

Status of Some Basic Antioxidants in Pre- and Postmalaria Treatment in Children

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Abstract

Malaria is the most common tropical disease to which infants and children are the most susceptible. It is caused by *Plasmodium* species and is associated with oxidative stress, which has an effect on body antioxidants. The relationship between the degree of parasitemia and copper (Cu), zinc (Zn), and uric acid was evaluated in this study.

Seventy children (mean age: 7.80 ± 0.38 years) microscopically diagnosed positive for malaria parasite were selected. Fifty-six apparently healthy children (mean age: 6.68 ± 0.48 years) served as the control group. The malaria group was classified into pretreatment and posttreatment groups. The pretreatment group was also subgrouped based on parasitemia into four as follows: group A, group B, group C, and group D. Serum Cu and Zn were studied. Uric acid, an abundant endogenous metabolic antioxidant, was also determined.

The serum levels of Cu and Zn were significantly lower in pretreatment malaria patients compared with control. Uric acid level was slightly raised in the malaria patients but not significant ($p > 0.05$). In the posttreatment malaria patient group, serum uric acid and Cu levels were significantly raised compared with control ($p = 0.008$). Also, serum Cu and uric acid were significantly higher in the malaria posttreatment compared with the pretreatment group. Serum Zn level though higher in the posttreatment group compared with the pretreatment group was not significantly different ($p > 0.05$). The result showed a negative correlation between serum Cu level and parasitemia, and serum Zn level and parasitemia while that of uric acid and parasitemia was positively correlated but none of these were significantly correlated with parasitemia.

The observed changes in Cu, Zn, and uric acid levels in this study could be a reflection of progressive upregulation of the antioxidant system to combat the associated oxidative stress in this condition.

Keywords

- antioxidant
- children
- free radicals
- malaria parasitemia
- oxidative stress

Introduction

Malaria is one of the most important parasitic diseases worldwide and approximately 250 million people are infected every year and up to one million die, most of them being young children and pregnant women.¹ Infants are more vulnerable to malaria from the age of approximately 3 months when immunity acquired from the mothers wears off. The disease is caused by a parasite of the genus

Plasmodium that is transmitted by female *Anopheles* mosquitoes. Malaria is characterized by the typical clinical features, such as fever, shivers, headache, and myalgia. These typical malaria symptoms—often referred to as acute or febrile malaria—are especially seen in young children and nonimmune adults.

The malarial infection has the ability to activate the immune system, which causes the release of reactive oxygen species (ROS) with the potency of inducing oxidative

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damage and cell destruction.² Free radicals (such as hydroxyl radical and superoxide anion), which are generated during the malaria episode will react rapidly and nonspecifically with most biological molecules.³ Thus, there are complex antioxidant metabolites present in organisms that work together to prevent oxidative damage to cellular components.⁴ Generally, antioxidants may either prevent or they may remove ROS formed prior their damage to cellular components. Though these may not be totally removed due to their useful functions in the cells but rather keep at an optimal level.⁵ There are different antioxidants present in the body fluids at a wide range of concentrations, for instance, uric acid (UA), which is more evenly distributed. Also, zinc (Zn) and copper (Cu), the antioxidant nutrients, are required by the antioxidant enzymes. Zinc plays a key role in immune competence⁶ and also in the linear growth of children.⁷ Copper is an important component of superoxide dismutase (SOD), an antioxidant enzyme that fights against the effect of ROS.⁸ UA is an antioxidant oxypurine produced from xanthine by the enzyme xanthine oxidase and is an intermediate product of purine metabolism.⁹ In almost all land animals, urate oxidase has the ability to further catalyze the oxidation of UA to allantoin, while in humans UA is not further broken down.^{10,11} UA has the highest concentration of any blood antioxidant¹² and provides over half of the total antioxidant capacity of human serum.¹³ The antioxidant activities of UA are complex assumed that it does not react with some oxidants, such as superoxide, but does act against peroxynitrite,¹⁴ peroxides, and hypochlorous acid.⁹ Introduction of UA in animal studies that investigated diseases accelerated by oxidative stress prevents the disease or reduces it.¹⁵ This finding was supported by UA antioxidant mechanism.¹⁶

Materials and Methods

Study Site and Population

Ethical approval was obtained from the University of Ibadan (UI)/University College Hospital (UCH) Ethical Committee before the commencement of the study. A written consent was obtained from the parents or guardians who agreed to participate and provide useful information about their children. The study was conducted at UCH, from May to August 2012 in Ibadan, Oyo State. Malaria is hyperendemic, with transmission peaking during the rainy season (June, July, and September). The hospital has a pediatrics unit and malaria research section where the children were examined. One hundred twenty-seven children aged 2 to 15 years were enrolled into the study.

Inclusion and Exclusion Criteria

Eligibility criteria were:

1. Children who visited UCH for malaria treatment.
2. Children within the age range of 2 to 15 years.
3. Parental consent for the child's participation.
4. Patients who did not receive antimalarial treatment before enrolment.

Exclusion Criteria

The exclusion criteria were:

1. Children below age 2 years.
2. Children above 15 years.
3. Children presenting with other illnesses.

Blood Collection

Blood samples were collected by finger prick for *P. falciparum* test. Three milliliters of blood was collected from each participant by venipuncture and dispensed into plain metal-free bottles. The samples were centrifuged at 5,000 rpm for 10 minutes and the supernatant (serum) was separated with a pipette and stored at -20°C until analysis of serum concentrations of Cu, Zn, and UA was done. The process was performed before and after treatment in children with conformed *P. falciparum* infection and once in uninfected subjects (control)

Confirmation of Malaria Parasite

Malaria thick and thin films were prepared by finger prick and the thin film was fixed with ethanol. Blood slides were stained with 5% Giemsa stain. The asexual parasites densities were estimated by counting parasites against 200 white blood cells, assuming a standard leukocyte count of 8,000 per μL .¹⁷ Parasites densities were classified into four groups: A (parasite density $< 1,000$ per μL); B (parasite density $< 2,000$ per μL); C (parasite density $< 10,000$ per μL); and D (parasite density $> 100,000$ per μL).

Drug Administration

Artesunate-amodiaquine was given orally to all children for 3 consecutive days according to their body weight. They had initial and day 3 follow-up evaluation.

Determination of Serum Uric Acid by Spectrophotometric Technique

- In 3 mL centrifuge tubes labeled standard, blank, and unknown, 0.2 mL working UA standard, water, and unknown sera were prepared, respectively.
- Trisodium phosphate reagent (0.2 mL) was added to each; mixed and left for 5 minutes. This alkaline treatment destroyed any ascorbic acid that was in the sample, a nonurate chromogen.
- Phosphotungstic acid (0.6 mL) was added to the solution, mixed well, and centrifuged for 5 minutes.
- The supernatant fluid (0.5 mL) was transferred to a 12×75 mm square cuvet of 10-mm light path.
- Phosphotungstic acid (0.2 mL) and 1.5 mL of carbon-urea-triethanolamine (CUTE) reagent were added and mixed well by inversion.
- After 20 minutes, the absorbance of the sample against the blank at 680 to 700 nm was read.
- Calculation:

$\text{Au/As} \times 5.0 = \text{mg/dL UA}$ (Au = absorbance of UA; As = absorbance of standard).

Determination of Serum Copper and Zinc by Atomic Absorption Spectrophotometry Technique

The frozen serum samples were thawed and brought to room temperature after which they were prepared for analysis. The samples were deproteinized with 2 M hydrochloric acid (HCl) (1:3). The digested samples were then centrifuged at $1,163 \times g$ for 5 minutes and the resulting supernatant was aspirated into the atomic absorption spectrophotometry (AAS). Three working standard solutions in parts per million (ppm) were prepared for each trace metal.

Statistical Methods

The data obtained from this study were subjected to statistical analysis using SPSS version 17.0 and Microsoft Excel Office. The descriptive statistics such as frequency, mean,

and standard error of mean (SEM) of sex, age, weight, and height, and the biochemical profile for the study groups were computed. Student's *t*-test (independent and paired) and analysis of variance (ANOVA) were used to compare means among variables. Correlation between serum Cu level and parasitemia, serum Zn level and parasitemia, and UA and parasitemia were examined using Pearson's product moment correlation. Results were considered significant at *p*-value of $< 5\%$.

Results

The background characteristics of patients who participated in the study are presented in **Table 1**.

The levels of Cu and Zn were significantly lower in the pretreatment group compared with control ($p < 0.05$). While UA was not significantly different (**Table 2**).

Parasitemia level was grouped into four and each group was compared with control. In group A, Cu and Zn were significantly lower compared with control ($p < 0.05$), while UA was not significantly different ($p > 0.05$) (**Table 3**).

Similarly, in group B, Cu and Zn were significantly lower compared with control ($p < 0.05$). UA was significantly higher compared with control ($p < 0.05$) (**Table 3**).

Copper and Zn were significantly lower in group C compared with control ($p < 0.05$). UA was higher than in control; however, it was not statistically significant ($p = 0.056$) (**Table 3**).

In group D, Cu and Zn were significantly lower compared with control ($p < 0.05$). Meanwhile, UA was higher in patients compared with control but not significant (**Table 3**).

Table 1 Background characteristics

	Control	Pretreatment	Posttreatment
Variable	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
Age (y)	6.64 \pm 0.48	7.87 \pm 0.37	8.14 \pm 0.45
Weight (kg)	19.77 \pm 1.29	22.83 \pm 1.42	24.48 \pm 2.06
Height (cm)	111.57 \pm 3.09	121.25 \pm 2.26	123.55 \pm 2.63
Sex	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)
Male	26 (49.06)	36 (52.17)	23 (45.10)
Female	27 (50.94)	33 (47.83)	28 (54.90)

Abbreviation: SEM, Standard error of mean.

Table 2 Copper, zinc, and uric acid in malaria pretreatment compared with control

Variables	Case (<i>n</i> = 70) Mean \pm SEM	Control (<i>n</i> = 56) Mean \pm SEM	<i>t</i> -Value	<i>p</i> -Value
Cu ($\mu\text{g/dL}$)	82.46 \pm 1.30	128.15 \pm 1.30	24.512	$< 0.05^a$
Zn ($\mu\text{g/dL}$)	85.90 \pm 8.10	109.86 \pm 1.84	2.600	$< 0.05^a$
Uric acid (mg/dL)	6.94 \pm 0.35	5.6 \pm 0.35	2.595	0.110

Abbreviation: SEM, standard error of mean.

^aMean values are significantly different compared with control ($p < 0.05$).

Table 3 Zinc, Copper, and uric acid in four groups of parasitemia compared with control

Variables	Control (<i>n</i> = 56) Mean \pm SEM	Parasitemia group A (pd $< 1,000$ per μL) (<i>n</i> = 11) Mean \pm SEM	Parasitemia group B (pd 1,000– $< 2,000$ per μL) (<i>n</i> = 30) Mean \pm SEM	Parasitemia group C (pd 2000– $< 10,000$ per μL) (<i>n</i> = 22) Mean \pm SEM	Parasitemia group D (pd 10,000– $< 100,000$ per μL) (<i>n</i> = 5) Mean \pm SEM
Cu ($\mu\text{g/dL}$)	128.15 \pm 9.68	82.25 \pm 2.65 ^a	81.93 \pm 2.20 ^a	81.84 \pm 2.33 ^a	88.14 \pm 5.22 ^a
Zn ($\mu\text{g/dL}$)	109.86 \pm 1.85	71.29 \pm 2.14 ^a	80.34 \pm 2.18 ^a	77.76 \pm 2.36 ^a	79.21 \pm 2.80 ^a
Uric acid (g/dL)	5.63 \pm 0.35	5.45 \pm 0.50	7.28 \pm 0.63 ^a	6.95 \pm 0.62	6.88 \pm 0.90

Abbreviations: pd, parasite density; SEM, standard error of mean.

^aMean values are significantly different compared with control ($p < 0.05$).

To evaluate the effect of the treatment on parasitemia and its outcomes on the parameters, pre- and posttreatments were compared. Copper and UA were significantly increased in posttreatment compared with pretreatment ($p < 0.05$) (►Table 4).

All the three groups were compared to assess the effect of the intervention on malaria patients compared with apparently healthy controls. In these groups, Cu and UA were significantly different ($p < 0.05$) while Zn was not ($p > 0.05$) (►Table 4).

A negative correlation existed in this study between parasitemia and pretreatment Cu level, and also between parasitemia and pretreatment Zn level. In contrast, the relationship between parasitemia and UA was positive. These three relationships were very weak (►Table 5).

Discussion

This study showed that the serum Zn levels in patients varied in all the four categories of parasitemia levels. This slight decrease of serum Zn in these malaria-infected children may be attributed to increased consumption of Zn resulting from the enhanced production of tumor necrosis factor and other free radicals generated in the course of increasing parasitemia. It was documented by Onyesom et al,¹⁸ that severe malaria enhances the production of free radicals. It has been suggested that lowered Zn level is mediated by exaggerated production of free radicals and may reflect a normal protective mechanism. In addition to increased utilization of Zn by free radicals and oxidants, another plausible explanation for lower Zn levels in malaria can be preexisting Zn deficiency making the child more susceptible to malaria due to impaired immunity.^{19,20} This implies that serum Zn tended to decline with increasing malaria parasitemia. It was reported by Veenemans et al²¹ that there was a reduction

in the episodes of malaria parasitemia in malaria patients on Zn supplement, suggesting that Zn might protect against severe forms of malaria.²⁰ Although most often, serum Zn is low in malaria episodes in children, this was recovered after the completion of the treatment (72 hours).²² This suggests that low serum concentration in acute malaria infection most likely reflects the redistribution of blood Zn to other organs during the acute phase response.

In this study, serum Cu was significantly lower in malaria patients compared with the control group. This is in contrast to Saad et al's study²³ that reported that serum Cu is most often raised as a component of ceruloplasmin, an acute phase reactant and antioxidant. Copper is also a component of the potent cytosolic antioxidant Cu–Zn SOD (Cu–Zn SOD). The reduced Cu in this study could be to scavenge the oxidant released by immune cells and in so doing prevented the host and lessened the oxidative effect of parasitemia.

Uric acid was slightly higher in pretreatment but not statistically different as compared with control. In addition, this was statistically significant at posttreatment. UA is a metabolic or endogenous antioxidant. The UA pool appears to expand to contend with the body burden of oxidants as an adaptive response. The elevated urate level in malaria patients could indicate an adaptive response or mechanism against the toxins released by the immune cells against the parasites. This is in line with earlier reports, of raised serum UA concentration as a result of a physiologic response to oxidative stress, providing a counterregulatory increase in antioxidant defences.^{24,25} According to Lopera-Mesa et al,²⁶ UA may be a consistent and reliable biomarker of significant generation of oxidants in malaria. UA also acts as a repair agent of oxidative damage to DNA bases,²⁴ this may be another reason why UA was elevated in malaria patients after the treatment. In addition, in posttreatment, serum UA

Table 4 Copper, zinc, and uric acid in pre- and posttreatment of malaria and control

Variables	Control (<i>n</i> = 56) Mean ± SEM	Pretreatment (<i>n</i> = 70) Mean ± SEM	Posttreatment (<i>n</i> = 50) Mean ± SEM
Cu (µg/dL)	128.15 ± 9.68	82.46 ± 1.30	114.78 ± 10.3 ^a
Zn (µg/dL)	109.86 ± 1.85	85.90 ± 8.10	108.87 ± 15.50
Uric acid (g/dL)	5.63 ± 0.35	6.94 ± 0.35	8.77 ± 0.56 ^{a,b}

Abbreviation: SEM, standard error of mean.

^aMean values are significantly different in posttreatment compared with pretreatment ($p < 0.05$).

^bMean values are significantly different in posttreatment compared with control ($p < 0.05$).

Table 5 Correlation of copper, zinc, and uric acid with parasitemia in the pretreatment group

	Parasite	Pretreatment	Pretreatment	Pretreatment
Variable	Count	Copper	Zinc	Uric acid
Pearson's correlation	1.000	−0.161	−0.155	0.166
<i>p</i> -Value		0.223	0.242	0.380

was higher than in pretreatment probably due to oxidative stress induced by the drug.

Conclusion

The study revealed low levels of Cu and Zn in the pretreatment phase, which may at least in part suggest depressed antioxidant level as these micronutrients are component of the antioxidant system (Cu–Zn SOD). The raised levels in the posttreatment phase may imply improved antioxidant status.

Conflict of Interest
None.

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