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Full Length Research Paper

Hematological and temperature profile in micronutrient based combination therapy of uncomplicated *falciparum* malaria in under five's

Iribhogbe O. I.[1]*, Agbaje E. O.[2], Oreagba I. A.[2], Aina O. O.[3], Emordi J. E.[1], Akaomen J. E.[4] and Nmorsi O. P. G.[5]

[1]Department of Pharmacology and Therapeutics College of Medicine, Ambrose Alli University Ekpoma, Nigeria.
[2]Department of Pharmacology, College of Medicine of the University of Lagos, Nigeria.
[3]Malaria Research Unit, Nigerian Institute of Medical Research, Yaba Lagos State, Nigeria.
[4]Faith-dome Medical Centre, Ekpoma Edo State, Nigeria.
[5]Department of Zoology, Parasitology Unit, Ambrose Alli University Ekpoma, Edo State, Nigeria.

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This study was conducted to elucidate the influence of some selected antioxidant micronutrient combination on hematological parameters in the course and therapeutics of malaria in early childhood. 150 participants (6 months to 5 years) diagnosed with uncomplicated *falciparum* malaria were recruited for the study from two Health Centres in Ekpoma, Edo State Nigeria, after obtaining ethical clearance from the ethical review Board of the Edo State Ministry of Health. The participants were randomized into 15 cohorts of 10 patients each. Additionally, 20 apparently healthy volunteers were used as control for the hematological parameters assessed. The patients in the active comparator group were administered with oral doses of standard artemisinin based combination therapy, while the interventional cohorts were administered varying combinations of antimalarials (Artesunate or Amodiaquine) and micronutrients (vitamin A, E, zinc and selenium). A comparative analysis between the total white blood cell (WBC) count on day 0 and day 28 post treatment revealed a significant increase in all the micronutrient treated groups on day 28 ($p<0.05$; $r = 0.76$; $P<0.01$). In addition, there was a negative correlation between the parasite density, packed cell volume (PCV) and hemoglobin concentration in the study population ($r = -0.102$; $P >0.01$; $r = -0.08$; $P>0.01$ respectively). Conclusively, the use of varying bi-combination of antioxidant micronutrients as adjuvants to standard antimalarial agents has immunomodulating potential that may be of benefit in malaria therapeutics.

Key words: Immunomodulation, hematological profile, adjuvant therapy, micronutrient combination, malaria.

INTRODUCTION

Micronutrients have a wide range of functions. More than one micronutrient may support a single function for example, antioxidant defense and a single micronutrient may act in more than one role, for example, iron is involved in both oxygen transport and immune function. Much of the mechanistic research on single micronutrients has been conducted in animal or cell models which may not be readily applicable to the usual human situation of multiple coexisting deficiencies. On the other hand, multiple micronutrient intervention studies do not permit easy identification of the mechanism of any effects (Friis, 2006).

Some authors have associated malaria acquisition and its severity to the concentrations of micronutrients in children viz a viz its protection against acute infection through a moderated deficiency in iron (Nyakeriga et al., 2004; Wander et al., 2009); the reduction of risk of fever and clinical malaria episodes through zinc supplementation (Seyrek et al., 2005; Zeba et al., 2008) and the reduction of the ratio copper/zinc in malaria infection (Mezzetti et al., 1998).

*Corresponding author. E-mail: osedeignis@gmail.com. Tel: +2348065794437.

However, information about the influence of these micronutrients on the course of malaria in developing countries where malnutrition and infection coexist need to be addressed. This study aims to elucidate the hematological and temperature profile following the use of varying micronutrient combinations as adjuvant in the treatment of uncomplicated *falciparum* malaria.

MATERIALS AND METHODS

Study area

The study was conducted in Ekpoma, Esan West Local Government Area of Edo State Nigeria. This community is a semi-urban community with an estimated population of over 125,842 inhabitants (NPC, 2006). Patients were recruited from two Medical Centers; Central Primary Health Center and Faith-Dome Medical Center both in Esan West Local Government Area of Edo State.

Sample size estimation

Sample size was estimated at 5% significance and 80% power using the method of Campbell et al. (1995). The formulae $M = 2 \times [Z_{(1-\alpha/2)} + Z_{(1-\beta)}]^2 \div \Delta^2$ and $\Delta = P_1-P_2/\sqrt{p \times (1-p)}$ was used for sample size estimation. Where $p = P_1+P_2/ 2$, $Z_{(1-\alpha/2)} = 5\% = 0.05 = 1.96$, $Z_{(1-\beta)}$ = power at 80% = 0.8 = 0.8416 and Δ = standardized difference = 2.1053. Therefore, the minimum sample size is approximately 5 patients per group (total of 75 participants).

Study participants

A total of 150 participants with uncomplicated *falciparum* malaria were recruited for the study. The participants were drawn from early childhood (6 months to 5 years of age). Additionally, 20 apparently healthy volunteers were used as control for hematological parameters assessed.

Study design

The study is a randomized controlled clinical trial with consecutive recruitment of eligible patients until the total sampling size was achieved. Specifically designed medical record forms were used to elicit biodata and clinical data from participants. Participants were admitted into the study after meeting the following inclusion criteria:

1. Age of ≥ 6months ≤ 5 years
2. Asexual parasitemia of between 1,000 and 200,000/µl
3. Acute manifestation of malaria (for example, history of fever in the preceding 24 h, a temperature of >37.5°C at baseline)
4. Body weight between 5 and 30 kg
5. Ability to tolerate oral therapy
6. informed consent by the legal representative of the Participants (the parents, if possible), oral agreement of the child if appropriate.
7. Resident in the study area for duration of at least 4 weeks.

The exclusion criteria for the study were as follows:

1. Adequate antimalarial treatment within the previous 7 days
2. Use of micronutrients in the last 2 weeks,
3. Use of herbal medications in the last 2 weeks
4. Antibiotic treatment for a concurrent infection
5. Haemoglobin level of <7 g/dl
6. Haematocrit of <25%
7. Leukocyte count of >15,000/µl
8. Mixed plasmodial infection
9. Severe malaria, any other severe underlying disease
10. Concomitant disease masking assessment of the treatment response
11. Inflammatory bowel disease and any other disease causing fever.

Ethical issues/considerations

The trial was registered with clinical trials.gov (registration number NCT01152931) and was conducted in accordance with the principle of Helsinki Declaration and its Hong Kong amendment and according to the principle of good clinical practice. Ethical permission was obtained from the ethical review Board of the Edo State Ministry of Health after submitting the research proposal for the study. Informed consent was procured from parents and guardian of study participants and or directly from the participants depending on their age group. The participants on micronutrient combinations without an appropriate response were scheduled to be given adequate treatment with artemisinin based combination therapy after 72 h of commencing micronutrient therapy.

Study drugs and administration

Drugs were administered daily at different doses depending on the age and weight of the participants. The intervention was based on the use of standard antimalarial combination therapy for uncomplicated malaria according to WHO recommendation (WHO, 2003). Micronutrient dosage was based on dietary reference intake values (DRI) adapted from Food and Nutrition Board (2010). The study participants were randomly grouped into 15 cohorts (A-O) of 10 patients

each after appropriate age and sex matching. Envelopes containing the letters (A1-10, B1-10, C1-10, D1-10, E1-10, F1-10, G1-10, H1-10, I1-10, J1-10, K1-10, L1-10, M1-10, N1-10 and O1-10) were placed in a basket. The content of the envelope picked after balloting, determined the arm of the study the participants were allotted to. The treatment groups are shown in Table 1.

Study flow and procedures

Patients were recruited consecutively until the sampling size was achieved. The study is a single blind study with concealment of the interventional groups using the sealed envelope system (David and Chris, 1999). The patients were seen by a study physician at 24 h intervals after each drug administration and/or until two consecutive negative blood smears occurred and subsequently on days 7, 14, 21, and 28 post-treatment or as otherwise indicated. At each visit during the treatment and follow-up phases, the medical history was taken, vital signs were checked, axillary temperature were measured with a standard mercury thermometer, and a thick blood smear was prepared from the finger prick for microscopic examination. Venipunctures were performed on study days 0 and 28 to monitor the hemoglobin level, hematocrit, and differential white blood cell count.

Laboratory procedures

Dried thick blood smears were stained with 10% Giemsa solutions at pH 7.2 for 10 min. Parasite species were identified using standard morphological characteristics, and the parasite density was calculated using standard procedure in which parasite were counted per 200 WBC multiplied by a standard count of 8,000 leukocytes/µl (O'Meara et al., 2007).

Data management and statistical analysis

Statistical analyses of the data were performed using statistical software package SPSS version 17.0. Student's *t* test and one way ANOVA were used to compare the mean of laboratory data between groups. Bonferroni correction was done for multiple comparisons. Pearson's correlation test was also used to establish the relationship between variables such as parasite density and packed cell volume, haemoglobin concentration, white blood cell count etc. The statistical significance level was set at 95% confidence interval and P value < 0.05 was considered significant.

RESULTS

Of the 150 participants recruited for this study, only 116 (77.33%) were successfully and completely followed up over a period of 4 weeks. The mean peak age of the participants in the study was 2.31 ± 0.11 years. Male: Female ratio being 1:1 (75 males and 75 females respectively). Mean weight of the study population at presentation was 12.97 ± 0.34 kg while the mean temperature, packed cell volume (PCV), hemoglobin concentration and Total white blood cell count (WBC) were 38.03 ± 0.37°C, 33.21 ± 0.37%, 11.01 ± 0.13 g/dL and 6,507.27 ± 217.00 /mm^3 respectively. The mean baseline parasite density in the study population was 17,677.67 ± 17,677.67 /µL. However, there was no significant difference (P>0.05) in base line parameters between groups (Tables 2 and 3).

As revealed in Table 4, the comparative analysis between the total WBC count on day 0 and day 28 post treatment revealed a significant increase in all the micronutrient treated groups on day 28 ($p<0.05$; $r = 0.76$; $P<0.01$). A comparatively significant increase ($p<0.05$) was also noted in the differential count of the micronutrient treated groups between day 0 and day 28 post treatment (Table 5).

As shown in Table 6, the mean temperature difference between groups on day 0/day 1 and day 0/day 2 was statistically significant ($p < 0.05$). On day 1 of treatment (Figures 1 to 4) there was a rapid decline in the mean temperature (38.03 ± 0.37°C) on day 0 in all the groups. Mean temperature was maintained below 37.5°C during the period of follow up. However, the amoiaquine + zinc combination group showed a more rapid decline in temperature when compared to the other groups. This was sustained through out the period of monitoring. There was a negative correlation between the parasite density, PCV and hemoglobin concentration in the study population ($r = -0.102$; $P >0.01$; $r = -0.08$; $P>0.01$ respectively). This was also the case between the parasite density and WBC count, which showed a negative correlation ($r = -0.004$; $P>0.01$) (Table 7).

DISCUSSION

Nutritional status plays an important role in humoral and cell-mediated immune function. Micronutrient deficiencies in human immunodeficiency virus (HIV)-infected women impair immune responses, increasing the risk of infection, HIV disease progression, and possibly vertical transmission. Systemic immune response to HIV infection in infants and children may also be impaired by maternal micronutrient deficiencies. Most evidence of the effects of micronutrients on immune function comes from studies that examined a single nutrient, often used pharmacologic doses, and did not involve HIV infection. Some authors have associated the acquisition of malaria infection with decreased serum micronutrient concentration in children (Nyakeriga et al., 2004). Additionally, the protection against acute malaria infection

Table 1. Drug administration in the study population.

Group	Drugs (tablets/gels)	Administration (oral route)
A	Amodiaquine + Artesunate	Amodiaquine 10 mg/kg dly x 3 days Artesunate 4 mg/kg daily x 3days
B	Lumefantrine +Artemether	Fixed combination 120/20 mg daily x 3days
C	Artesunate + Vitamin A	Artesunate 4 mg/kg daily for 4 days + Vitamin A 5000IU dly x 4 days if ≤ 1yr. 10,000IU dly if > 1 yr x 4 days
D	Artesunate + Vitamin E	Artesunate 4 mg/kg daily for 4 days + vitamin E 100 mg dly x 4 days
E	Artesunate + Zinc	Artesunate 4 mg/kg daily for 4 days + zinc 50 mg dly x 4 days if > 1 yr. 25 mg dly x 4 days if ≤ 1yr
F	Artesunate + Selenium	Artesunate same dose as above + selenium 100 ug dly x 4days if > 1 yr. 50 ug dly x 4 days if ≤ 1yr
G	Amodiaquine +Vitamin A	Amodiaquine 10 mg/kg dly x 3 days + vitamin A same schedule as above for 4 days
H	Amodiaquine+ Vitamin E	Amodiaquine 10 mg/kg dly x 3 days+ vitamin E 100 mg daily for 4 days
I	Amodiaquine+Zinc	Amodiaquine 10 mg/kg dly x 3 days + zinc same schedule as above for 4 days.
J	Amodiaquine+Selenium	Amodiaquine 10 mg/kg dly x 3 days + selenium same schedule as above for 4 days.
K	Artesunate+ Vit A+ Vit E	Artesunate 4 mg/kg daily for 4 days + Vitamin A + E same schedule as above for 4 days
L	Artesunate+ Vit A+ Zinc	Artesunate same schedule as above + Vitamin A + zinc same schedule as above for 4 days
M	Artesunate+ Vit A+ Selenium	Artesunate same schedule as above + Vitamin A + selenium same schedule as above for 4 days
N	Artesunate+ Vit E+ Zinc	Artesunate same schedule as above + Vitamin E + Zinc same schedule as above for 4 days
O	Artesunate+ Vit E+Selenium	Artesunate same schedule as above + Vitamin E + selenium same schedule as above for 4 days

Table 2. Base line characteristic of the study population (mean ± SEM).

Group N= 10	Age (Years)	Weight (kg)	Temperature (°C)	Parasite Density (/µL)
Amodia + Arte	1.95 ± 0.42	13.20 ± 1.58	38.06 ± 0.07	8694.00 ± 1668.20
Artemet +Lume	2.16 ± 0.44	12.80 ± 1.17	37.96 ± 0.09	22459.00 ± 5214.01
Arte + Vit A	2.55 ± 0.35	14.40 ± 0.87	38.10 ± 0.09	21445.00 ± 8628.96
Arte + Vit E	1.70 ± 042	10.10 ± 1.57	38.06 ± 0.12	15390.00 ± 6040.90
Arte + Zinc	2.30 ± 0.53	12.00 ± 1.02	38.01 ± 0.06	20196.10 ± 7336.57
Arte + Sele	2.29 ± 0.47	13.50 ± 1.57	38.01 ± 0.05	36260.00 ± 9067.43
Amodia + Vit A	2.48 ± 0.53	13.98 ± 1.78	38.04 ± 0.08	15608.00 ± 4872.51
Amodia + Vit E	2.17 ± 0.47	13.20 ± 1.55	38.06 ± 0.14	20156.00 ± 6007.27
Amodia + Zinc	2.61 ± 0.41	13.80 ± 1.08	37.95 ± 0.05	16242.00 ± 5421.19
Amodia + Sel	2.44 ± 0.48	13.40 ± 1.31	38.15 ± 0.14	14070.00 ± 4643.83
Arte + Vit A + Vit E	2.15 ± 0.47	12.40 ± 1.67	37.98 ± 0.07	10202.00 ± 2959.02
Arte + Vit A+ Zinc	2.44 ± 0.31	12.60 ± 1.01	38.15 ± 0.09	16712.00 ± 3463.18
Arte + Vit A + Sel	2.56 ± 0.25	13.60 ± 1.36	38.08 ± 0.11	24619.00 ± 4940.08
Arte + Vit E + Zinc	2.44 ± 0.51	13.90 ± 1.18	37.91 ± 0.05	9060.00 ± 2103.47
Arte + Vit E + Sel	2.33 ± 0.57	11.60 ± 1.15	37.93 ± 0.06	14052.00 ± 4901.90
One way ANOVA	F = 0.30 P > 0.05	F = 0.67 P > 0.05	F = 0.73 P > 0.05	F = 1.46 P > 0.05

Values are expressed as Mean±SEM. df = 149, $P < 0.05$ is considered significant.

Table 3. Base line hematological profile of participants (mean ± SEM).

Group N= 10	Hb (g/dL)	PCV (%)	WBC (/mm^3)	Neutrophil (%)	Lymphocyt (%)	Monocyte (%)	Basophils (%)	Eosinophils (%)
Amodia + Arte	10.84 ± 0.60	33.30 ± 1.41	8130.00 ± 1127.64	48.50 ± 2.29	48.80 ± 2.12	2.20 ± 0.81	0.10 ± 0.10	1.00 ± 0.54
Artemet +Lume	11.85 ± 0.33	35.60 ± 1.05	5450.00 ± 529.41	46.60 ± 1.67	52.00 ± 1.78	0.90 ± 0.23	0.10 ± 0.10	0.40 ± 0.40
Arte +Vit A	11.09 ± 0.50	33.10 ± 1.52	6810.00 ± 954.92	49.60 ± 3.02	48.10 ± 3.23	1.30 ± 0.30	0.40 ± 0.22	0.60 ± 0.34
Arte + Vit E	10.52 ± 0.36	31.80 ± 1.04	7468.00 ± 1008.80	52.40 ± 2.24	44.70 ± 2.31	1.70 ± 0.75	0.20 ± 0.13	0.90 ± 0.53
Arte + Zinc	11.61 ± 0.43	35.00 ± 1.26	5429.00 ± 482.03	50.50 ± 1.96	48.70 ± 1.87	0.80 ± 0.20	0.10 ± 0.10	0.10 ± 0.10
Arte + Se	11.56 ± 0.43	34.70 ± 1.38	7840.00 ± 1482.88	54.40 ± 1.83	44.50 ± 1.85	0.80 ± 0.25	0.20 ± 0.13	0.10 ± 0.10
Amodia + Vit A	10.28 ± 0.58	31.60 ± 1.38	6510 ± 768.32	53.20 ± 1.99	46.00 ± 2.01	0.40 ± 0.22	0.20 ± 0.20	0.20 ± 0.20
Amodia + Vit E	10.86 ± 0.52	32.80 ± 1.51	5870.00 ± 410.43	55.20 ± 2.58	43.70 ± 2.56	0.60 ± 0.22	0.20 ± 0.13	0.30 ± 0.21
Amodia + Zinc	10.72 ± 0.43	32.00 ± 1.34	7272.00 ± 845.37	47.50 ± 1.92	51.30 ± 1.90	0.60 ± 0.22	0.40 ± 0.22	0.10 ± 0.10
Amodia + Se	11.08 ± 0.55	33.80 ± 1.60	5740.00 ± 412.09	50.80 ± 1.95	47.50 ± 2.41	0.30 ± 0.15	0.20 ± 0.13	0.20 ± 0.20
Arte + Vit A + Vit E	11.19 ± 0.38	33.70 ± 1.17	6360 ± 1246.08	51.40 ± 2.73	47.90 ± 2.64	0.50 ± 0.17	0.10 ± 0.10	0.20 ± 0.13
Arte + Vit A+ Zinc	11.06 ± 0.61	33.50 ± 2.15	6240 ± 1831.33	53.80 ± 1.43	45.50 ± 1.22	0.70 ± 0.42	0.00 ± 0.00	0.00 ± 0.00
Arte + Vit A + Se	11.20 ± 0.69	32.70 ± 1.61	6260 ± 288.75	50.30 ± 1.54	49.10 ± 1.62	0.40 ± 0.16	0.00 ± 0.00	0.20 ± 0.13
Arte + Vit E + Zinc	10.66 ± 0.48	32.00 ± 1.48	6430 ± 728.17	46.90 ± 1.14	52.50 ± 1.10	0.20 ± 0.13	0.30 ± 0.15	0.10 ± 0.10
Arte+ Vit E+ Se	10.68 ± 0.6	32.50 ± 1.65	5800 ± 728.77	48.50 ± 2.77	49.60 ± 2.59	1.20 ± 0.36	0.20 ± 0.13	0.50 ± 0.27
One way ANOVA	F = 0.71 P > 0.05	F = 0.68 P > 0.05	F = 1.01 P> 0.05	F = 1.66 P > 0.05	F = 1.61 P > 0.05	F = 2.27 P < 0.05	F = 0.75 P > 0.05	F = 1.22 P > 0.05

Values are expressed as mean ± SEM. df = 149, P < 0.05 is considered significant.

Table 4. Comparative profile of total WBC (/mm^3) count on day 0 of treatment and day 28 of follow up.

Group	WBC_0 (/mm^3)	WBC_{28} (/mm^3)
Apparently Healthy Control (N10)	6400 ± 1017.64	6985 ± 1212.88
Amodia + Arte (N0,10,N28,8)	8130.00 ± 1127.64	7110.00 ± 443.83
Artemet + Lume (N0,10,N28,8)	5450.00 ± 529.41	6410.00 ± 507.38
Arte + vit A (N0,10,N28,8)	6810.00 ± 954.92	9236.00 ± 660.60
Arte + vit E(N0,10,N28,)	7468.00 ± 1008.80	9609.00 ± 746.76
Arte + Zinc (N0,10,N28,8)	5429.00 ± 482.03	8648.00 ± 338.12
Arte + Se (N0,10,N28,8)	7840.00 ± 1482.88	10051.00 ± 1025.41
Amodia + VitA (N0,10,N28,8)	6510 ± 768.32	8463.00 ± 616.93
Amodia + VitE (N0,10,N28,8)	5870.00 ± 410.43	8759.00 ± 451.68
Amodia + Zinc (N0,10,N28,8)	7272.00 ± 845.37	8925.00 ± 708.34
Amodia + Se (N0,10,N28,8)	5740.00 ± 412.09	8570.90 ± 449.04

Table 4 Contd.

Arte+VitA+VitE (N0,10,N28,8)	6360 ± 1246.08	9488.00 ± 819.86
Arte+VitA+Zic (N0,10,N8,8)	6240 ± 1831.33	9498.00 ± 490.32
Arte+VitA+Se (N0,10,N28,8)	6260 ± 288.75	9002.00 ± 513.22
Arte+VitE+Zic (N0,10,N28,8)	6430 ± 728.17	9290.00 ± 459.58
Arte+VitE+Se (N0,10,N28,8)	5800 ± 728.77	9176.00 ± 413.20
One way ANOVA	F = 1.01 P > 0.05	F = 2.47 P < 0.05
Paired T-test WBC_0 /WBC_{28}	P < 0.05	
Pearson's correlation	0.76, P < 0.01	

WBC_0 = white blood cell count on day 0 of treatment; WBC_{28} = white blood cell count on day 28 of follow up, P < 0.05 is significant; P < 0.01 is strongly significant.

Table 5. Comparative profile of WBC differential count on day 0 of treatment and day 28 of follow up.

Group	N_0 (%)	L_0 (%)	M_0 (%)	B_0 (%)	E_0 (%)	N_{28} (%)	L_{28} (%)	M_{28} (%)	B_{28} (%)	E_{28} (%)
Amodia + Arte (N0,10,N28,8)	48.50 ± 2.29	48.80 ± 2.12	2.20 ± 0.81	0.10 ± 0.10	1.00 ± 0.54	53.80 ± 1.11	45.90 ± 1.22	0.20 ± 0.13	0.10 ± 0.10	0.00 ± 0.00
Artemet +Lume (N0,10,N28,8)	46.60 ± 1.67	52.00 ± 1.78	0.90 ± 0.23	0.10 ± 0.10	0.40 ± 0.40	54.40 ± 1.10	44.80 ± 0.84	0.30 ± 0.15	0.10 ± 0.10	0.30 ± 0.15
Arte +Vit A (N0,10,N28,8)	49.60 ± 3.02	48.10 ± 3.23	1.30 ± 0.30	0.40 ± 0.22	0.60 ± 0.34	38.70 ± 1.23	60.80 ± 1.27	0.40 ± 0.22	0.10 ± 0.10	0.00 ± 0.00
Arte + Vit E (N0,10,N28,)	52.40 ± 2.24	44.70 ± 2.31	1.70 ± 0.75	0.20 ± 0.13	0.90 ± 0.53	39.90 ± 0.92	59.40 ± 0.79	0.50 ± 0.22	0.10 ± 0.10	0.10 ± 0.10
Arte+Zinc (N0,10,N28,8)	50.50 ± 1.96	48.70 ± 1.87	0.80 ± 0.20	0.10 ± 0.10	0.10 ± 0.10	42.80 ± 1.23	57.00 ± 1.18	0.20 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
Arte+Se (N0,10,N28,8)	54.40 ± 1.83	44.50 ± 1.85	0.80 ± 0.25	0.20 ± 0.13	0.10 ± 0.10	41.60 ± 1.51	57.70 ± 1.50	0.30 ± 0.15	0.30 ± 0.15	0.20 ± 0.13
Amodia+VitA (N0,10,N28,8)	53.20 ± 1.99	46.00 ± 2.01	0.40 ± 0.22	0.20 ± 0.20	0.20 ± 0.20	40.20 ± 1.46	59.80 ± 1.46	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Amodia+VitE (N0,10,N28,8)	55.20 ± 2.58	43.70 ± 2.56	0.60 ± 0.22	0.20 ± 0.13	0.30 ± 0.21	37.30 ± 1.87	61.90 ± 1.68	0.50 ± 0.22	0.10 ± 0.10	0.20 ± 0.13
Amodia+Zinc (N0,10,N28,8)	47.50 ± 1.92	51.30 ± 1.90	0.60 ± 0.22	0.40 ± 0.22	0.10 ± 0.10	37.30 ± 1.68	62.00 ± 1.67	0.70 ± 0.30	0.00 ± 0.00	0.00 ± 0.00
Amodia+Se (N0,10,N28,8)	50.80 ± 1.95	47.50 ± 2.41	0.30 ± 0.15	0.20 ± 0.13	0.20 ± 0.20	41.50 ± 1..56	58.50 ± 1.56	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Arte+VitA+VitE (N0,10,N28,8)	51.40 ± 2.73	47.90 ± 2.64	0.50 ± 0.17	0.10 ± 0.10	0.20 ± 0.13	40.00 ± 1..50	59.50 ± 1.46	0.30 ± 0.21	0.10 ± 0.10	0.10 ± 0.10
Arte+VitA+Zinc (N0,10,N28,8)	53.80 ± 1.43	45.50 ± 1.22	0.70 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	38.40 ± 2.02	61.40 ± 1.96	0.20 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
Arte+VitA+Se (N0,10,N28,8)	50.30 ± 1.54	49.10 ± 1.62	0.40 ± 0.16	0.00 ± 0.00	0.20 ± 0.13	41.00 ± 1..51	59.00 ± 1.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Arte+VitE+Zinc (N0,10,N28,8)	46.90 ± 1.14	52.50 ± 1.10	0.20 ± 0.13	0.30 ± 0.15	0.10 ± 0.10	37.70 ± 1.76	62.80 ± 1.51	0.40 ± 0.22	0.00 ± 0.00	0.10 ± 0.10
Arte+VitE+Se (N0,10,N28,8)	48.50 ± 2.77	49.60 ± 2.59	1.20 ± 0.36	0.20 ± 0.13	0.50 ± 0.27	37.40 ± 1..22	61.90 ± 1.03	0.60 ± 0.27	0.00 ± 0.00	0.10 ± 0.10
One way ANOVA	F = 1.66 P>0.05	F=1.61 P>0.05	F=2.27 P>0.05	F=0.75 P>0.05	F=1.22 P>0.05	F=13.52 P<0.05	F=15.01 P<0.05	F=1.38 P>0.05	F=1.23 P>0.05	F=1.40 P>0.05
Paired T-test	P <0.05	P<0.05	P<0.05	P<0.05	P<0.05					
Pearson's correlation	-0.07, P>0.05	-0.03, P>0.05	0.06, P>0.05	-0.04, P>0.05	0.01, P>0.05					

P < 0.05 is significant.

Table 6. Mean temperature level (mean ± SEM) in different groups from day 0 to day 2 (D0-D2) of treatment.

Group	T0 (°C)	T1 (°C)	T2 (°C)	Paired t-test T0/T1	Paired t-test T0/T2	Paired t-test T1/T2
Amodia + Arte (N0,10,N1,8,N2,8)	38.06 ± 0.07	36.98 ± 0.10	36.89 ± 0.08	P < 0.05	P <0.05	P >0.05
Artemet + Lume (N0,10,N1,9,N2,9)	37.96 ± 0.09	36.81 ± 0.07	36.96 ± 0.07	P < 0.05	P <0.05	P >0.05
Arte + Vit A (N0,10,N1,6,N2,6)	38.10 ± 0.09	37.02 ± 0.10	37.05 ± 0.12	P < 0.05	P <0.05	P >0.05
Arte + Vit E, (N010,N1,7,N2,7)	38.06 ± 0.12	37.10 ± 0.14	36.89 ± 0.08	P < 0.05	P <0.05	P >0.05
Arte + Zinc, (N010,N1,8,N2,8)	38.01 ± 0.06	37.03 ± 0.12	36.99 ± 0.06	P < 0.05	P <0.05	P >0.05
Arte + Se, (N0,10,N1,8,N2,8)	38.01 ± 0.05	37.16 ± 0.14	36.89 ± 0.08	P < 0.05	P <0.05	P >0.05
Amodia + Vit A, (N010,N1,8,N2,8)	38.04 ± 0.08	37.05 ± 0.15	36.99 ± 0.06	P < 0.05	P <0.05	P >0.05
Amodia + Vit E, (N0,10,N1,8,N2,8)	38.06 ± 0.14	37.05 ± 0.13	36.89 ± 0.08	P < 0.05	P <0.05	P >0.05
Amodia + Zinc, (N0,10,N1,7,N2,7)	37.95 ± 0.05	36.84 ± 5.31	36.75 ± 0.08	P < 0.05	P <0.05	P >0.05
Amodia + Se (N0,10,N1,9,N2,9)	38.15 ± 0.14	36.81 ± 0.08	36.695 ± 5.28	P < 0.05	P <0.05	P >0.05
Arte + Vit A + Vit E (N0,10,N1,7,N2,7)	37.98 ± 0.07	37.20 ± 0.13	37.01 ± 0.07	P < 0.05	P <0.05	P >0.05
Arte + Vit A+ Zinc (N0,10,N1,6,N2,6)	38.15 ± 0.09	36.87 ± 0.12	37.03 ± 0.17	P < 0.05	P <0.05	P >0.05
Arte + Vit A + Se (N0,10,N1,8,N2,8)	38.08 ± 0.11	37.01 ± 0.12	36.85 ± 0.06	P < 0.05	P <0.05	P >0.05
Arte + Vit E + Zinc, (N0,10,N1,7,N2,7)	37.91 ± 0.05	37.08 ± 0.10	36.94 ± 0.05	P < 0.05	P <0.05	P >0.05
Arte + Vit E + Se (N0,10,N1,10,N2,10)	37.93 ± 0.06	36.87 ± 0.87	36.97 ± 0.05	P < 0.05	P <0.05	P >0.05
One way ANOVA	F = 0.73 P > 0.05	F = 1.07 P > 0.05	F = 1.11 P > 0.05			
Bonferroni correction				P > 0.05	P > 0.05	P > 0.05

Values are expressed as mean ± SEM. T0/TI df = 115, T0/T2 df = 115, T1/T2 df = 115. P < 0.05 is considered significant. Key: N0=number of participants on day 0, N1= number of participants on day 1, N2=number of participants on day 2.

and the reduction in the risk of fever and malaria episode have been observed in children who received zinc supplementation (Seyrek et al., 2005; Zeba et al., 2008). Vitamin A has an important regulatory role in systemic immune function (Ross and Stephenson, 1996; Semba, 1998). Vitamin A deficiency impairs cytotoxic T lymphocyte activity (Sijtsma et al., 1990) and neutrophil function in animals (Twining et al., 1997). Vitamin A supplementation improves natural killer cell cytotoxicity in rats (Zhao and Ross, 1995) and increases the number of natural killer cells in HIV-infected children (Hussey et al., 1996). Antibody responses to tetanus toxoid (Semba et al., 1992) and measles vaccines (Coutsoudis et al., 1992) are also enhanced by vitamin A supplementation. In epidemiologic studies, mega dose vitamin A supplementation of children reduced the severity of infectious morbidity such as measles (Hussey and Klein, 1990), malaria (Shankar et al., 1999), and diarrhea, and increased overall survival (Fawzi et al., 1993).

Antioxidant vitamins are important enhancers of immune function. Vitamin E deficiency is associated with impairment of cell-mediated immune functions, such as the delayed type hypersensitivity (DTH) skin response, neutrophil phagocytosis, and lymphocyte proliferation in human and animal studies (Bendich, 1988). Vitamin E supplementation has immunostimulatory benefits in acquired immune deficiency syndrome (AIDS)-infected mice, including increased IL-2 production and natural killer cell cytotoxicity and a reduced production of inflammatory cytokines such as tumor necrosis factor α and IL-6 (Wang et al., 1994; Wang et al., 1995). Short-term, high-dose vitamin E supplementation in elderly subjects significantly

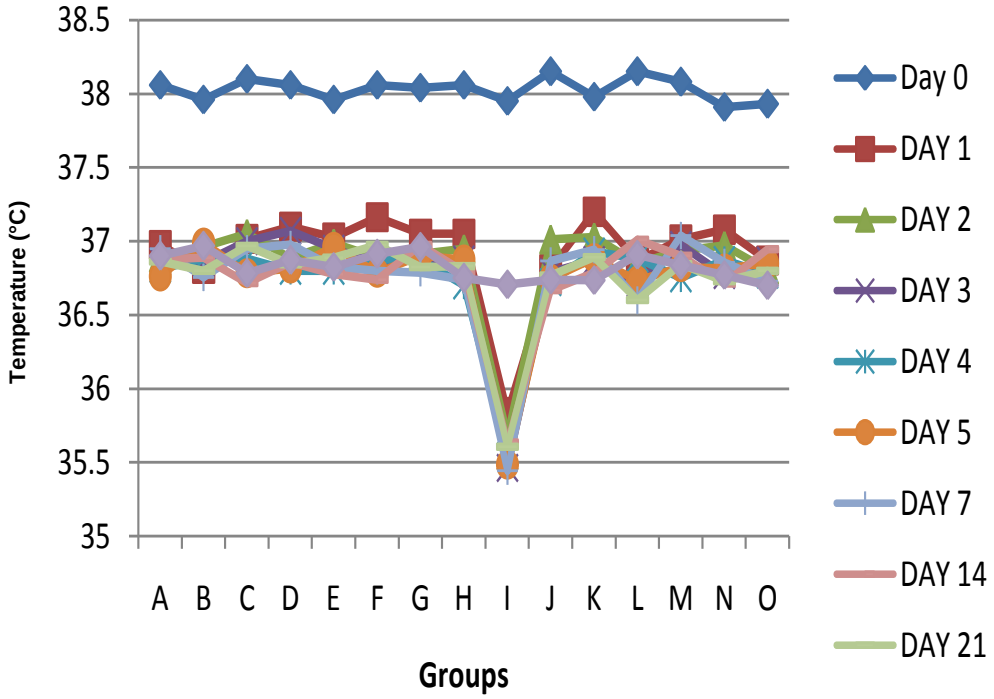

Figure 1. Temperature profile in the treated groups from day 0 to day 21. Key: A= artesunate+amodiaquine, B= artemether+lumefantrine, C= artesunate+vitA, D=artesunate+vitE, E= artesunate+zinc, F = artesunate + selenium, G=amodiaquine+vitA, H= amodiaquine+vitE, I= amodiaquine+zinc, J= amodiaquine+selenium, K=artesunate+vitA+vitE, L=artesunate+vitA+zinc, M =artesunate+vitA + selenium, N = artesunate+vitE+zinc, O =artesunate+vit E+selenium, PCR= Parasite Clearance Rate (in percentage).

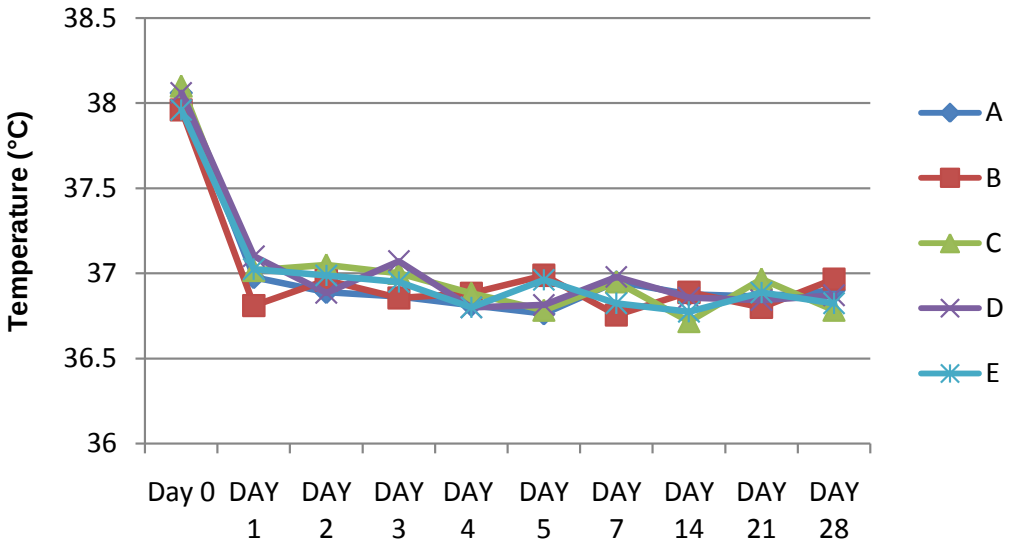

Figure 2. Temperature profile in the treated groups from day 0 to day 28. Key: A=artesunate+amodiaquine, B=artemether+lumefantrine, C=artesunate+vitA, D=artesunate+vitE, E= artesunate+zinc.

increased lymphocyte proliferation from mitogen stimulation, IL-2 production, and the DTH response (Meydani et al., 1996). Longer-term vitamin E supplementation at lower dosages also increased the DTH response and improved the antibody response to T cell–dependent vaccines (Meydani et al., 1997). Vitamin

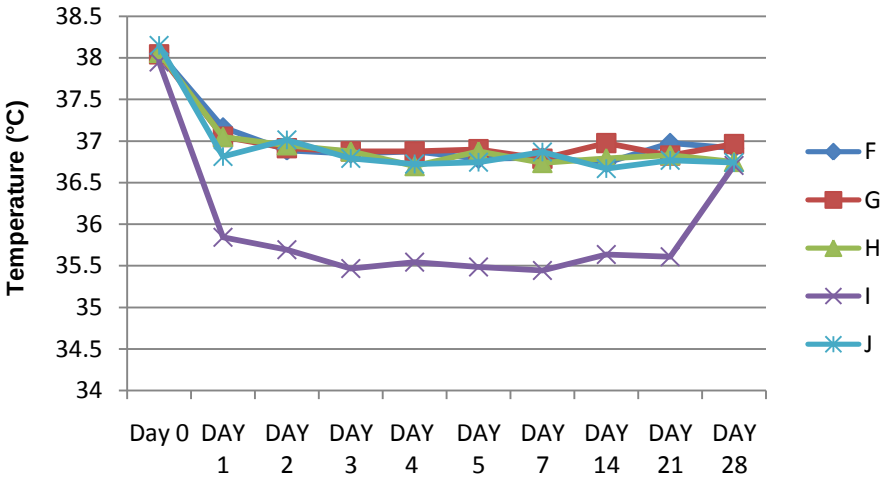

Figure 3. Temperature profile in the treated groups from day 0 to day 28. Key: F = artesunate + selenium, G=amodiaquine+vitA, H= amodiaquine+vitE, I= amodiaquine+zinc, J=amodiaquine+selenium.

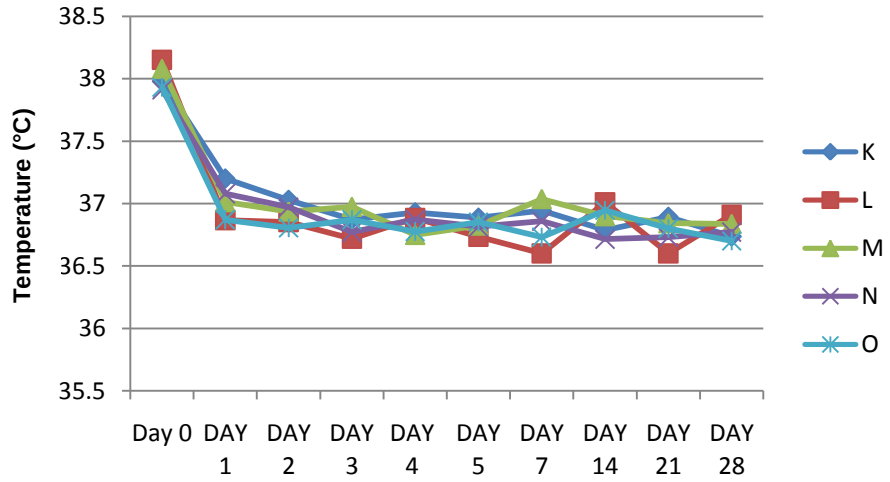

Figure 4. Temperature profile in the treated groups from day 0 to day 28. Key: K=artesunate+vitA+vitE, L=artesunate+vitA+zinc, M=artesunate+vitA+selenium, N= artesunate+vit E + zinc, O =artesunate+vit E+selenium.

Table 7. Correlational relationship between parasite density, packed cell volume, hemoglobin concentration and white blood cell count on day 0.

Parameter	PCV (%) (r value)	Hb (g/dl) (r value)	WBC (/mm^3) (r value)
Parasite density (day 0)	-0.102*	-0.084*	-0.004*
Pearson's correlation (2 tailed)	P=0.019	P=0.025	P=0.016

P < 0.05 = significant.

C deficiency negatively affects cellular immune responses in animal studies (Bendich, 1988).

Zinc deficiency has widespread negative effects on immunity and increases the risk of infections because of zinc's central role in many aspects of immune function (Shankar and Prasad, 1998). Zinc is necessary for the normal function of neutrophils, natural killer cells, and macrophages and for the production and activity of T and B lymphocytes. Zinc supplementation trials in children showed significant reductions in diarrheal and respiratory

infections and malaria (Black, 1998). Zinc is also crucial for the normal function of cells which mediate nonspecific immunity, such as neutrophils and natural killer cell. B lymphocyte development and antibody production, particularly immunoglobulin G, is compromised by zinc deficiency. The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiency. These can dysregulate intracellular killing, cytokine production, and phagocytosis.

The effects of zinc on these key immunologic mediators is rooted in the myriad roles for zinc in basic cellular functions such as deoxyribonucleic acid (DNA) replication, ribonucleic acid (RNA) transcription, cell division, and cell activation. Apoptosis or programmed cell death is potentiated by zinc deficiency. Zinc also functions as an antioxidant and can stabilize membranes.

Selenium is an essential structural component of the antioxidant enzyme glutathione peroxidase, and it has numerous important functions in the maintenance of humoral and cell-mediated immunity (Kiremidjian-Schumacher and Stotzky, 1987). Deficiency inhibits neutrophil function, the cytoxicity of T lymphocytes and natural killer cells, lymphocyte proliferation in response to mitogens, the DTH response, antibody production, and resistance to pathogens (Kiremidjian-Schumacher and Stotzky, 1987). In a small study of selenium depletion and supplementation in patients with gut failure and receiving parenteral nutrition, 2 to 4 months of supplementation with a moderate dose of selenium improved lymphocyte responses to various mitogens and antigens (Peretz et al., 1991).

In a related animal study, we observed a significant increase in total WBC count in the artesunate, chloroquine, vitamin A, vitamin E, zinc and selenium groups when compared with apparently healthy uninfected control. Although there was a non significant increase in total WBC count in the negative control and vehicle group, this increase is a reflection of the elevation in WBC count associated with malaria infection as reported by Kamga et al. (2010). The marked elevation of total WBC count in the antioxidant micronutrient treated group as observed in the present study is a reflection of the additional immunostimulating and immunomodulatory role of the micronutrients in malaria infection. Recent studies among children with *falciparum* malaria revealed that a fairly low lymphocyte and monocyte counts were independently associated with morbidity (Kamga et al., 2010). However, in the present study no significant change was observed in lymphocyte and monocyte count in the micronutrient treated groups. This suggests that antioxidant micronutrients may actually be involved in the modulation of lymphocyte and monocyte activity. A platelet count of less than $150 \times 10^3/mm^3$ of blood was found in 13% of the subjects with *falciparum* malaria in the study of Kamga et al. (2010). However, the finding in the present study revealed a markedly significant elevation of absolute platelet count in the micronutrient group. This suggests that antioxidant micronutrient may also be involved in the modulation of platelet activity in malaria.

Conclusion

Conclusively, combination of antioxidant micronutrients (vitamin A, E, selenium and zinc) with standard antimalarials has immunomodulating potential in the management of uncomplicated *falciparum* malaria in early childhood.

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