

## Determination of malondialdehyde, Uric Acid, Bilirubin And Total Antioxidant Status, in Children Under 5 Years Suffering From Malaria in Osogbo, Nigeria

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### Abstract

**Background:** Children are prone to malaria infection. Peroxidation of membranes lipids, hemolysis and oxidative stress due to invasion of erythrocytes by malaria parasites could worsen the disease and increase mortality. **Aim:** Evaluation of Malondialdehyde (MDA), Total Antioxidant Status (TAS), uric acid and bilirubin in children suffering from malaria.

**Materials and Method:** Thirty malaria patients (age 1-5 years) were divided into two groups based on density of parasitaemia: Group1 = thirteen children scored as (+) and group2 = seventeen children scored as (++) . Thirty apparently healthy children (age 1-5 years, parasitaemia score= -) served as control. Serum from groups 1, 2 and control were used to determine levels of uric acid, TAS, MDA and bilirubin by standard spectrophotometric methods. **Results:** Results showed that mean uric acid in group1 ( $5.28 \pm 0.19$ ) and group2 ( $5.96 \pm 0.25$ ) as well as MDA levels (group1= $6.36 \pm 0.17$ ; group2=  $7.97 \pm 0.44$ ) were higher than means of corresponding control (uric acid= $3.69 \pm 0.10$ , MDA= $1.02 \pm 0.06$ ) respectively. Lower TAS level (group1=  $1.16 \pm 0.02$  group2= $0.92 \pm 0.06$ ) than control ( $1.44 \pm 0.01$ ) was observed. **Conclusion:** Malaria leads to increased peroxidation, haemolysis and oxidative stress possibly resulting in depletion of the body's antioxidant defenses and decreased TAS. Children, generally not enthusiastic about intake of fruits and vegetables may benefit from antioxidant supplementation during malaria bouts.

**Keywords:** Malaria, Haemolysis, Oxidative Stress, Lipid Peroxidation, Antioxidants

### I. Introduction

Malaria is a mosquito-borne infectious disease of humans caused by eukaryotic protists of the genus *Plasmodium* are responsible for more than 500 million clinical cases of malaria globally each year<sup>[1]</sup>. About 1.5 – 3.5 million deaths from malaria have been reported to occur annually and of these deaths the overwhelming majority is among children aged <5 years old Several studies showed that physicochemical changes in the membrane of the erythrocyte induced by oxidative stress is responsible for membrane lipid peroxidation and increased haemolysis seen in malaria patients<sup>[3,4,5]</sup>. Orengo and colleagues showed that after haemolysis, uric acid derived from hypoxanthine accumulated by *Plasmodium falciparum*-infected erythrocytes is a major contributor to the inflammatory response triggered in human peripheral blood mononuclear cells (PBMCs)<sup>[6]</sup>. The immune system of the body is activated by infections, including malaria, thereby causing the release of reactive oxygen species. The accumulation of organic peroxides and oxidation of membrane lipids place a stress on cellular vitality ultimately leading to destructive effects on the cell. The production of malondialdehyde is used as a biomarker to measure the level of oxidative stress in an organism<sup>[7]</sup>.

Antioxidants are substances which when present in low concentration compared to the oxidizable substrate, significantly delay or inhibit the oxidation of that substrate<sup>[8]</sup>. The physiological role of antioxidants is to prevent damage to cellular components arising from the activity of chemical reactions involving free radicals seen in oxidative stress. There are three classes of antioxidants namely: primary antioxidants<sup>[9]</sup>, secondary antioxidants<sup>[10, 11]</sup> and tertiary antioxidants<sup>[12]</sup>. All these antioxidants in the body together form the total antioxidant status (TAS) of an individual. Bilirubin is the yellow breakdown product of normal heme catabolism after haemolysis. Heme is found in hemoglobin, a principal component of red blood cells. So far few studies have been carried out on the extent of oxidative stress, lipid peroxidation and haemolysis in malarious children living in Southwestern Nigeria. Therefore this study is designed to determine possible associations and relationships between malaria infection and oxidative stress by assessing changes in uric acid, total antioxidant status (TAS), malondialdehyde (MDA) and bilirubin in children under 5 years of age in Osogbo, Osun state, Southwest Nigeria.

## II. Materials

### 2.1 Subject selection

The study involved thirty (30) malariapatients (age range 1-5 years) diagnosed on the basis of clinical findings and positive peripheral blood smear for malaria parasite and thirty subjects, who were found to be negative for *P.falciparum* in their peripheral blood served as control. These subjects were children that attended Osun State University Teaching Hospital (OSUTH), Asubiaro, Osogbo and Maternity Center Olorunsogo, Egbedore Local Government Area, Osogbo, Osun State, Nigeria. The malaria patients were further subdivided into two groups on the basis of the presence and density of *P. falciparum* in each blood sample (group 1= + and group 2= ++)

### 2.2 Collection and storage of Blood Samples

5ml of venous blood was obtained from each malaria patient and control subjects by venopuncture before they were administered with any antimalaria therapy at the time of hospitalization. Blood was allowed to clot at room temperature and serum was obtained by centrifugation at 3000rpm for 5 minutes. Serum total and conjugated bilirubin concentrations were determined immediately while the remaining serum was stored at -20 °C until used for the measurements of uric acid, MDA and TAS.

### 2.3 Parasitology

The presence and density of *P. falciparum* in each blood sample was determined from Giemsa-stained thick blood films. A slide was scored as negative if 100 high power fields (at 100x objective) had been examined carefully without seeing any parasites. The amount of parasites in positive smears was counted to determine the intensity of infection. Positive smears were grouped into 2 (high field power 1-10= MP+, high field power 11-100= MP++) based on the criteria described by Cheesbrough<sup>[13]</sup>

## III. Methods

### 3.1 Methods of Assay of uric acid, total antioxidant status, malondialdehyde and bilirubin

Assay for serum uric acid was carried out according to the protocol described by Fossati and coworkers<sup>[14]</sup>, Malondialdehyde (MDA) level was estimated according to the protocol outlined by Gutteridge and Wilkins<sup>[15]</sup> and total antioxidant status was measured according to the spectrophotometric method of Koracevic and colleagues<sup>[16]</sup>. Serum bilirubin was assayed by the modified method of Malloy and Evelyn<sup>[17]</sup>. Results obtained for MDA was calculated as described by Buege and Aust<sup>[18]</sup>.

### 3.2 Statistical Analysis

Results obtained from this study were reported as mean ( $\pm$  SE). Means of the parameters were analyzed using the analysis of variance (ANOVA) test and pairwise t-test was used to compare significant variables. Pearson's correlation coefficient (r) was used to determine the relationship between means of the parameters in the groups. Results were regarded as significant at  $p < 0.05$ .

## III. Results

Results from the present study showed that the means of MDA, TAS, uric acid and bilirubin were higher in the test group when compared with the corresponding control ( $p < 0.001$ ) (Table 1)

When the test group was categorized into two groups based on the density of the malaria parasite (MP+ and MP++), mean MDA, TAS, uric acid and bilirubin were significantly higher than respective means of the corresponding control group (table 2). However, mean TAS in MP++ was higher than the value of TAS in the control group (Table 3). When means of MDA, TAS, uric acid and bilirubin of MP+ were compared with respective means of MP++, a significantly higher MDA and uric acid values were observed in MP++ group while a lower Mean TAS was seen in MP++ group when the mean value was compared with the corresponding value in MP+ group (Table 4). Negative Pearson's correlation coefficient (r) was observed between uric acid and TAS ( $r = -0.567$ ,  $p < 0.05$ ) and between total bilirubin and TAS ( $r = -0.716$ ,  $p < 0.01$ ) (Table 5)

**Table 1** Means (SEM), p-values of biochemical parameters in test and control groups

Parameters	Test (n=30)	Control (n=30)	t-value	p-value
MDA $\mu\text{mol/l}$	7.05 $\pm$ 0.26	1.02 $\pm$ 0.06	22.863	0.001
TAS $\mu\text{mol/l}$	1.05 $\pm$ 0.04	1.44 $\pm$ 0.01	-10.211	0.001
Uric acid mg/dl	5.58 $\pm$ 0.16	3.69 $\pm$ 0.10	9.887	0.001
TB $\mu\text{mol/l}$	20.77 $\pm$ 0.26	12.27 $\pm$ 0.37	18.727	0.001
CB $\mu\text{mol/l}$	7.13 $\pm$ 0.22	3.67 $\pm$ 0.19	11.876	0.001

TAS= total antioxidant status, MDA= Malondialdehyde, TB= total bilirubin, CB= conjugated bilirubin

**Table 2.** Means (SEM), p-values of biochemical parameters in test group I (MP +) and control

Parameters	MP (+),n=13	Control,n=30	t-value	p-value
MDA $\mu\text{mol/l}$	6.36 $\pm$ 0.17	1.02 $\pm$ 0.06	-29.344	0.001
TAS $\mu\text{mol/l}$	1.16 $\pm$ 0.02	1.44 $\pm$ 0.01	13.309	0.001
Uric acid mg/dl	5.28 $\pm$ 0.19	3.69 $\pm$ 0.10	-8.223	0.001
TB $\mu\text{mol/l}$	20.47 $\pm$ 0.35	12.27 $\pm$ 0.37	-14.510	0.001
CB $\mu\text{mol/l}$	7.00 $\pm$ 0.32	3.67 $\pm$ 0.19	-9.617	0.001

TAS= total antioxidant status, MDA= Malondialdehyde, TB= total bilirubin, CB= conjugated bilirubin  
As shown in table 2, when the test group was divided based on parasite density,

**Table 3.** Means (SEM), p-values of biochemical parameters in test group I (MP ++) and control

Parameters	MP (++) ,n=17	Control,n=30	t-value	p-value
MDA $\mu\text{mol/l}$	6.36 $\pm$ 0.17	1.02 $\pm$ 0.06	-15.652	0.001
TAS $\mu\text{mol/l}$	1.16 $\pm$ 0.02	1.44 $\pm$ 0.01	8.293	0.001
Uric acid mg/dl	5.28 $\pm$ 0.19	3.69 $\pm$ 0.10	-8.339	0.001
TB $\mu\text{mol/l}$	20.47 $\pm$ 0.35	12.27 $\pm$ 0.37	-14.404	0.001
CB $\mu\text{mol/l}$	7.00 $\pm$ 0.32	3.67 $\pm$ 0.19	-10.416	0.001

TAS= total antioxidant status, MDA= Malondialdehyde, TB= total bilirubin, CB= conjugated bilirubin

**Table 4.** Means (SEM), p-values of biochemical parameters in test group based on density of malaria parasites

Parameters	MP (+) n=13	MP (++) n=17	t-Value	p-Value
MDA $\mu\text{mol/l}$	6.36 $\pm$ 0.17	7.97 $\pm$ 0.44	-3.419	0.004
TAS $\mu\text{mol/l}$	1.16 $\pm$ 0.02	0.92 $\pm$ 0.06	3.772	0.002
Uric Acid mg/dl	5.28 $\pm$ 0.19	5.96 $\pm$ 0.25	-2.218	0.035
TB $\mu\text{mol/l}$	20.47 $\pm$ 0.35	21.15 $\pm$ 0.36	-1.336	0.192
CB $\mu\text{mol/l}$	7.00 $\pm$ 0.32	7.31 $\pm$ 0.31	-0.676	0.505

TAS= total antioxidant status, MDA= Malondialdehyde, TB= total bilirubin, CB= conjugated bilirubin

**Table 5.** Pearson's Correlation coefficient (r) of biochemical parameters in MP(+) and MP (++)groups

Parameters	Parasite density group	Pearson's correlation coefficient (r)	p-value
Uric acid and TAS	+	-0.567	<0.05
TB and TAS	++	-0.716	<0.01
TB and CB	++	0.614	<0.01
TB and uric acid	++	0.719	<0.01
TB and MDA	++	0.683	<0.05

TAS= total antioxidant status, MDA= Malondialdehyde, TB= total bilirubin, CB= conjugated bilirubin

#### IV. Discussion

Asignificantly higher uric acid level in malarious children seenin this study is similar with the reports from previous studies <sup>[19, 20, 21]</sup> which showed an elevated level of uric acid in the blood of malaria infected humans and mice. A suggested mechanism for this observation was the presence of hypoxanthine and xanthine in both erythrocytes and the Plasmodium parasite. Hypoxanthine and xanthine is metabolized by the activities of the enzyme xanthine oxidase but the activity of xanthine oxidase has not been detected in erythrocytes or in the Plasmodium parasite. It is possible that the Plasmodium parasite imports excessive hypoxanthine and xanthine into the infected erythrocytes <sup>[22]</sup>. Since the enzyme is absent, imported hypoxanthine/xanthine would not be degraded into uric acid in erythrocytes. However, upon haemolysis, the accumulated hypoxanthine/xanthine would be released into the extracellular medium where they are degraded by xanthine oxidase to form uric acid. This process also results in the production of reactive oxygen species<sup>[23]</sup>. In humans, uric acid is the final product of hypoxanthine degradation because uricase enzyme was mutated throughout evolution and its activity cannot be found in human serum<sup>[24]</sup>. High concentrations of soluble uric acid induces the release of inflammatory mediators from different cell types including immune cells<sup>[25, 26, 27]</sup> suggesting that the soluble uric acid formed via hypoxanthine degradation could also contribute to the malaria induced inflammatory response.

Total antioxidant status (TAS) level observed in children with malaria infection was significantly lower than the level for healthy children. A previous study by Akpotuzor and colleagues[28], also reported a significantly reduced level of TAS in malaria infected children when compared to healthy childrenin Calabar, South –South Nigeria. The lower value observed in TAS in malaria may be attributed to increased utilization of the host's plasma antioxidants by the malaria parasites to counteract oxidative damages. Additionally, increased consumption and degradation of antioxidants and antioxidant enzymes as well as haemoglobin by malaria parasite to produce its own proteins has been reported by Koracevic and coworkers<sup>[16]</sup>.

The significant increase in malondialdehyde (MDA) concentration observed in malarious children seen in this present study might reflect the extent of lipid peroxidation and is supported by previous reports<sup>[29, 30]</sup>

where the authors reported an increased MDA concentration in malarious children both in Eku metropolis and Benin City, both in Mid-Western Nigeria. Some other studies<sup>[31, 32]</sup> also showed increased lipid peroxidation in malaria. Invasion of human erythrocytes by malaria parasite results in vulnerability to damage of the erythrocytes due to toxic metabolites derived from both the host cells and parasites. Reactive oxygen species (ROS) generated in the host-parasite interaction which could lead to the lysis of erythrocytes and possible alterations in the antioxidant defense system<sup>[33]</sup>.

Increased bilirubin level was observed in malarious children compared with healthy children in this study. A similar observation was reported in the study of Adeosun and colleagues<sup>[34]</sup> in Ile-Ife, South Western Nigeria which showed that hyperbilirubinemia occurred in malarious children compared with the controls. In another study, authors noted that the possible causes of hyperbilirubinemia were multi-factorial and include intravascular haemolysis of parasitized RBCs as well as haemolysis of non-parasitized RBCs<sup>[35]</sup>.

Increased uric acid and bilirubin concentrations probably indicate increased haemolysis arising as a result of increased lipid peroxidative damage to the red blood cells. An indication for increased lipid peroxidation was given by the significant increase in MDA level seen in the children suffering from malaria.

## V. Conclusion

In conclusion, Increased MDA, uric acid and bilirubin in malarious children may be attributed to the increased peroxidation, haemolysis and oxidative stress resulting in depletion of the body's antioxidant defenses and decreased TAS. Children, generally not enthusiastic about intake of fruits and vegetables may benefit from antioxidant supplementation during malaria bouts.

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