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

M. Kokori[1], Turaki, Z. G .S.[2] Ahmed, [3] A.T., Faisal, A.[4]

[1]Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria

[2]Lake Chad Research Institute, Maiduguri, Borno State, Nigeria

[3]Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria

[4]Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria

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 x10^9/µl in AQ+SP group compared to AT+SP (55281 x 10^9 / 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 x10^9/µl in AQ+SP as against 37,956 x10^9/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 platelets 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 x10^9/µl) moderate (<500,000 to >20,000 x10^9/µl) severe (Severe (<20,000 x10^9/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 hypersensiblity 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 parasites/µl of blood. Others include, absence of concomitant infections and severe malnutrition as defined by [30]. Measured axillary temperature ≥ 37.5 °C with the ability to attend the stipulated follow-up visits, and easy access to the health facility. Informed consent provided by patient or parent/guardian. Absence of history of hypersensitivity reactions to any of the drugs being evaluated. A history of adverse reactions to anti-malarial or other drugs used. Signs and symptoms of acute uncomplicated *Plasmodium falciparum* malaria [32]. Absence of severe illness like cardiac, renal or hepatic diseases and a Mean Packed Cell volume greater than 15% at enrollmen in concordance with [31].

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 leucocytes. 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 $x10^9$⁄µl and 37,956 $x10^9$⁄µl in AT+SP and AQ+SP, respectively.

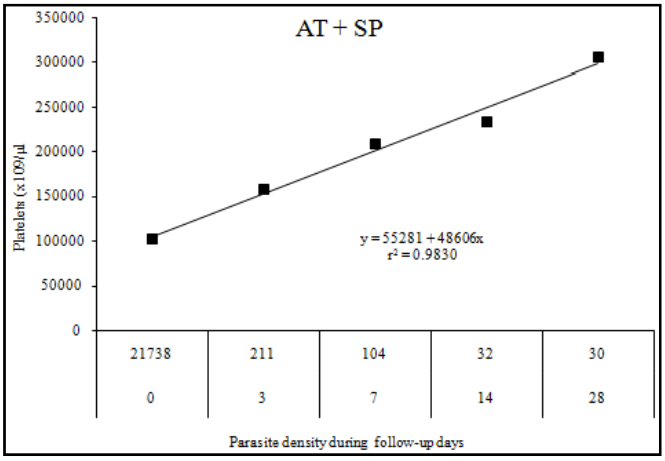

Figure 1: Recovery trends of thrombocytes and parasitaemia of *Plasmodium falciparum* treated children with AT+SP during follow-up period

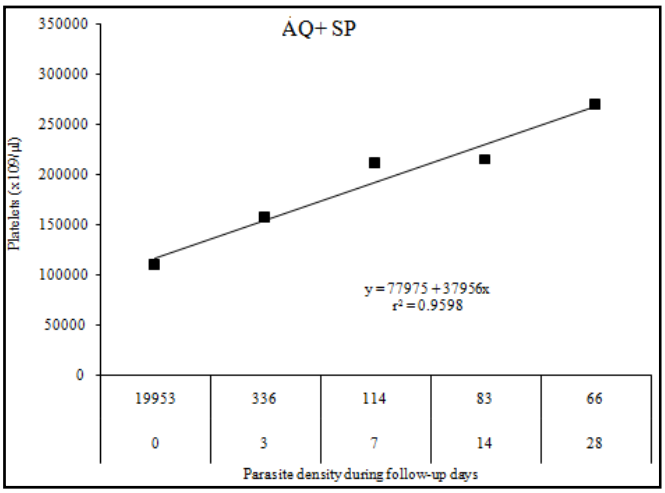

Figure 2: Recovery trends of thrombocytes and parasitaemia of *Plasmodium falciparum* treated children with AQ+SP during follow-up period.

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 platelets 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 enrollmen in AT + SP (21,738 per µl) compared to AQ + SP (19,953 per µl), parasitiaemia 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 falcifarum* parasitaemia with inverse relationship with platelets distribution in malaria infected children[5] the advantage of the Sulphadoxine-Pyrimethamine component of the two combinatuion 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 x109⁄µl) than AT+SP (55281 x 109 ⁄ 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 platetes in relation to parasitaemia 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 plaelets 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 parasitaemia 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].

5. Acknowledgement

The authors wish to express their gratitude to the staff and management of University of Maiduguri Teaching Hospital, Maiduguri, Borno State. Special thanks go to the pediatrics unit of the Teaching Hospital where the laboratory procedures were carried out.

References

[1] D, Adedapo, C.O., Falade, R.T. Kotila, G.O., Ademowo,"Age as a risk factor for thrombocytopenia and anaemia in children treated for acute uncomplicated falciparum malaria" Jou Vector Borne Dis, 44:266-271, 2007.

[2] G. O. Adjei, A.J. Kurhals, O.P. Rodriguez, M. Alifrangis, L. G.Hoeberg, E. D. Kitcer, E.V. Badoe, L. Roberto,. and B. O.Goka," Amodiaquine+Artesunat vs Arthemether-Lumefantrine for uncomplicated malaria in Ghanian children a randomized efficacy and safety trial with one year follow-up", Malaria Journal. 7: 2875-2877,2008..

[3] M. Adjuik, A. Babiker, P. Garner, P. Olliaro, W. Taylor, and N. White, "Artesunate combinations for treatment of malaria: Meta-analysis". Lancet. 363: 9-17, 2004.

[4] P. U.Agomo, R. A.S. Mustapha, B.G. Omoleye, A.N. Okechukwu, A.G. Mafe, S.I. Ijale, Y. Olukori, H.I. Okoh, O. O.Aina, C.A. Agomo, S.K. Akindele, A. Akinyele, I. I. E. Egbuna, and V.A. Ezeire, "Efficacy and safety of Artesunate + Mefloquine in the treatment of uncomplicated falciparum malaria in Ijede community, Ikorodu local government area, Lagos State", Nigeria Journal of Medical Science. 7(5): 816-824, 2007.

[5] S. O. Bello, B. Y.I. Muhammad, A.Y. Bello, A.I. Ukatu, B.M. Ahmad, A. A. I. deneye, J.Y. Cherima, "The pattern of infection and in vivo response to chloroquine by uncomplicated" Plasmodium falciparum malaria in northwestern Nigeria. African Journal of Biotechnology. 4(1): 79-82, 2005.

[6] F. Boehlen, "Thrombocytopenia during pregnancy. Importance, diagnosis and management" Hamostaseologie, 26: 72-4, 2006.

[7] H. Bukirwa, A. Yeka, M. R. Kamya, A.Talisuna, K. Banek, N.Bakyaita,J.B. wakimari, P.J. Rosenthal, F. Wabwire-Mangen, G. Dorsey, and S. G. Staedke, "Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda". Public library of Science and Clinical Trials. 1: 233-236, 2006.

[8] M. Cheesbrough,. Laboratory Diagnosis of Malaria Parasite: In District Laboratory Practice in Tropical Countries. Cambridge University Press, 246-250, 1998.

[9] J. Crawley, "Burden of clinical management and anaemia in African children" American Journal of Tropical Medicine Hygiene.71: 25-34,2004.

[10] L. M. Erhart, K. Yingyuen, N. Chuanak, N. Buathong, A. Laoboonchai, R. Miller, S.R. Meshwick, R.A. Gasser, C.Wongsrichanalai, "Hematologic and clinical indices of malaria in a semi-immune population of Western Thailand", American Journal of Tropical Medicine and Hygiene 70: 8-14, 2004.

[11] A.F. Gasasira, G. Dorsey, B. Nzarubara, S.G. Staedke, A. Nassali, P.J. Rosenthal, and M.R. Kamya, " Comparative efficacy of Aminoquinoline-Antifolate combinations for the treatment of uncomplicated Plasmodium falciparum malaria in Kampala, Uganda", 68: 127-132,2003.

[12] A.F. Gasasira, M.R. Kamya, J. Achan, T. Mebrahtu, J.N. Kalyango, T. Ruel, E. Charlebois, S.G. Staedke, A. Kekitiinwa, P.J. Rosenthal, D. Havlir, and G. Dorsey, "High risk of neutropenia in HIV-infected children following treatment with artesunate plus amodiaquine for uncomplicated malaria in Uganda" Clinical and Infectious Diseases. 46: 985-991, 2008.

[13] H. Gilles, Diagnostic methods in malaria. In: H. M Gilles and D. A. Warrell (Eds) Essential malariology, 3rd ed. P. Edwards Arnold London, United Kingdom.pp342, 1993.

[14] P. Imbert "Criteria of severity in childhood falciparum malaria,"Arch Pediatr. 2003; 10 suppl 5:532s-38s.

[15] U.M. Jadhav, V.S. Patkar, N. N. Kadam, N. N."Thrombocyto-penia in Malaria Correlation with Type and Severity of Malaria" .JAPI, 52: 615-618, 2004.

[16] Kreil, C. Wenisch, G. Brittenham, S. Looareesuwan M. Peck-Radosavljevic Thrombopoietin in Plasmodium falciparum malaria.Br J Hematol; 109: 534-6, 2000.

[17] T.B. Lathia and R. Joshi, "Can hematological parameters discriminate malaria from non malarious acute febrile illness in the Tropics?" Indian Journal Medical Science. 58: 239-244, 2004.

[18] A.M.B. Layla, A. Ahmed, A.A. Mandil, M. Bahnassy, and A. Ahmed, "Malaria haematological aspects". Annals of Saudi Medicine. 22: 5-6, 2002.

[19] A.R. Memoni and S. Afsar, "Thrombocytopenia in hospitalized malaria patients", Pakiatani Journal of Medical Scenience. 22(2): 141-143,2006.

[20] F. Mendez, A. Munoz, G. Carrasquilla, D.Jurado, M. Arevalo-Herrera, J.F. Cortese, and C.V. Plowe, "Determinants of treatment response to sulfadoxine-pyrimethamine and subsequent transmission potential in falciparum malaria". American Journal of Epidemiology. 156: 230-238, 2002.

[21] N. B. Molta, I. M. Watila, and S. Oguche, "Responses of Plasmodium falciparum infections to antimalarial drugs in north eastern Nigeria part 1-1988-1995", Journal of Phermacy and Bioresources. 1(1): 51-60, 2004.

[22] Nithish, G. S. Vikram, S. Hariprasad," Thrombocytopenia in Malaria: A clinical study", Biomedical Research; 22 (4): 489-491, 2011.

[23] L.H. Perrin, L.J. Mackey, and P.A. Miescher, "The hematology of malaria in man". Semin Hematol. 19: 70-82, 1982.

[24] S. L. Pitmang, T.D. Thacher, J. K. A.Madaki, D.Z. Egah, and P. R. Fischer,"Comparison of sulfadoxine-pyrimethamine with and without chloroqunie for uncomplicated malaria in Nigeria", American Journal of Tropical Medicine and Hygiene. 72: 263-266, 2005.

[25] M. W. Richards, R.H. Behrens, and J.F. Doherty, "Short report: Hematologic changes in acute, imported" Plasmodium falciparum malaria. American Journal of Tropical Medicine and Hygiene, 59: 859, 1998.

[26] S. Rojanasthien, V. Surakamolleart, S. Boonpucknavig, P. Isarangkura, "Hematological and coagulation studies in malaria" Journal of Medical Association of Thailand, 75: 190-194, 1992

[27] T.W. Scott, W. Takken, B.G.J. Knols, and C.Boete, "Ecology of genetically modified mosquitoes", Science. 298: 117-119, 2002.

[28] L. Wattan. A, T. Noppadon, T. Sai Kaung, N. Souwanit, S. Siripun, K. Shigeyuki, W. Polrat, and K. Srivich, "Changes in plateles in uncomplicated and severe falciparum malaria", Sotheast Asian Journal of

Tropical Medicine Public Health, Vol 41 No. 5 September , 2010
[29] World Health Organization "Basic Malaria Microscopy". (part I and II) (WHO-OMS), 72 pp, 1991.
[30] World Health Organization, "WHO Experts Committee Report on Malaria". 20th report. World Health Organization, Technical Report Series, 892.pp1-71, 2002.
[31] World Health Organization (2003), Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated Falciparum Malaria. Geneva, Switzerland: WHO; 2003. Technical document, WHO/ RBM/HTM/2003.50.
[32] World Health Organization "WHO Guidelines for the Treatment of Malaria". Geneva, Switzerland: Technical document, WHO/HTM/MAL/2006.1108, 2003.
[33] S. Yamaguchi, T. Kubota, T. Yamagishi, K. Okamoto, T. Izumi, M. Takada, S. Kanou, M. Suzuki, J. Tsuchiya, T. Naruse, "Severe thrombocytopenia suggesting immunological mechanisms in two cases of vivax malaria" American Journal of Hematology, 56: 183-186, 1997.
[34] Zongo,G. Dorsey, N. Rouamba, H.Tinto,C. Dokomajilar, R.T. Guiguemde, P. J. Rosenthal, and J. B.Ouedraogo, "Artemether-lumefantrine versus amodiaquine plus sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Burkina Faso: a randomised non-inferiority trial", Lancet. 369: 491-498, 2007