Recovery Trends of Thrombocytes in the Parasitaemia of *Plasmodium Falciparum* Treated Children

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Abstract: This study was conducted in the malaria endemic settlements of Lake-Alau, Borno State, Nigeria, between August to December, 2012. The relationship between the mean parasite densities on days 0, 3, 7, 14 and 28 during follow-up and the trends of recoveries from thrombocytopenia in children (≤59 months) was conducted on *Plasmodium falciparum* malaria using the Standard therapeutic guidelines protocols. A sample size of 313 children was enrolled for the study, all suffered from uncomplicated *Plasmodium falciparum* malaria and treated with either Artesunate + Sulphadoxine - Pyrimethamine (AT+SP) and Amodiaquine + Sulphadoxine - Pyrimethamine (AQ+SP). The results shows if the study showed that there was a higher initial platelet count of 77975 x10⁹/µl in AQ+SP group compared to AT+SP (55281 x 10⁹/µl) patients, and also the influence of parasitaemia on platelet distribution was relatively higher (98.30%) in AT+SP compared to AQ+SP (95.98%) combinations. Both drugs significantly acted in a similar pattern but the trend of mean daily reduction in parasite density per µl of blood which caused a mean recovery in platelets by 48,606 x10⁹/µl in AQ+SP as against 37,956 x10⁹/µl for AT+SP over 28 days of follow-up.

Keywords: recovery, trends, thrombocytes, parasitaemia.

1. Introduction

Malaria remains a leading cause of ill health, causing an estimated 243 million cases of clinical malaria and 863 thousand deaths [31]. More than 85% of malaria cases and 90% of malaria deaths occur in Africa, south of Sahara [32]. In Africa, the vast majority of cases and deaths occur in young children [31]. Malaria due to *Plasmodium falciparum* remains one of the most prevalent and significant health threats as well as a major cause of pre- and post-natal mortality with low birth weights [9]. Platelet abnormalities in malaria are both qualitative and quantitative and the association of platelet count and malaria has previously been described as strong [1], [28]. Studies conducted on the linkage of malaria to platelet distribution among children indicated that over 50% had high parasitaemia (> 10%) with a platelet count of <50,000/uL and these observations imply that thrombocytopenia could be a marker of parasite burden and disease severity [24].

Hematological changes in general are reported in association with malaria and lower platelets counts or thrombocytopenia are identified as one of the common haematological abnormalities [18],[6] and changes are the most common complications in malaria and they play a major role in malaria pathology. Platelet count is the only parameter in the malaria-infected group that shows a decreasing trend across quartiles of parasite density [22]. Malaria is strongly associated with various degrees of thrombocyte counts, such as mild (<150,000 to >50,000 x10⁹/µl) moderate (<50,000 to >20,000 x10⁹/µl) severe (Severe (<20,000 x10⁹/µl) thrombocytopenia and strongly in association with malaria infections [19]. The causes of thrombocytopenia is linked to immune-mediated lyses, sequestration in the spleen and dyspoietic process in the marrow with diminished platelet production, such are features postulated by [16], [20]. Thrombocytopenia is a common finding in malaria and its correlation with the type of malaria and parasitaemia are of prognostic value and significant in health care deliveries [15] and also considered an important predictor of severity in childhood falciparum malaria [14].

2. Materials and Methods

2.1 Study site

The study took place in the peri-urban outpatient primary Health Center of Kayamla village around Lake-Alau in Konduga Local Government Area of Borno State Nigeria. It is located at Lat: 120N and 130N; Long: 110E and 130E (Fig. 1). The primary Health Center caters for 63 village settlements with a combined population of 114,224 heads (National Population Commission, 1991).

Patient’s enrollment criteria

2.2 Recruitment and Treatment Allocation Procedures

2.2.1 Ethical clearance

Prior to the commencement of the project, ethical clearance was sought from the Health Department of Konduga Local Government Area and letter of consent were served to the respective village and the district heads of the affected settlements.
2.2.2 Inclusion and exclusion procedures
Complete physical examination was performed and full history obtained by qualified medical personnel. Detailed information concerning the history of present illness, past and present drug history such as hypersensibility to 4-aminoquinolines was recorded into case record form (CRF).

Admission into the study was based on WHO (2003) guideline for evaluating anti-malarial drugs in children (6 - 59 months), with clinically apparent uncomplicated malaria, devoid of danger signs like inability to drink or breastfeed, vomiting, recent history of convulsions, lethargy, unconsciousness and inability to sit or stand up. Mono - infection with a slide - confirmed infection by *Plasmodium falciparum* with asexual blood stage parasitaemia (i.e. no mixed infections) with initial parasite density requirement of > 2,000 and < 200,000 asexual parasitaemia and present drug history such as hypersensibility to 4-aminoquinolines was recorded into case record form (CRF).

2.2.3 Treatment Allocation
All patients were assigned study numbers and groups at enrollment prior to drug administration and referred to the clinicians for treatment as assigned either AT + SP or AQ +SP treatment groups based on protocols by [32]. Group one - Each of the children orally received 4 mg/ kg body weight artesunate daily for three days and a combined 25 mg/kg body weight sulphadoxine and 1.25 mg/kg body weight pyrimethamine as single oral dose on the first day of treatment. Similarly, Group two- Each of the children orally received 10 mg/kg body weights of amodiaquine daily for three days and also a combined 25 mg/kg body weight sulphadoxine and 1.25 mg/kg body weight pyrimethamine as a single oral dose on the first day of treatment.

2.3 Experimental Procedure

2.3.1 Blood Sampling Techniques
The techniques of the [29] were followed for blood sampling and finger prick and venipuncture (syringe) methods were both employed for blood sampling on days 0, 1, 2, 3, 4, 7, 14 and 28 for the assessment of parasite densities and other platelet counts.

2.3.2 Parasite density count (per µl)
Two slides of thick and thin blood films were prepared for each patient consistent with [13].

2.3.3 Thick film
The blood was stained using 10% Giemsa (pH 7.1 – 7.2) for 10 - 15 minutes for rapid appraisal of the patient for inclusion in the study at the field.

2.3.4 Thin film
Laboratory procedures [31] for parasite densities determination were employed. Sampled blood was stained for 30 - 45 minutes using 3% Giemsa for the assessment of parasite density. The slides were examined using objectives of a research microscope (x100) for asexual parasites counted alongside with 200 leukocytes. In an even that parasite count was < 10 parasites/200 leukocytes; count was continued per 500 leukocytes. The parasite density was expressed as the number of asexual parasites per ml of blood by assuming a mean normal leukocyte count of 8000/µl of blood.

Parasitaemia (per µl) = number of parasites x 8000 / number of leucocytes (200/500)

2.3.5 Platelet count (x109/µl):
Micropipette was used to measure out 20 µl of EDTA anticoagulated blood sample. The blood sample was then diluted in ammonium oxalate 10 g/l (1% w/v) which haemolyzed the red blood cell. Platelets in the small squares of the haemocytometer were then counted under the objectives of the microscope (x40) using hand tally counter. The actual platelet count was then directly reflected as the platelet count x 109 per liter of blood [8].

2.4 Data Management and Analysis
Data collected were subjected to descriptive statistics using the analytical software Statistics equations. Means, standard deviations (SD) and ranges: Means, standard deviations (SD) and ranges were computed for Version 8.0 (Microsoft, 2003). Charts were drawn using Microsoft Excel (2003) and the regression for the rates in parasite and platelet count determined.

3. Results
Figures 1 and 2 shows the trends of parasite densities influence on platelets concentrations in the two experimented drugs during follow-up periods. The initial/intercept platelet count was relatively higher in AQ+SP (77975 x109/µl) compared to AT+SP (55281 x 109 / µl) patients at inception; however, the effect was higher in the former (98.30%) than later group (95.98%). This correlated by a daily reduction in each parasite density per µl caused mean recovery in platelets by 48,606 x109/µl and 37,956 x109/µl in AT+SP and AQ+SP, respectively.
4. Discussion

A decreasing trend of platelet count with corresponding increase in parasitemia was observed in the present study which is consistent with [26], [2]. Many studies have evaluated platelet counts in malaria infection, but few have followed counts up to 28 days of post-treatment in uncomplicated and falciparum malaria [31] and it is often accompanied by thrombocytopenia and very rarely symptomatic [16],[22]. The result of this study shows that both parasites biomass and thrombocytopenia at different follow-up periods are identified as strong indicators of drug performance in malaria treated patients [24] and reports by[10],[17]drew parallel trends between thrombocytes counts and parasitaemia in malaria patients.

The present study showed a rapid parasitological cure at the early days (days 0-3) post drug administration (figs 1 and 2) as indicated by 99.44% clearance by AT+SP (fig 1) compared to 99.38% AQ+SP (fig 2), which is in corroborates with [5], [7], [12]. Similarly, in spite of the higher parasitaemia at enrollment in AT + SP (21,738 per µl) compared to AQ + SP (19,953 per µl), parasitaemia cleared by 99.9% (fig 1) and 99.7% (fig 2) at the end of 28 days follow-up period. Previous reports have also expressed higher efficacies of AT + SP against *Plasmodium falciparum* parasitaemia with inverse relationship with platelets distribution in malaria infected children[5] the advantage of the Sulphadoxine-Pyrimethamine component of the two combination therapies was to improved parasitological clearance [34],[2] and longer half life [11]. There was a decreasing platelet count concentration with increasing parasitemia observed which has been previously noted for *P. falciparum* [23], [26] [10].

The present study found 95.9% cases of thrombocytopenia at enrollment in a similar with 93% [19], lower range of 89.0% [27] and 60% [22]. In a similar trend, [10], [24] related the platelet count density as a strong index for predicting malaria severity among all blood parameters after Packed cell Volume. On the recovery pattern in respect to parasite densities, platelet count at inception was relatively higher in AQ+SP (77975 x10^9/µl) than AT+SP (55281 x 10^9 / µl) patients however higher influence in the former drug (98.30%) was observed in the later treated group (95.98%). The result in relation to the speed of recoveries of platelets in relation to parasitemia showed a higher influence of recovery was ascribed to AT+SP with the mean daily reduction in each parasite density per µl of blood caused recovery in platelets by 48,606 x10^9/µl for AT+SP as against 37,956 x10^9/µl for AQ+SP, thus the speed of recovery of platelets was faster in AT+SP, with a difference of 10,650 x10^9/µl parasite density per µl of blood in favor of AT+SP[3] giving the former drug combination (AT+SP) an edge over the later (AQ+SP), which is in conformity with [12] though parasite clearance was faster in AT+SP (99.44%) than AQ+SP (99.38%), there was higher marginal daily parasite clearance of (1785 x10^9/µl) by AT+SP over AQ+SP combination, this finding is consistent with [28] and [19].

In summary, there was a decreasing trend of platelet/thrombocyte count with increasing levels of parasitemia as was observed by [33], [25] and [21] and AT+SP had a faster recovery from lower thrombocyte count even at inception() than AT+SP combination. A normal platelet count in a healthy person is between 150 – 400 x 10^9/l and 95% of healthy people will have platelet counts in this range. Low platelet counts are consistently associated with malaria endemic areas [10].

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References


