Anaemia and Thrombocytopenia among Children of African Descent with Acute Plasmodium Falciparum Malaria in Sokoto, North Western Nigeria

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Abstract

Introduction
Malaria is one of the most important tropical infectious diseases. The annual worldwide incidence is estimated to be 300–500 each year with a mortality of between one and three million people.

Method
This study was carried out to determine the effect of Plasmodium falciparum parasitaemia on the of the indices of anaemia (Haemoglobin, red cell count, packed cell volume, MCV, MCH and MCHC) and thrombocytopenia (platelet count) among 60 malaria parasitized children of African descent aged 6 month-5 years visiting the children emergency unit and paediatric unit of Sokoto State Specialist Hospital in Sokoto, North Western, Nigeria. Controls included 30 non-parasitized children. Haemoglobin, red cell count, packed cell volume, red cell indices (MCV, MCH and MCHC) and platelet count were analyzed using the Mythic 22 CT 5-part differential Haematology analyzer (Orphée, Switzerland). Data were analyzed using SPSS 22.0 statistical package. A p-value ≤0.05 was considered significant in all statistical comparisons.

Results
The mean haematocrit, haemoglobin, red cell count and platelet count of plasmodium parasitized children was significantly lower among the Plasmodium parasitized subjects compared to non-infected controls (p= 0.006, 0.016, 0.005 and 0.000) respectively. The mean corpuscular haemoglobin and mean corpuscular volume count, mean cell haemoglobin concentration and red cell distribution width of plasmodium parasitized children were not significantly different when compared to non-infected controls. Plasmodium parasitaemia was more prevalent among children in the 6 months-30 months age group (61.67%) compared to children in the 31 months -60 months age group (38.33%) (p<0.05). Male children were more predisposed to malaria (71.67%) compared to female children (28.33%) (p<0.05). Based on the level of educational attainment of the mothers, Plasmodium parasitaemia was more prevalent among mothers with no formal education. Plasmodium falciparum was the specie responsible for all the cases of malaria observed among the plasmodium parasitized subjects.

Conclusion
This study has shown that malaria infection has a significant negative effect of the indices of anaemia and thrombocytopenia among parasitized children of African descent. There is need for routine monitoring of some haematological parameters that are indices for anaemia and thrombocytopenia in plasmodium parasitized children. Educational enlightenment on the need to seek medical attention following potential malaria infection as well as to keep their environment clean to eradicate the vector and use of insecticide-treated nets are advocated.

Key Words: Anaemia; Thrombocytopenia; Children; African; Plasmodium Falciparum; Sokoto; Nigeria

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Implication Statement

Research on the effect of malaria infection has been largely done among adults. The aim of this study was to investigate the effect of acute Plasmodium falciparum on anaemia and thrombocytopenia among children of African descent in Sokoto, North Western Nigeria. Apart from adding to scientific literature on malaria, result generated can potential be used to improve the care offered to children with malaria.

Introduction

Malaria is a global public health problem particularly in tropical and subtropical regions. World Health Organization report that approximately 216 million cases of clinical malaria occur worldwide, with deaths of about 655,000 occurring mostly among African children [1]. It is sad to note that every 45 seconds, a child in Africa dies of malaria infection [2]. Nigeria accounts for a quarter of all malaria cases in the 45 malaria endemic nations in African continent [3].

Malaria is endemic and a major cause of morbidity and mortality. It is responsible for 25% of infant and 30% childhood mortality (FMH, 2005). The disease is responsible for 30% -40% of out-patient consultations and paediatric admission [4-5]. Children under 5 years of age are one of most vulnerable group affected by malaria. There were an estimated 438,000 malaria-related deaths around the world in 2015, of which approximately 69% were among children under 5 years of age. In malaria endemic countries, partial immunity to disease is acquired during childhood. In such areas, majority of the severe disease with rapid progression to death, occur in young children without acquired immunity [6].

A major challenge associated with the clinical management of malaria is that the symptoms are varied and frequently mimic other common childhood illness; fever, gastroenteritis, meningitis, nausea, vomiting, pneumonia and headache may be the sole symptoms. However, children with malaria are more likely to have high fever (>40°C), which can potentially lead to febrile convulsion [7].

Malaria is known to have a negative impact on full blood count (FBC) parameters, of which the most prominent are anaemia and thrombocytopenia [8]. Anaemia is one of the most common complications in malaria infection especially in younger children and pregnant women in endemic areas [9]. It has been reported that over half of malaria-related deaths are attributed to severe anaemia [10]. The pathogenesis of anaemia during malaria infection is not clearly understood. However, it is believed to result from the destruction and fast-tracked removal of parasitized and non-parasitized red cells [11] and bone marrow dysfunction [12].

Anaemia is frequent in developing countries and its etiology is usually multifactorial. The most important factors that contribute to anaemia include parasitic infections, HIV infection, chronic inflammatory disorders, micronutrient deficiencies and genetic disorders [13]. Malaria-related anaemia is associated with many factors which involve increased destruction and reduced production of red blood cells [14].

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 red cells and thrombocytes [15-19]. Malaria infected patients tend to have significantly lower platelets, and Haemoglobin (Hb0 level compared to non-malaria infected patients [20-21]. The most common complication during malaria infection is thrombocytopenia [19]. Individuals with platelet counts <150,000/μL are 12-15 times more likely to have malaria infection than persons with platelet counts >150,000/μL [19].

Evaluation of some haematological parameters may be beneficial for monitoring malaria-infected children as well as determining the relationship between clinical and haematological changes among children with Plasmodium falciparum malaria in Sokoto, North Western Nigeria in particular and Nigeria in general. This study focused on the determination of anaemia parameters (HB, RBC count, PCV, MCV, MCH and MCHC) and thrombocytopenia parameter (platelet count) among children with acute Plasmodium falciparum malaria in Specialist Hospital, Sokoto. Result from this study apart from adding to scientific knowledge, will provide valuable information that can potentially optimize the care offered to children with malaria in the area.

Materials and Methods

Study Area

The study was conducted Specialist Hospital, Sokoto State, Nigeria. The hospital serves as a referral center for people from Sokoto, Kebbi and Zamfara and the neighboring Niger and Benin Republic, in the West African sub region. The study area is located in Sokoto State, which is in the extreme North-Western part of Nigeria between longitude 05° 11’ to 13° 03’ East latitudes 13° 00’ to 13° 06’ North. The State share borders with the Republic of Niger to the North, Kebbi State to the West and South East and Zamfara to the East. The State covers a land area of about 32,000 square kilometers and with a population of 4.602298 million based on the United Nation Population Fund projection [22]. Sokoto is, on a whole, a very hot area. However, maximum daytime temperatures are most of the year generally under 40°C (104°F). The warmest months are February to April. The raining season is from June to October during which showers are a daily occurrence, although rarely last long compared to that of the Wet tropical regions. The indigenous inhabitants of the area are the Hausas and Fulani. Other ethnic groups resident in the State includes; Igbo, Ebira, Yoruba, Igala as well as Buzus from the neighboring Niger Republic. Farming and crop production are the major occupation of the people living in the study area. The major crops grown in the area includes millet, sorghum, ground nuts, cowpea and tobacco. Livestock reared includes cattle, sheep, goat, donkey, camel, horses and poultry [23].

Subjects

The subjects for this study included 60 malaria parasitized children aged 6 month-5 years. Controls included 30 non-parasitized children. Subjects
were recruited consecutively among the children visiting the Emergency Unit and Paediatric Department while controls were recruited from among children visiting the immunization unit of Specialist Hospital, Sokoto North-Western Nigeria.

Inclusion Criteria
The following children that met the inclusion criteria; aged between 6 months – 5 years with confirmed Plasmodium parasitaemia visiting the Paediatric Outpatient Department of Specialist Hospital Sokoto, Nigeria and children who parents and guardian give a written informed consent for their ward to be included in the study were consecutively recruited into the study.

Exclusion Criteria
The following children who did not meet the inclusion criteria (children aged < 6 months and > 5 years and children who parents or guardian refused to give a written informed consent for their ward to participate in the study) were excluded from the study.

Study Design
The research was a case-control study involving malaria parasitized children (subjects) and non-malaria parasitized controls. The aim of the study was to assess some full blood parameters that are indices of anaemia and thrombocytopenia among 60 Plasmodium parasitized children aged (6month-5 years). Thirty (30) age - matched non-parasitized children will be monitored as controls. Subjects were recruited from among the children visiting the Paediatric Clinic of Specialist Hospital, Sokoto. Blood samples were collected (from both subjects and controls) and tested for HB, RBC count, PCV, MCV, MCH, MCHC and platelet count. Results of these parameters will be analyzed using appropriate data analysis instrument.

Ethical Consideration
Ethical approval for this study will be obtained from the Ethics Committee of Specialist Hospital, Sokoto.

Principle of FBC
The haemoglobin, red cell count, packed cell volume, red cell indices (MCV, MCH and MCHC) and platelet count were analyzed using the Mythic 22 CT 5- part differential Haematology analyzer (Orphée, Switzerland). The analyser is based on impedance principle on the basis that red or white cells are poor conductor of electricity compared to the diluents. When cells pass through an aperture the diluent is displaced by a cell and produces a measurable change resistance. Cells passing through the aperture displace diluent and being bad conductors of electricity, produces as increased resistance which is counted as a voltage pulse. The cell suspension is drawn through the aperture with help of vacuum pump into a system of tubing. When a cell passes through the aperture, it displaces an equal volume of the conducting solution and increases the electrical resistance, creating a voltage pulse. The height of the pulse (impedance) is proportional to the volume of the cell [24].

Statistical Analysis
The data analysis was performed using Statistical Package for Social Sciences (SPSS) version 22.0. Data was presented as mean ± standard error of mean (SEM) and percentage. Student t-test for mean comparison between two groups was used. A p-value of less than or equal to 0.05 (p≤0.05) was considered as statistically significant in all statistical analysis.

Results
The result of 60 malaria parasitized subjects and 30 non-parasitized apparently controls were analyzed. A structured interviewer administered questionnaire was used to obtain socio-demographic information of the subjects. Blood samples were analyzed for red blood cell, total, haematocrit, haemoglobin concentration, mean cell volume, mean cell haemoglobin concentration and platelet counts in the haematology laboratory in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria.

Table 1 shows the socio-demographic characteristics of the parasitized subjects and the non-parasitized controls. Majority of the subjects 37 (61.67%) and control group 17 (56.7%) were in the age range of 31-60 months. The distribution of patients and control group based on ethnicity shows that majority of the subjects and control participants were Hausa 59 (98.3) and 27 (90%) respectively. The distribution of the patients based on maternal education status shows that none of the mothers were educated to tertiary level, 5 (8.33%) had secondary education, 16 (26.67%) had primary education and 39 (65%) had informal education as compared with control where none of the mothers were educated to tertiary level, 11 (36.67%) had secondary education, 9 (30%) had primary education and 10 (33.3%) had informal education. The distribution of subjects and control based on gender shows that majority were male 43 (71.67%) and 17 (28.33%) respectively.

Table 2 shows a comparison between some haematological parameters among parasitized subjects and non-parasitized controls. The mean Red blood cell, haematocrit, haemoglobin, and platelet count was significantly lower among malaria-parasitized children compared to non-parasitized controls (p<0.05). There were no statistically significant differences in the MCV, MCH, MCHC and RDW of malaria-parasitized and non-parasitized controls (p>0.05).
Table 1: The socio-demographic characteristics of subjects and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (N=30)</th>
<th>Group II (N=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-30 month</td>
<td>13 (43.33%)</td>
<td>37 (61.67%)</td>
</tr>
<tr>
<td>31-60 month</td>
<td>17 (56.7 %)</td>
<td>23 (38.33%)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (26.65%)</td>
<td>17 (28.33%)</td>
</tr>
<tr>
<td>Male</td>
<td>22 (73.33%)</td>
<td>43 (71.67%)</td>
</tr>
<tr>
<td><strong>Maternal education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>9 (30 %)</td>
<td>16 (26.67%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>11 (36.67 %)</td>
<td>5 (8.33%)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Informal</td>
<td>10 (33.3%)</td>
<td>39 (65%)</td>
</tr>
</tbody>
</table>

Key: Group 1=Control group and Group 11= Subjects

Table 2: Mean Comparison of Some Haematological Parameter in Children with Malaria and Controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>4.58 ± 0.09</td>
<td>3.46 ± 0.09</td>
<td>7.44</td>
<td>0.005 (s)</td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>10.50 ± 0.22</td>
<td>8.74 ± 0.23</td>
<td>4.72</td>
<td>0.016 (s)</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>32.69 ± 0.52</td>
<td>26.62 ± 0.68</td>
<td>587</td>
<td>0.006 (s)</td>
</tr>
<tr>
<td>MCV</td>
<td>72.41 ± 1.49</td>
<td>77.21 ± 1.07</td>
<td>-2.59</td>
<td>0.303</td>
</tr>
<tr>
<td>MCH</td>
<td>23.64 ± 0.64</td>
<td>25.20 ± 0.49</td>
<td>-1.87</td>
<td>0.237</td>
</tr>
<tr>
<td>PLT</td>
<td>322.5 ± 21.16</td>
<td>132.75 ± 6.18</td>
<td>10.99</td>
<td>0.000 (s)</td>
</tr>
<tr>
<td>RDW</td>
<td>19.31 ± 0.69</td>
<td>16.21 ± 0.41</td>
<td>4.07</td>
<td>0.333</td>
</tr>
</tbody>
</table>

Key: Group 1=Control, Group 11= Subjects, RBC=Red blood cell, HBG=Haemoglobin, HCT=Hematocrit WBC= White Blood Cell count, PLT= Platelet and RDW=Red Cell Distribution Width

Figure 1: Film showing Trophozoites of P. falciparum from one of the parasitized subjects

Figure 2: Film showing Hypochromic Red Cells from one of the parasitized subjects
In this study the indices used for the diagnosis of anaemia (RBC, PCV and HB) were significantly lower among plasmodium parasitized subjects when compared to controls. Our finding is also consistent with previous report [34] which indicated a higher incidence of anaemia among parasitized children compared to controls. Anaemia is one of the most common complications in malaria infection especially in younger children and pregnant women in high transmission areas [35]. The pathogenesis of anaemia during malaria infection is not clearly understood. However, it is thought to result because the parasite's primary target is the red blood cell resulting in RBCs destruction, accelerated removal of both parasitized and non-parasitized cells [10] and bone marrow dysfunction [36].

This study, reported a significant reduction of Hb level, PCV and RBC count plasmodium parasitized subjects compared to non-parasitized controls. Haematological abnormalities have been observed in patients with malaria, with anaemia, and thrombocytopenia being the most common [37-38]. Anaemia is a known complication of malaria. It has a profound effect on the quality of life of people by inducing such symptoms as loss of stamina, rapid heart rate and shortness of breath [39]. Malaria is a significant risk factor for the development of anaemia in children [40]. Malaria significantly affected the prevalence of anaemia [41]. During their blood stages of infection, both *P. falciparum* and *P. vivax* induce anaemia. Severe malarial anaemia (SMA) caused by *P. falciparum* is responsible for approximately a third of the deaths associated with disease [42]. Severe anaemia (defined as haemoglobin concentration of < 5 g/dL) remains is a major health problem in endemic areas among parasitized children and pregnant women and a main cause of the infant mortality associated with malaria. Malarial anaemia appears to be multi-factorial. It involves increased removal of circulating erythrocytes as well as decreased production of erythrocytes in the bone marrow. In addition to removal of infected and uninfected erythrocytes, decreased erythrocyte production and/or suppression of the erythropoietic response cause SMA. Deficient erythropoietin production does not appear to be the cause of inadequate erythropoiesis in malaria [43]. Co-infections with worms or bacteria further complicates the malaria-associated anaemia particularly in children [44-45]. Genetic disorders and nutritional deficiencies were also associated, suggesting that there clearly were multiple causes of anaemia [46].

In this study, we observed a higher male gender predisposition to malaria infection. This conforms with previous reports which indicated that *Falciparum* malaria was profound in males than female [27-28]. Our finding is also consistent with the report of a previous study in Nigeria which indicated that the prevalence of malaria is higher among male children under five years than their female counterpart [29]. This also conforms with previous reports which showed that male children generally suffered more malaria compared to their female counterpart [30-31].

It was observed in this study that the majority of the mothers of the plasmodium parasitized subjects (65%) had no formal education; the remaining (26.6%) had primary education and (8.33%) had secondary education. This finding is in agreement with other previous studies [32-32] which found strong statistical association between child health, survivorship, improved nutritional status and increased immunization uptake with maternal education levels. A study in Kenya found that women reporting some higher level of education were more likely to own and use insecticide- treated bed net obtained from retail sector outlets than those without any formal education [33].

Discussion

Malaria is endemic throughout most of the tropics. Ninety-five countries and territories have ongoing transmission with approximately 1.2 billion at high risk [25].

The subjects included in this study were aged 6 months- 5 years. This finding is consistent with previous reports which indicate that children under 5 years of age are one of the most vulnerable groups affected by malaria. In endemic countries, partial immunity to disease is acquired during childhood. In such settings, the majority of malarial disease, and particularly severe disease with rapid progression to death, occur in young children without acquired immunity [26].

In this present study, we observed a higher male gender predisposition to malaria infection. This conforms with previous reports which indicated that *Falciparum* malaria was profound in males than female [27-28]. Our finding is also consistent with the report of a previous study in Nigeria which indicated that the prevalence of malaria is higher among male children under five years than their female counterpart [29]. This also conforms with previous reports which showed that male children generally suffered more malaria compared to their female counterpart [30-31].

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Figure 3: Blood Film showing Thrombocytopenia from one of the Thrombocytopenic parasitized subjects with Blood Film showing Thrombocytopenia from one of the Thrombocytopenic parasitized subjects with
finding is in agreement with previous studies conducted in Kenya [47] and Thailand [48] which reported that patients with malaria are likely to have thrombocytopenia. Thrombocytopenia has been observed in malaria infected children in earlier reports [49-50]. The pathophysiology of malaria associated thrombocytopenia is multifactorial. It has been associated with sequestration and pooling of the platelets in the spleen, immune-mediated destruction of circulating platelets, and platelets mediating the clumping of P. falciparum-infected erythrocytes [49-51]. Thrombocytopenia is commonly seen in Plasmodium vivax malaria, but its prognostic value has not been addressed in children [52]. Thrombocytopenia is one of the most common complications of both Plasmodium vivax and Plasmodium falciparum malaria [53].

The proportion of patients having severe manifestations including thrombocytopenia is significantly high in association with P. vivax mono-infection [54]. Out of 130 cases detected with vivax malaria in a previous report [55], 100 (76%) cases had thrombocytopenia. Patients with severe falciparum malaria had a statistically significant lower platelet count (P=0.01) compared to non-severe falciparum malaria [56]. Some but not all studies have shown that there is strong association between thrombocytopenia and severity of malaria [57-58]. Thrombocytopenia has been reported in the majority of malaria studies [59]. Gerardin et al. [60] have studied the utility of thrombocytopenia as a prognostic marker in falciparum malaria alone. In an African study, the mean platelet count in falciparum malaria was 115×10^9/l [61]. The mean platelet count in vivax malaria in the study by Jadhav et al. was also 115×10^9/l [62].

The exact mechanism of thrombocytopenia in malaria is unknown. Previous report [63] demonstrated that malaria parasite has a direct lytic effect on the platelets. Both non-immunological destruction as well as immune mechanism [64] involving specific platelet associated IgG antibodies that bind directly to malarial antigen in the platelets have been recently reported to play a role in the lysis of platelets [65]. Oxidative stress damage of platelets has also been implicated in the etiopathogenesis based on the finding of low levels of platelet superoxide-dismutase and glutathione peroxidase activity and high platelet lipid peroxidation levels in malaria patients, when compared to those of healthy subjects [66]. Decreased thrombopoiesis has been ruled out, because platelet forming megakaryocytes in the marrow are usually normal or increased [67-68]. A good tolerance of low platelet count is well-known in malaria. This could be explained by platelet activation and an enhanced aggregability [69]. The hyperactive platelets may enhance haemostatic responses and this may be the reason why bleeding episodes are very rare in acute malarial infections, despite significant thrombocytopenia [70]. Recognizing the disease in the early stages can prevent the disease from becoming fatal.

Anaemia and thrombocytopenia are the most frequent malaria-associated haematological complications [71].

Anaemia and thrombocytopenia can become a diagnostic indicator for malaria among children presenting with acute febrile illness in Nigeria [72]. The presence of thrombocytopenia in acute febrile travelers returning from tropical areas to non-endemic regions has become a highly sensitive clinical marker for malaria diagnosis [73].

In this study we observed that there were no significant differences between the RDW and MCV of malaria parasitized children and non-parasitized controls. Red cell distribution width (RDW) is a measure of population dispersion of red cell volume or variation in size of red blood cells. Our finding is at variance with a previous report [74] which indicated that red cells were enlarged after malarial invasion and that RDW values were higher in malaria infected subjects compared to non-parasitized controls. Our finding is however consistent with the findings of Lathia et al. [69]. The opinions of authors seem divided on the sensitivity and specificity of RDW as diagnostic marker in malaria diagnosis. While a previous author considered high RDW a poor marker [69] another does not [74]. The role of RDW in the diagnosis of malaria seem debatable. However, the presence of increased RDW correlated well with the percentage of macrocytes in a previous report [75]. The variation in RDW in a previous report was attributed to infection of red cells by malaria parasites (particularly P. vivax), where the cells become enlarged. This is however not a common finding in P. falciparum malaria, were they retain their original size [74].

In this study we observed that Plasmodium falciparum was the predominant specie among the plasmodium parasitized children. Our finding is consistent with previous reports [34,40,59,61] in Nigeria which indicated that Plasmodium falciparum is the predominant specie responsible for cases of malaria in Nigeria.

Conclusion and Recommendations

This study indicates that plasmodium parasitaemia has a significant impact on the indices of anaemia (haemoglobin, packed cell volume, Red blood cell count) and thrombocytopenia (platelet count) of malaria parasitized children in Sokoto. There was no significant impact on the MCV, RDW and MCH. Malaria is more prevalent among the lower socio-economic class among children whose mothers have no formal education. We recommend that there is need to routinely monitor the full blood count among plasmodium parasitized children in Sokoto, Nigeria, promote the use of insecticides- treated bed net and the use of mosquito repellent to reduce the incidence of malaria among children in Sokoto, Nigeria. Preventive treatment with antimalarial drugs and effective follow up is required to prevent the development of anaemia and thrombocytopenia and to prevent the development of drug resistant strains. There is need for the enrollment of more females in school to improve their access to formal education. Community awareness program to strengthen the malaria prevention program by educating parents on the benefits of effective environmental sanitation aimed at destroying the breeding sites of Anopheles mosquito -the vector of malaria is advocated.
References


