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Does Artesunate Treatment normalize Depressed Immunological Parameters Following Plasmodium falciparum Infection?

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Abstract : *Plasmodium falciparum is the predominant cause of malaria infection and death in Nigeria and Africa. It perturbs the normal process of immune cells, of which CD 4^+ T cells have a crucial relevance for anti-malarial immunity. The study aims at determining the effect of artesunate treatment on immunological parameters following Plasmodium falciparum malaria. A randomized non-block experimental design was conducted for the study at Bayelsa State College of Health Technology, Otuogidi-Ogbia, Bayelsa State, Nigeria, between February 2015 and April 2017. Out of 90 subjects enrolled into the study, data collected from 80 subjects (38 males, 42 females, and age range 18-59 years) were analyzed. 40 of the subjects had P. falciparum malaria and the other 40 were negative and were used as controls. 3 ml of blood was collected from the antecubital vein of each enrollee without stasis using a disposable plastic syringe and dispensed into K-EDTA bottles. Immuno-phenotyping was done using the AuRICA system. 20 of the malaria subjects had lymphopenia at presentation. The white cell count was also reduced (P = .05) among the malaria subjects compared with the controls. There was a significant increase in WBC count on completion of the 5-day treatment with the drug (P = .05). Although an increase in the CD4 count after treatment was observed, the mean CD4 count of 1008.1/µl for normal subjects was significantly higher (P = .05) than 889.76/µl for the malaria patients. We conclude that Plasmodium falciparum infection is associated with strong decrease in CD4+ cell activation and depletion of WBC counts. Artesunate in addition to other effects possess immune reconstituting properties.*
Keywords: *Antimalarial Treatment, Artesunate, CD4 Count, Immunophenotying, and Malaria*

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I. Introduction

Malaria is caused by the intracellular protozoan *Plasmodium*. Currently, there are five known species: *P. falciparum, P. malariae, P. ovale, P. vivax* and *P. Knowlesi* [1]. However, *P. falciparum* is the cause of about 80% of infections and 91% of deaths [2]. According to the WHO, malaria causes about 596,000 deaths annually in Africa [2]. Malaria parasites are known to perturb the normal profile of immune cells. Immunity against malaria can be acquired after infection and its protective efficacy depends on host characteristics, place of stay, number of infections suffered etc. This acquired immunity has been graded as anti-disease immunity (that protects against clinical disease), anti-parasite immunity (protects against high parasitemia), and sterilizing immunity (protects against new infections by maintaining a low-grade, asymptomatic parasitemia). The acquired anti-malaria immunity has been demonstrated to be strain and stage specific, with cross reactivity [3]. Cells concerned with anti-malarial immune response comprise neutrophils, activated macrophages, Natural Killer (NK) cells, dendritic cells, and T-cells. $CD4^+$ T cells (of both the T_H1 and T_H2 subtypes) have a crucial relevance for anti-malarial immune protection against asexual blood stage infections [4]. The naturally acquired immunity against *P. falciparum* takes years to develop, at least partly due to a very effective immune evasion strategy mediated by naturally occurring variants of the same antigen epitopes which are capable of inhibiting memory T cells [5]. It can also be explained by the fact that the parasite actively modulates the immune system of the host, preventing the development of specific immune responses [6]. In the absence of re-infection for a long period, this acquired immunity can be lost. Thus, malaria infection does not induce long-term immunity, and antimalarial drug treatment is expedient.

Artesunate is the most rapidly acting and potent antimalarial drug [7]. It is a semi-synthetic derivative of Artemisinin which is the principal active constituent of the plant *Artemisia annua.* In addition to their activity against multi-drug resistant *P. falciparum*, artemisinin and its derivatives possess antiviral [8], antifungal [9] and anti-inflammatory effects [10]. They can be administered once every day and are safer and easier than quinine. Artesunate or artemether given orally are an essential component of the combination treatment of uncomplicated falciparum malaria, which is now accepted as the treatment of choice [11]. We therefore aimed at determining the effect of artesunate treatment on depressed immunological parameters following Plasmodium falciparum malaria.

II. Materials And Methods

Subjects /Setting

A randomized non-block experimental design was conducted for the study, between February 2015 and April 2017. 90 subjects of both sexes who were serologically negative for HIV-I and HIV-II, without known underlying chronic illnesses such as diabetes mellitus and tuberculosis but had a clinical history of fever, shivering, joint pain, general weakness and/or nausea, were enrolled for this study. Pregnant women were ruled out and the elderly (60 years and above) were also exempted. Data collected from 80 of the subjects (38 males, 42 females, age range 18-59 years) was analyzed. 40 of the subjects had *P. falciparum* malaria and the other 40 were negative for *P. falciparum* malaria and used as controls. The control subjects were sampled once during the study. The study was carried out at the Bayelsa State College of Health Technology, Otuogidi-Ogbia, Bayelsa State, in Southern Nigeria. Informed consent was obtained from all subjects. Ethical approval was obtained from the College Ethical Committee. Subjects were treated with the Lever® brand of artesunate 50 mg tablets (NAFDAC Registration Number: 04-5865) at the rate of 600 – 800mg (12mg/kg), for 5 days [12]. Parameters were checked 3 times as follows: before treatment, day 2 of the treatment and day 6 (24 hours after completing the 5-day treatment).

Sample Collection

3 ml of blood was collected from the antecubital vein of each subject without stasis using a disposable plastic syringe and dispensed into K-EDTA bottles.

Microscopy

Parasite detection was done manually as described by Lewis, Bain and Bates [13].

Immunophenotyping

Immuno-phenotyping was done using the AuRICA system. This reports CD4 T-lymphocyte counts and percents in conjunction with White Blood Cell (WBC) and total lymphocyte count information, based on software analysis of light scatter measurements with the use of proprietary non-fluorescent reagents. All on-board aspiration and dispensing steps were precise and volumetric and permit direct cellular enumeration [14].

Data reporting

Results of WBCs were expressed in absolute members of cells ($\times 10^9$/l). Relative (differential) WBC Counts were converted to their absolute counts by multiplying the respective WBC differential by the total WBC count. $CD4^+$ counts are expressed in absolute number of cells per µl.

Statistical analysis

Statistical analysis was done with Statistical Package for Social Sciences software (version 10, SPSS Inc. Chicago, IL, USA). Data was grouped into Control (No malaria), untreated, day 2 treatment, and after treatment. Pairwise comparisons were done between: control and untreated, untreated and day 2 treatment, and after treatment and untreated groups; using t – test. An error probability (*P* value) $\leq .05$ was considered significant.

III. Results

Demographic characteristics of enrolled subjects

A total number of 90 adults participated in the study; data of 80 subjects were included in the analysis. Data from 10 enrollees were not included in the analysis, 2 out of the 10 were HIV positive, and 3 were lost to follow-up while 5 withdrew their consent. The demographic characteristics of those included in the study are shown in Table1.

Immunological results

The baseline Mean ± SD for the parameters of the subjects showed a statistically significant difference from that of the controls (WBC, $P = .05$; $CD4^+$, $P = .001$), as shown in Table 2.

The results showed that there was no statistically significant difference on all the parameters investigated at day 2 of treatment. At day 6, the results show an increase in both WBC and $CD4^+$ counts ($P = .05$).

Table 1: Demographic Characteristics of Enrolled Subjects

Feature	Statistics
Number of subjects	80
Males	38
Females	42
Age range (in years)	18-59
18-20	10
20-29	27
30-39	19
40-49	19
50-59	5

Table 2: Baseline Immunological Values of Subjects

Parameter	Mean ± SD of subjects (n=25)	Mean ± SD of control (n=25)	Significance level
WBC ($\times 10^9$/l)	4.75 ± 1.07	5.56 ± 1.44	$P = .05$*
$CD4^+$ (/µl)	848.32 ± 103.85	1008.10 ± 180.1	$P = .001$*

*significant difference observed, using independent *t-test*

Table 3: Effect of Treatment with artesunate on Immunological Parameters at day 2

Parameter	Mean ± SD of subjects before treatment	Mean ± SD f subjects at day 2	Significance level
WBC ($\times 10^9$/l)	4.75 ± 1.07	4.96 ± 1.11	$P > .05^{\#}$
$CD4^+$ (/µl)	848.32 ± 103.85	860.64 ± 97.09	$P > .05^{\#}$

$^{\#}$No significant difference observed, using dependent *t-test*

Table 4: Effect of Treatment with artesunate on Immunological Parameters on day 6

Parameter	Mean ± SD of subjects Before treatment	Mean ± SD of subjects After treatment	Significance level
WBC ($X10^9$/l)	4.75 ± 1.07	5.624 ± 1.1271	$P = .05$*
$CD4^+$ (/µl)	848.32 ± 103.85	889.76 ± 95.73	$P = .05$*

*Significant difference observed, using dependent *t-test*

IV. Discussion

The mean counts of total white blood cells were generally lowered in patients with *Plasmodium falciparum* than in the controls ($P = .05$, table 2). The decrease in total white blood cell count obtained in this study tallies with reports from earlier studies [15, 16]. Of particular interest in acute malaria are the white cell subsets. Toure-Balde *et al* [17], observed cases of monocytosis, neutrophilia and eosinophilia which could be attributed to migration, sequestration and destruction of the cells during acute malaria. However, changes in the values of monocytes and neutrophils are basically as a result of such factors as rate of bone marrow release of the cells into the general circulation, the proportion of circulatory cells to the cells adherent to the endothelial cells and the rate at which the cells exit the general circulation to body tissue. Utoh-Nedosa *et al.* [18], reported an increase in neutrophils count after treatment with dihydroartemisinin, this portrays an immune reconstituting potential for the artemsinin. This may be accountable for the increase in WBC at the end of treatment ($P = .05$, table 4).

The reduction in total lymphocyte count as noted in this study could result in the occurrence of leucopaenia (a total white cell count < 4,000/µl) which is commonly reported in malarial studies [15, 19]. Although the lymphocyte profiles in the circulatory blood are generally affected, the type of lymphocyte subsets affected and the extent of such decreases differ in different geographic locations. This has been attributed to several factors: differences in the immune status of the study subjects compared to the level of malaria endemicity [15], differences in parasite strains which itself may cause differences in the activation of the immune system [20], differences in the baseline values of the absolute counts of the immune cells of the study subjects [21] or to the impact of geographical locations.

In this study, only the $CD4^+$ subset of the total leucocytes was analyzed. The mean count of 1008.10/µl for the control subject was significantly higher ($P = .001$, Table 2) than that of the untreated subjects (848.32/µl). There was a slight increase from 848.32/µl to 863.04/µl at the second day of treatment, a significant increase to 889.76/µl was obtained after completion of treatment ($P = .05$, table 4). Thus the mean $CD4^+$ count of patients after treatment was still lower than those of control subjects. The reduced $CD4^+$ as observed in this study is consistent with previous findings [22].

Artemisinins have been reported to possess immune boosting properties [18, 23], this may account for the sudden rise in CD4 cell count as seen after the completion of treatment with artesunate (P = .05, table 4). This finding hence corroborates the immune reconstituting properties of artesunate.

V. Conclusion

Plasmodium falciparum infection is associated with reduction in CD4+ Lymphocyte and WBC counts. Artesunate in addition to other effects possess immune reconstituting properties. WBC counts return to normal after treatment with artesunate while $CD4^+$ require more time to return to normal. It is necessary to carry out further studies using artesunate in its combined form with other drugs as used in the treatment of malaria.

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