0.05). Multiple comparison between post treatment and control show that post treatment had significantly (p<0.05) higher mean ± SD of absolute platelet, relative lymphocyte and absolute monocyte compared to mean ± SD in control while control had significantly (p<0.05) higher mean ± SD of absolute platelet, relative neutrophil and absolute monocyte compared to mean ± SD in post treatment. The mean ± SD of relative eosinophil was higher in post treatment compared to control; comparisons show no statistical significant difference (p>0.05). However, Figure 1 showed the prevalence of P. falciparum malaria parasite infection in sex distribution for both malaria infected subjects and control. The frequency for male and female were 129 and 73 respectively in malaria infected subjects while the frequency of control (non malaria infected individual) were 58 and 44 for male and female respectively.

Malaria parasite counts is the most important aspects of reporting malaria infections, it estimate the level of parasitaemia present in the blood. In this present study, the mean value of malaria parasite count in pre-treatment was significantly higher compared to post- treatment; this was due to effect of anti-malaria drug used during treatment. McElroy et al.,[17] and Murthy et al.,[18] supported that, patients with high parasite count have more severe and complicated course, also stated that 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. Blood cell parameters which include absolute platelet counts, platelet distribution width, mean platelet volume, total white blood cell count, relative and absolute neutrophil, lymphocyte, monocyte and

<table>
<thead>
<tr>
<th>Groups</th>
<th>MPC L/μL</th>
<th>PLT X10^9</th>
<th>PDW</th>
<th>MPV X10^9</th>
<th>WBC</th>
<th>NEU %</th>
<th>LYMP %</th>
<th>MONO %</th>
<th>EOSIN %</th>
<th>NUE X10^9</th>
<th>LYM X10^9</th>
<th>MONO X10^9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Treatment (N=202)</td>
<td>2628.20 ± 415.22</td>
<td>168.62 ± 50.05</td>
<td>13.60 ± 2.44</td>
<td>9.37 ± 0.80</td>
<td>6.69 ± 1.67</td>
<td>57.43 ± 9.99</td>
<td>38.27 ± 9.34</td>
<td>3.67 ± 2.18</td>
<td>0.96 ± 1.36</td>
<td>3.89 ± 1.37</td>
<td>2.49 ± 0.76</td>
<td>0.24 ± 0.18</td>
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<tr>
<td>Post Treatment a= (202)</td>
<td>2305.20 ± 538.65</td>
<td>179.78 ± 57.41</td>
<td>13.74 ± 2.46</td>
<td>9.61 ± 0.65</td>
<td>5.85 ± 1.96</td>
<td>52.90 ± 8.56</td>
<td>44.80 ± 7.98</td>
<td>1.81 ± 1.51</td>
<td>0.49 ± 0.89</td>
<td>3.14 ± 1.37</td>
<td>2.57 ± 0.38</td>
<td>0.12 ± 0.03</td>
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<tr>
<td>Control N= 102</td>
<td>2585.65 ± 538.65</td>
<td>220.1 ± 95.51</td>
<td>12.01 ± 2.5</td>
<td>9.51 ± 0.33</td>
<td>4.42 ± 4.66</td>
<td>57.28 ± 4.66</td>
<td>41.56 ± 4.44</td>
<td>0.93 ± 1.05</td>
<td>0.31 ± 0.67</td>
<td>2.54 ± 0.29</td>
<td>1.84 ± 0.25</td>
<td>0.42 ± 0.47</td>
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<tr>
<td>F (p-value) (Pre Treatment VS (Post Treatment) p-value)</td>
<td>1369.00</td>
<td>184.38</td>
<td>20.81</td>
<td>5.97</td>
<td>65.26</td>
<td>15.50</td>
<td>33.55</td>
<td>101.14</td>
<td>15.53</td>
<td>45.69</td>
<td>40.32</td>
<td>88.98</td>
</tr>
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<td>Analysis of variance (ANOVA)</td>
<td>0.00*</td>
<td>0.10</td>
<td>0.84</td>
<td>0.06</td>
<td>0.00*</td>
<td>0.00*</td>
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<td>0.60</td>
<td>0.00*</td>
</tr>
<tr>
<td>Analysis of variance (ANOVA) (Pre Treatment VS (Control) p-value)</td>
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<tr>
<td>Analysis of variance (ANOVA) (Post Treatment VS (Control) p-value)</td>
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Table 1. Mean ± SD Of Blood Cells and Platelet Parameters in Pre, Post Anti-Malaria Treatment in Malaria Infected and Control

Figure 1. Sex Distribution of Malaria Infected Subject and Control

Figure 2. Age Distribution for Malaria Infected Subject And Control

5. Discussion
eosinophil were significant in pre-treatment, post-treatment and control subject. The mean values of absolute platelet counts, platelet distribution width, relative and absolute lymphocyte in pre-treatment were lower compared to post-treatment while mean platelet volume, total white blood cell count, relative and absolute neutrophil, monocyte and eosinophil were higher compared to post-treatment; the blood cell parameters in control subjects were within normal range. Among the various haematological changes in malaria infection; thrombocytopenia is the most consistent one, which occurs in more than half of the patients [7,8]. In endemic areas, malaria has been reported as the major cause of low platelet counts [9]. In this present study, thrombocytopenia was observed in the patient at presentation; a decrease in thrombocytopenia was observed in post-treatment. Increase in absolute platelet count observed in this present study during malaria treatment was supported by Dhanshri et al., [19], who stated that absolute platelet count rises rapidly during the recovery of the patient after the anti malaria treatment. In other study, thrombocytopenia usually disappears with the treatment of malaria infection and requires no treatment for it [20]. However, contrary to this fact, Sumbele et al., [21] reported a decrease in platelet count was observed following treatment. Mechanism of thrombocytopenia in malaria infection stated that platelets engulf malaria parasites, in the process of engulfing platelets are damaged and thus being removed from circulation [22]. Reduced platelet counts during malaria infection result from platelet activation; splenic pooling and the clumping of platelets have also been suggested as the reasons for thrombocytopenia [7,12,23]. Mean platelet volume (MPV) vary with platelet production; younger platelets are larger than older ones, low mean platelet volume (MPV) indicates average size of platelets; older platelets are generally smaller than younger ones; also low mean platelet volume (MPV) may indicate that a condition is affecting the production of platelets by the bone marrow. High mean platelet volume (MPV) indicates a high number of younger platelets in the blood; this may be due to the bone marrow producing and releasing platelets rapidly into circulation however, low platelet distribution width (PDW) indicates uniformity in size of platelets and high platelet distribution width (PDW) indicates increased variation in the size of the platelets, these facts are in support of what we obtained in the present study. In the present study, total white blood cell counts are within the normal range, although the mean values of differential leukocyte counts are in normal range. Inconsistent with Sumbele et al., [21] reported that, total and differential white blood cell counts were assessed at the onset and compared to the treated and untreated malaria groups at the end of the study, 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 [21]. Although neutrophil count positively correlated with parasitaemia density following treatment. On the contrary, Ladhani et al., [26] reported that neutrophil counts were not raised by hyperparasitaemia Molynieux et al., [27] reported that, the positive association of neutrophil and parasitaemia may have been influenced by intercurrent bacterial infection which was not investigated. Monocytes are also important in developing immunity against P. falciparum malaria, monocytes have been reported to act against the malaria parasite through several mechanisms; the higher WBC, lymphocyte and granulocyte counts during the progress of malaria could be associated with severe or acute malaria. Similarly, Facer, [22] reported that; leukocytosis is typically in a fraction of cases and may be associated with concurrent infections and/or poor prognosis. He reported that, total WBC count findings showed the vast majority to be in the normal range unlike some studies which showed that leukopenia appears to be a common finding in both non-immune patients with falciparum malaria and semi-immune children living in malaria-endemic regions, where WBC may be as low as 1-2x10⁹/L. Differential leukocyte count showed neutrophil counts are in the normal range, which differs from other studies that reported either neutropenia or neutrophilia among malaria infections [6]. About half the patients had low lymphocyte counts (lymphopenia), while others had lymphocyte counts in the normal range. Monocytes, eosinophils and basophils were in the normal range in the majority of the cases which is in conformity with this present study. Although monocytosis has been reported to occur [5,6]. Bashawri reported that the majority of malaria patients had a normal monocyte count. There was also no increase in eosinophils, which agrees with previous reports [22]. However, eosinophilia has been reported to occur after initiation of anti-malaria treatment. Facer, [22] is contrary to this present study. 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 [6,22,28]. Erythrophagocytosis of infected and uninfected red cells by monocytes/macrophages has also been observed [6,26]. In consistent with this present report, anti-malaria drug may be associated with a decline in the total white cell and neutrophil counts; Neutropenia is related to increased splenic sequestration. Leukocytosis is associated with more severe disease and is a poor prognostic marker. In a study conducted by Penali in Côte d’Ivoire where WBC and neutrophil counts were assessed on Day 0 (pre-treatment) and Day 7 (post treatment). The values at each data-point were compared for changes after treatment with anti malaria drugs. He reported that, there was a general
trend of a slight decrease in both total WBCs and neutrophil counts after treatment. In a similar study, WBC and neutrophil counts were assessed at pre-treatment and Day 14 after start of treatment. The values at each data-point were compared across treatments for changes after treatment with anti malaria drugs. There were minimal changes in WBC counts after treatment and a slight decrease in neutrophil counts in all groups [29]; their findings were similar to this present study. However, the prevalence of malaria parasite infection in male and female in this present study was supported by Akanbi et al., [30] reported the prevalence of Plasmodium infection was reportedly higher in male than in female malaria infected patients, it was concluded that the cause could be due to the fact that males expose their bodies more than females when the weather is hot and thus increases their chances of being bitten by the mosquito. Also, females are usually not naked and tend to stay indoors, helping out with household chores. This reduces their contact with the mosquito vector. Also, studies have shown that females have better immunity to parasitic diseases and this was attributed to genetic and hormonal factors [31].

6. Conclusion

The study demonstrated that *p. falciparum* malaria is an important health problem in West African countries. High parasite density was associated with severe clinical illness and complications. Parasite density was significantly reduced after taking anti-malaria drug. However, there is an indication of drug resistance in some patients manifested by increase in malaria parasite count during treatment; this was due to high parasitaemia, low immune response to malaria infection and incompliance to directive in taking anti-malaria drugs. Thrombocytopenaemia is one of the haematological abnormalities observed in patients with malaria parasite infection which usually disappears with the treatment of malaria infection and requires no treatment for it. However, changes in the white blood cells were less dramatic in this study.

References
