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Eosinophilic Responses to the Parasitaemia of *Plasmodium Falciparum* Treated Malaria In Children, Lake-Alau, North Eastern Nigeria

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Abstract: ***This study assessed the recovery patterns of children (6-59 months) from malaria using the relationship between eosinophils concentration and parasite densities trends. Two groups where were treated for malaria infections; namely, Artesunate + Sulphadoxine-Pyrimethamine (AT+SP) or Amodiaquine + Sulphadoxine-Pyrimethamine (AQ+SP) in uncomplicated Plasmodium falciparum for 28 days, using the standard therapeutic protocols of the World Health Organization. The results shows a weak association between eosinophil concentrations and parasite densities over segments of the study period of days 0 - 28 using the two combination drugs. The results show r^2-values of 0.2848 versus 0.1207 for AT+SP versus AQ +SP, respectively. There was higher mean eosinophillic elicitation in AQ+SP (b-values = 0.4137) compared to AT+SP (b-value = 0.2037). The results also shows an eosinophilic reduction with parasite depletion in general but concentration at enrollments were close and marginal for both AT+SP (8.8959%) and AQ+SP (8.4545%) patients. The effect of parasitaemia on eosinophils was generally low for both drugs which ranged from 7.12% for AT+SP to 12.07% for AQ+SP patients.***

Keywords: *Eosinophils, Parasitaemia, Plasmodium falciparum, Artesunate, Amodiaquine*, Lake-Alau.

1. Introduction

Malaria severity concurs most often with persons with decreased immunities. This includes all residents of areas with low or no malaria transmission, young children and pregnant women in areas with high transmission. In all areas, severe malaria is a medical emergency should be treated urgently and aggressively. Severe malaria is almost exclusively caused by *P. falciparum* infection and usually arises between 6 - 14 days after infection [1] **.** In severe malaria, fatality rates may range from 10 - 20% even with intensive care and treatment **[3]; [4]**. Chronic malaria is seen in both *P. vivax* and *P. ovale*, but it is rare in *P. falciparum*. Severe malaria could relapse for months or years and the longest incubation period reported for a *P. vivax* infection is thirty years **[4],** however, in respect of *P. vivax* relapses begin after the mosquito bite **[5]** . According to **[6],** children with complicated malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage. Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria [4]. Eosinophils are white blood cells of the immune system that are responsible for combating infection in vertebrates and they also control mechanisms associated with allergic reactions. Eosinophils ('acid-loving' cells) make up about 1 - 6% of the white blood cells, and are about 12 - 17 micrometers in size [7]; [8]. Chemical mediators, such as histamine and proteins such as eosinophil peroxidase, RNase, DNases, lipase, plasminogen and major basic protein are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues. Eosinophils persists in the circulation for 8 - 12 hours, and can survive in tissue for an additional 8 - 12 days in the absence of stimulation [7]. Eosinophils play a role in protection against malaria by inducing parasite elimination and was associated with hastened recovery from the effects of malaria infection and interpreted as signifying the onset of protective response in malaria infected individuals [9] ; [10] . Acute malaria in adults with or without limited previous exposure to *Plasmodium* infection is usually associated with high eosinophil counts, followed by persistent eosinophilia [11]. A study conducted in Thailand showed that eosinophils counts were elevated in 11% of patients with acute malaria at presentation and 93% had elevated eosinophil counted by day 7 after treatment [12] . In contrast, reports on children from endemic areas of Africa, malaria is similarly associated with decreased eosinophil concentrations in peripheral blood, although at the same time the bone marrow is rich in eosinophil precursors [14]**.** [15] observed a marked reduction of eosinophil counts by day 14, followed by a sudden increase on day 28. From the foregoing, it is apparent that the pattern of eosinophilic changes are inconsistent in malaria infected children.

2. Materials and Methods

2.1 Description of the 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: 12^0N and 13^0N; Long: 11^0E and 13^0E).

2.2 Ethical clearance

Ethical clearance was obtained through ethical committee of Borno State Ministry of Health and a letter of introduction and consent to conduct the research was obtained from the Konduga Local Government health unit authorities addressed to the respective village heads was also obtained[20].

2.3 Patients Enrollment and Recruitment procedure

Recruitment procedure[20] was employed to screen the 500 malaria suspected malaria children. Criteria like complete physical examination by qualified medical personnel. Detailed information on history of present illness, past and present drug history were obtained based on a questionnaire data sheet entered into the case record form.

2.4 Inclusion criteria

Admission into the study was based on [20] guideline for evaluating anti-malarial drugs on children between 6 and 59 months. Observations were made on clinical conditions in an uncomplicated malaria, devoid of danger signs like inability to drink or breastfeed, vomiting, recent history of convulsions, lethargy or unconsciousness and inability to sit or stand up. Mono-infection with a slide-confirmed infection by *Plasmodium falciparum* with asexual blood stage parasitaemia (no mixed infections). Initial parasite density requirement for areas of high transmission of between 2,000 and 200,000 asexual parasites/µl of blood.

2.5 Sample Size Determination and Drug Regiments

A total of 313 children that finally satisfied the inclusion criteria were assigned randomly into two groups and antimalarial therapy administered based on body weight:

Group 1, -Artesunate + Sulphadoxine- pyrimethamine (AT+SP). This group was made up of 152 children treated with the anti-malarial drug, Artesunate + Sulphadoxine-Pyrimethamine. Each of the children orally received 4 mg⁄kg body weight Artesunate daily for three days orally and a combined 25 mg⁄kg body weight Sulphadoxine and 1.25 mg⁄kg body weight Pyrimethamine as single dose on the first day of treatment.

Group 2, -Amodiaquine + Sulphadoxine – pyrimethamine(AQ+SP): Amodiaquine + Sulphadoxine - pyrimethamine were orally administered to the second group of 161 children at the dose of 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. Drug formulation, dose and treatment regiments are as shown below:

2.5.1 Drug Regiments

Patients were assigned study numbers at enrollment and referred to the study nurses for treatment group assignment during which they were randomly assigned to either Artesunate + Sulphadoxine-Pyrimethamine or Amodiaquine +Sulphadoxine-Pyrimethamine treatment groups. Randomization was done according to a predetermined randomization list and treatment in accordance to [19] . Allocation and administration of medications were performed by a study nurse not involved in the assessment of treatment outcomes, while the other members of the team were blinded to the treatment regimen in order to remove bias.

2.6 Blood Sampling

The technique outlined by[18] was followed for blood sampling. Finger prick and venipuncture (syringe) methods were both employed for blood sampling from patients on days 0, 1, 2, 3, 4, 7, 14 and 28 for the assessment of parasite densities.

2.7 Blood smear preparation

2.7.1. Thin film: The protocol of [18] was employed for thin film preparation. A drop of blood sample, equivalent to 1.5 µl and 3 - 4 mm in diameter, was collected on one end of a slide. The edge of a second slide, held at an angle of 45^0 with the first slide was then used to spread the blood thinly on the other slide for the determination of eosinophils concentrations.

2.7.2. Thick film: The procedure of [17] was adopted in the preparation of thick blood film. Three drops (4.0 µl) of blood sample was used for the preparation of the thick film. Three triangular blood drops were placed on the slide which was then gently mixed for 20-30 seconds using the corner of a second slide to defibrinate the blood to obtain a round smear of about 1 cm in diameter for the assessment of parasite densities.

2.8 Data and statistical analysis

Data collected were subjected to statistical analysis using the analytical software Staistix Version 8.0 (Microsoft, 2003) to determine percentages and correlation coefficients using regression analysis on the relationships between eosinophil concentrations and parasite densities during the 28 days of the therapeutic protocols.

3. Results

Figures 1 and 2 shows the comparative effects on *Plasmodium falciparum* parasite densities of treated children with AT+SP and AQ+SP on eosinophilic concentrations in children. The result shows a weak relationship between eosinophilic to parasitaemia during follow up periods as revealed by the r^2-values of 0.2848 versus 0.1207 for AT+SP versus AQ +SP, respectively. The intercepts suggests that at take off, eosinophil levels were almost at par for AQ+SP (49.00) and AT+SP (48.903), but later, there was a slightly higher eosinophillic concentration for AQ+SP (b-values = 0.4137) as compared to AT+SP (b-value = 0.2037).

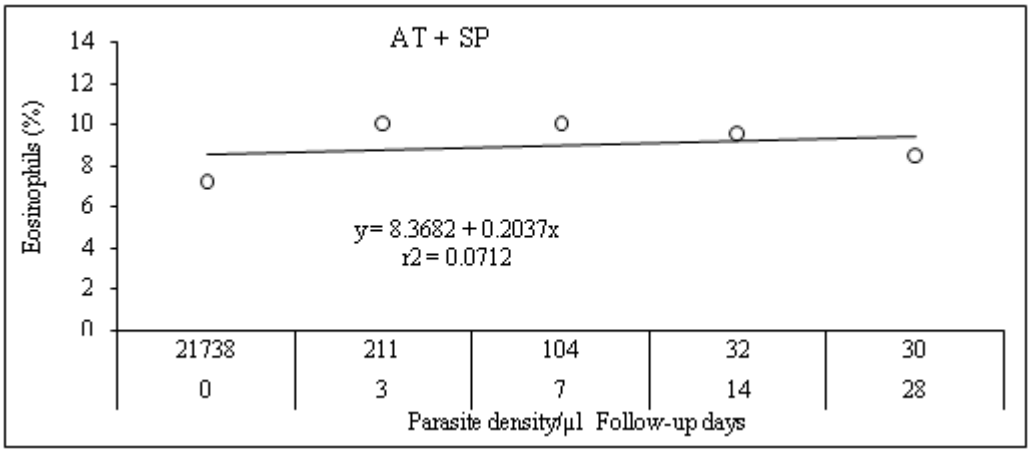

Figure 1: Effects of parasitamia on Eosionophils concentration in *P. falcifarum* Infected children treated with AT+SP during follow-up period

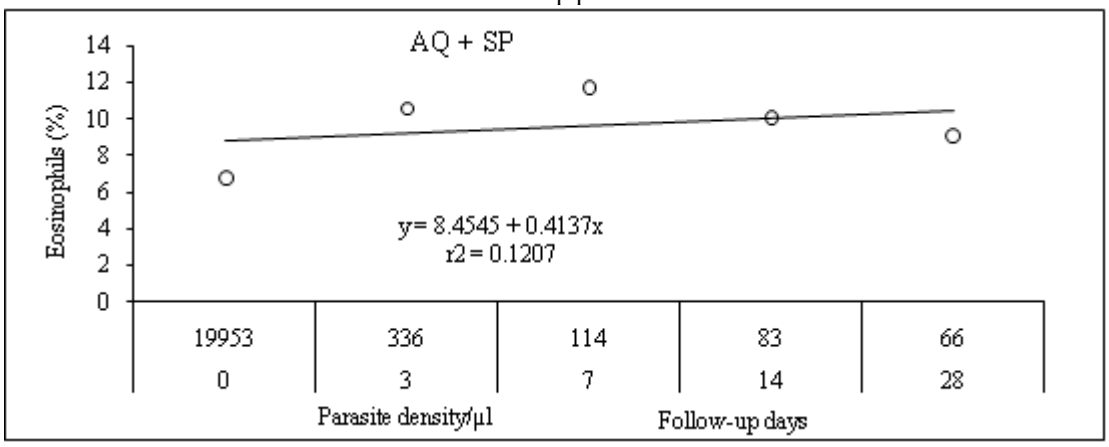

Figure 2: Effects of parasitamia on Eosionophils concentration in *P. falcifarum* infected children treated with AQ+SP during follow-up period

4. Discussion

The response of eosinophilia to parasite densities in children malaria before and after therapy to *Plasmodium falciparum* normally corresponds with eosinophil concentrations with parasitaemia are of absolute significance to malaria control programmes[12] . The study shows eosinophil concentrations decreased over time in the two administered drugs. However, higher effect was observed among AQ+SP treated patients by day seven (12%). This exaggerated eosinophilic concentrations at that point could be attributed to the influence of 4-aminoquinoline component of AQ+SP on eosinophils concentrations and not parasite densities [21], since Amodiaquine is known to elicit higher eosinophilic reactions that results in allergic clinical reactions [1]. Previous studies have clearly suggested as eosinophilia did not developed until between days 7-14 of post drug administration for malaria treatment and that it peaks at 4–8 weeks[16]. In contrast to earlier reports, present study observed peak eosinophilia by the end of the first weeks of post therapy (days 1-7). Similarly, observation of early eosinophilia is of interest in the context of the present study which suggests that eosinophils might have exerted an anti plasmodial action [9]. The correlation between the peak early eosinophilia and recovery from eosiniphilia by day 28 in the present study suggests that the early eosinophilia is part of a healthy immune response that facilitated full haematological recovery from malaria infection, since the production of eosinophils under normal circumstances is stimulated by T-helper cell type 2 (Th-2) immune cytokines such as interleukin-4 and interleukin-5 and apparently by other immune pathways [1].

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References

[1] Kain, K. C., Harrington, M. A., Tennyson, S. and Keytone, J. S., Imported malaria; prospective analysis of problems in diagnosis and management, *Clin. Infect. Dis*. **27:** 142-149, 1998.

[2] Kurtzhals, J. A. V., Adabayeri, B. Q., Goka, B. D., Akanmori, J. O., Oliver-Commey, F. K., Nkrumah, C. and Behr, L. H., Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. *Lancet* **351:** 1768-1772, 1998.

[3] Mockenhaupt, F. P., Ehrhardt, S., Dzisi, S. Y. T., Bousema, J., Wassilew, N., Schreiber, J., Anemana, S. D., Cramer, J. P., Otchwemah, R. N., Sauerwein, R. W., Eggelte, T. A., Bienzle, U. , A randomized, placebo-

controlled, double-blind trial on sulfadoxine-pyrimethamine alone or combined with artesunate or amodiaquine in uncomplicated malaria. *Trop Med Int Health*. **10:** 512-520, 2005.

[4] Carter, J. A., Ross, A. J., Neville, B. G., Obiero, E., Katana, K., Mung'ala-Odera, V., Lees, J. A. and Newton, C. R., "Developmental impairments following severe falciparum malaria in children". *Tropical Medicine and International Health*. **10**: 3-10.2005.

[5] Adak, T., Valecha, A and Sharma, N. V. P., *Plasmodium vivax* polymorphism in a clinical drug trial. *Clin. Diag. Lab. Immunol*. **8:** 891-894.2001.

[6] Idro, R., Bitarakwate, E., Tumwesigire, S. and John, C. C., Clinical Manifestations of severe malaria in the highlands of of southwestern Uganda. *American Journal of Tropical Medicine and Hygiene*. **72:** 561-567.2005.

[7] Richards, M.W., Behrens, R. H. and Doherty, J. F., Short report: Hematologic changes in acute, imported *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **59:** 859.1998.

[8] Verhage, D. F., Telgt, D. S. C., Bousema, J. T., Hersen, C. C., Van Gemert, G. J. A., Vander-Meer, J. W. M. and Sauerwein, R. W.,Clinical outcome of experimental human malaria induced by plasmodium infected mosquitoes *Neterlands journal of Medicine.* Vol: 63. 20- 21.2005.

[9] Waters, L. S., Taverne, J., Tai, P. C., Spry, C, J. F., Targett, G. A. T., Playfair, J. H. L., Killing of *Plasmodium falciparum* by eosinophil secretory products. *Infect Immun*. **55:** 877-881.1987.

[10] Rwagacondo, C., Karema, C., Mugisha, V., Erhart, A., Dujardin, J., Van, O. C., Ringwald, P. and D'Alessandro, U., "Is amodiaquine failing in Rwanda? Efficacy of amodiaquine alone and combined with artesunate in children with uncomplicated malaria". *Trop Med Int Health*. **9(10):** 1091-1098.2004.

[11] Davis, T.M. E., Safety evaluations of drugs containing artemisinin derivatives for the treatment of malaria. *Clin. Infect. Dis*. **36:** 1627-1628. 2003.

[12] Camacho, L. H., Wilairatana, W. G., Mercader, M. A., Brittenham, G. M., Looareesuwan, S. and Gordeuk, V. R., The eosinophilic response and haematological recovery after treatment for *Plasmodium falciparum* malaria. *Tropical Medicine and International Health*. **4(7)**: 471-475.2002.

[13] Davis, T. M. E., Ho, M., Supanaranond, W., Looareesuwan, S., Pukrittayakamee, S., White, N. J., Changes in the peripheral blood eosinophil count in falciparum malaria. *Acta Trop*. **48:** 243-245.1991.

[14] Abdalla, S. H., Peripheral blood and bone marrow leucocytes in Gambian childrenwith malaria: numerical changes and evaluation of phagocytosis. *Annals of Tropical. Paediatirics*. **8:** 250-258. 1998.

[15] Nosten, F., van Vugt, M., Price, R., Luxemburger, C., Thway, K. L., Brockman, A., McGready, R., ter Kuile, F., Looareesuwan, S. and White, N. J., Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet*. **356:** 297-302.2000.

[16] Adjei, G. O., Kurhals, A. J., Rodriguez, O. P., Alifrangis, M., Hoeberg, L. G., Kitcer, E. D., Badoe, E. V., Roberto, L. and Goka, B. O., 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.

[17] Gilles, H., 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.

[18] World Health Organization, Basic Malaria Microscopy. (part I and II) (WHO-OMS), 72 pp.1991.

[19] World Health Organization , Assessment of therapeutic efficacy for uncomplicated falciparum malaria in areas with intense transmission. Geneva: World Health Organization. Unpublished document, *WHO/MAL/96.1077*.PP-32.1996.

[20] World Health Organization, 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*. 2003.

[21] Fanello, C. I., Karema, C., van Doren, W., van Overmeir, C., Ngamije, D. and D'Alessandro, U., A randomised trial to assess the safety and efficacy of artemether-lumefantrine (Coartem) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwanda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **101:** 344-350.2007.