Oxidative Stress and Diminished Total Antioxidant Capacity in Malaria Patients Correspond to Increased Parasitemia and Severity of the Disease

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http://dx.doi.org/10.20455/ros.2019.855
(Received: April 4, 2019; Revised: April 16, 2019; Accepted: April 17, 2019)

ABSTRACT | Malaria is a leading cause of morbidity and mortality. Of the five Plasmodium species that cause malaria, P. falciparum is the deadliest. Oxidative stress might be increased in malaria patients and contribute to severity and complications. This may originate from intracellular parasitized erythrocytes and extra-erythrocytes as a result of hemolysis and host immune response. Oxidative stress-induced oxidation of hemoglobin to methemoglobin may cause further complications in malaria patients. The aim of this study was to estimate the total oxidative stress and non-enzymatic antioxidant levels in malaria patients. The study was undertaken with 60 malaria patients and 40 healthy controls. Severity of malaria was determined by the density of parasitemia. Out of the 60 malaria patients, 32 had low, 16 moderate, and 12 high parasitemia. Levels of total oxidative stress (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), uric acid, albumin, and total bilirubin and direct bilirubin levels were measured in the serum of malaria patients and healthy controls. Our results showed that TOS, OSI, uric acid, and total bilirubin and direct bilirubin levels were significantly increased in the serum of malaria patients compared to healthy control subjects. On the other hand, TAC and serum albumin levels were significantly reduced in malaria patients compared to control cases. The changes of oxidative stress and antioxidant status also correlated to the severity of parasitemia. Oxidative stress might thus contribute to the pathophysiology of malaria.

KEYWORDS | Albumin; Bilirubin; Malaria; Oxidative stress index; Oxidative stress; Total antioxidant capacity; Total oxidative stress; Uric acid

ABBREVIATIONS | FP, ferriprotoporphyrin IX; NO, nitric oxide; OSI, oxidative stress index; RNS, reactive nitrogen species; ROS, reactive oxygen species; TAC, total antioxidant capacity; TOS, total oxidative stress

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1. Introduction
2. Materials and Methods
1. INTRODUCTION

As indicated by the World Health Organization, malaria remains a major public health problem in the world. Despite efforts made to control malaria, 214 million people were infected by malaria around the world in 2015, leading to 438,000 deaths [1]. Nearly 90% of these cases were in Africa followed by South-East Asia (7%) and Eastern Mediterranean (2%) [2]. In Ethiopia, 75% of the zones are malarious and around 68% of the population lives in these territories [3–5]. Malaria transmission is occasional and seasonal in Ethiopia, with the transmission varying with altitude and weather [6]. The pinnacle malaria transmission season in the nation is from September to December. P. falciparum and P. vivax are the most prevailing species in Ethiopia [5, 7]. In Ethiopia, malaria remained to be the main infectious disease seen at health facilities [8]. It represented up to 14% of outpatient consultations and 9% of health facility affirmations. It has been estimated that there were 5 to 10 million clinical malaria cases and around 70,000 deaths every year in the nation [9].

Malaria actuates the immune system of the body causing the release of reactive oxygen species (ROS) like superoxide, hydrogen peroxide, hydroxyl radicals, lipid peroxides, and other related species. The parasite additionally fortifies certain cells and creates ROS via hemoglobin degradation [10–12]. The role of oxidative stress during malaria infection is yet vague. A few researchers recommend a defensive job, while others attribute a connection to the pathophysiology of the sickness [13]. The production of free radicals, which is associated with oxidative stress, is implicated in the complications caused by malaria. Malaria infection instigates the formation of hydroxyl radicals in the liver, which is most likely the cause of oxidative stress and apoptosis [14, 15]. A potential source of free radicals in malaria infection is the host’s hemoglobin since the parasite utilizes hemoglobin for its own nutrition, liberating a lot of circulating heme. These heme groups can prompt intravascular oxidative stress, causing changes in erythrocytes and endothelial cells and encouraging the entry of the parasite in tissues, like the liver and cerebrum [16].

A free radical species which seems by all accounts to be engaged in this malady is nitric oxide (NO) [12, 17]. It has been suggested that NO is created in abundance and kills the Plasmodium parasite [18] and low availability of NO causes cerebral malaria sickness [19]. On the other hand, with its harmful side effects, NO oxidatively damages tissues, alters the signal in the brain [20], and also causes anemia [21]. Moreover, host-parasite interactions advance consistent changes in the sensitive harmony between prooxidant and antioxidant molecules. In addition, even anti-malarial drugs comprise a share of oxidation via acting as inducers of free radical production [22–24].
Oxidative stress is believed to be a key factor in the pathogenesis of malaria; however, its role in the pathogenesis of the disease in Ethiopia still remains to be elucidated. Therefore, the current study seeks to examine changes in levels of total oxidative stress, total antioxidant capacity, uric acid, bilirubin, and albumin in malaria patients along with age-matched controls.

2. MATERIALS AND METHODS

2.1. The Study Area, Design, and Period

The study was conducted in Logia Health Center and Dubti Referral Hospital, Afar, Ethiopia. Dubti Referral Hospital is one of the oldest referral hospitals in Afar. It is found in zone one, Dubti wereda in Dubti town which is located 620 km East of Addis Ababa. A case-control study design was applied to estimate total oxidative stress among malaria patients during the period between October 2017 and April 2018.

2.2. Study Population and Ethical Considerations

The source population was all malaria patients who visited the Dubti Referral Hospital and Health Center of Dubti, Afar, Ethiopia, during the study period. The patients who were willing to participate in the study and who filled the consent form were included. Ethical clearance was obtained from the research and ethics committee of the Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University. Malaria patients, who were pregnant, HIV-positive, diabetic, had liver disease, smoke cigarettes, drink alcohol, or were with tuberculosis, were excluded from the study.

2.3. Sample Size Determination and Sampling Method

A convenience sampling technique was used to determine the sample size. When calculating the sample size requirements for the study, a number of factors were taken into consideration including effect size, cooperation and attrition, practical constrains such as time, subject availability and finance, subgroup analysis, and sensitivity of the measurement performed. The required sample size for the study was calculated using a single population proportion formula through assumption of 95% confidence interval (CI), 5% margin of error, and the sample size was calculated based on an estimated 50.8% prevalence of malaria in Ethiopia. A structured questionnaire was used to record patient information and demographic data.

2.4. Study Variables

Total oxidative stress (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), uric acid, total bilirubin, direct bilirubin, and albumin were considered as dependent variables. Sociodemographic characteristics like age, sex, presence and severity of malaria, and body mass index (BMI) were considered as independent variables.

2.5. Data and Specimen Collection Handling and Storage

Data were collected by professional laboratory technicians, nurses, and/or physicians with the involvement of the principal investigator. Under preceding instructions, malaria patients and healthy controls were checked for fasting in the morning of the examination day by the physician or nurse. Malaria patients were selected based on the microscopic examination of blood taken from their fingertips using Giemsa-stained thin blood smears. Patient selection was done by a simple random sampling. Appropriate information was obtained from standardized clinical files designed for the program. Five milliliters of fasting venous blood samples were obtained by vein puncture from the vein of the arm of each participant using sterile syringes and was transferred into anti-coagulant-free blood collection vacuum tubes and allowed to stand for 30 min to coagulate and centrifuged at 671 g for 10 min to separate the serum. Serum was collected by a sterile pipette and transferred to 3 ml Eppendorf tubes and stored at –20°C until analyzed.

2.6. Malaria Parasite Density Determination

*P. falciparum* parasitemia was determined in various blood smears stained with Giemsa stain. Parasitemia was assessed based on the method of Mohamed et al. [25] as: low, 1–10/100 fields; mild, 11–100/100 fields; moderate, 1–10/one field; and high parasitemia, > 10/one field.
2.7. Biochemical Assays

TOS and OSI were determined according to the method of Erel [26]. TAC was measured according to Koracevic et al. [27]. Serum uric acid was measured according to Piero et al. [28]. Determination of total bilirubin and direct bilirubin in the serum was done according to Cheesbrough [29].

2.8. Quality Assurance

There was a well-prepared data collection questionnaire to assess participant’s demographic information. There was quality control for the clinical chemistry analyzers which was run daily in the morning before the actual sample running. A well-prepared working protocol was in place for every parameter.

2.9. Data Processing and Statistical Analysis

All data were checked, cleared, and fed into EpiData (version 3.5.1, 2008, Denmark) and then exported to SPSS (version 22.0, 2012, Chicago, IL, USA) software for statistical analysis. Descriptive analysis, Spearman correlation and linear regression, independent sample t-test, and one-way ANOVA followed by post-hoc analysis were performed for this study. All data are expressed as mean ± standard deviation (SD) and p ≤ 0.05 was considered as statistically significant.

3. RESULTS

3.1. Sociodemographic Profile

This study enrolled 100 participants i.e., 60 malaria patients (38 males and 22 females) with a mean age of 21.63 ± 9.30 years, and 34 (56.7%) were married. Most of the patients were living in rural areas. The educational status of patients is also shown in Table 1. Most of the malaria patients did not use bed nets and have lower income. Controls were age-and sex-matched apparently healthy subjects. Out of these, 24 were males and 16 were females with a mean age of 21.85 ± 7.90 years. Clinical severity was determined according to the intensity of parasitemia. Malaria patients were further graded according to the parasitemia as low (32 patients), moderate (16 patients), and high risk (12 patients).

3.2. Oxidative Stress in Malaria Patients

As indicated in Table 2, TOS and OSI were significantly higher in malaria patients compared to control subjects.

3.3. Antioxidant Levels in Malaria Patients

Both TAC and serum albumin were significantly lower in malaria patients compared to control subjects. On the other hand, uric acid and total and direct bilirubin levels were significantly increased in malaria patients compared to control subjects (Table 3).

3.4. Oxidative Stress and Antioxidant Levels in Low, Moderate, and High Parasitemia Cases

Changes in serum oxidative stress and antioxidant parameters were compared with the severity of parasitemia. As shown in Table 4, there was no significant difference in TOS between moderate parasitemia and low parasitemia cases; however, high parasitemia cases showed significantly increased TOS compared to low and moderate parasitemia cases. In contrast, the TAC in moderate and high parasitemia malaria patients was significantly lower than that in low parasitemia cases. Like TOS, OSI was significantly increased in high parasitemia patients compared to low and moderate parasitemia cases. Serum uric acid, direct bilirubin, and total bilirubin levels were significantly increased as parasitemia severity increases. On the other hand, the serum albumin level was significantly decreased as parasitemia severity increases.

3.5. Correlations between TOS, TAC, and OSI in Malaria Patients

TOS showed a negative correlation with TAC, but the association was not statistically significant. Conversely, TOS showed a positive and statistically significant correlation with OSI among malaria patients (Table 5).

3.6. Correlations of TOS, OSI, and TAC with Parasitemia Severity

TAC negatively correlated with OSI among the different parasitemia severity stages. Among the low
and high parasitemia cases, correlation was statistically significant, whereas, among moderate malaria cases, it is not statistically significant. On the other hand, a positive correlation was demonstrated between TOS and OSI among the three severity stages of parasitemia with statistically significant correlation in low and moderate cases, but not in high severity cases (Table 6).

### TABLE 1. Demographic and clinical characteristics of malaria patients and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Malaria patients (n = 60)</th>
<th>Control cases (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male/Female</td>
<td>38/22</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>21.63 ± 9.30</td>
</tr>
<tr>
<td>Residence</td>
<td>Urban</td>
<td>16 (26.7%)</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>44 (73.3%)</td>
</tr>
<tr>
<td>Educational level</td>
<td>Illiterate</td>
<td>49 (81.7%)</td>
</tr>
<tr>
<td></td>
<td>High school or less</td>
<td>7 (11.7%)</td>
</tr>
<tr>
<td></td>
<td>College and above</td>
<td>4 (6.7%)</td>
</tr>
<tr>
<td>Marital status</td>
<td>Single</td>
<td>23 (38.3%)</td>
</tr>
<tr>
<td></td>
<td>Married</td>
<td>34 (56.7%)</td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Income</td>
<td>Low</td>
<td>48 (80%)</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>12 (20%)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Use of bed nets</td>
<td>Yes</td>
<td>21 (35%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>39 (65%)</td>
</tr>
<tr>
<td>Severity of malaria</td>
<td>Low (&lt; 1000)</td>
<td>32 (53.3%)</td>
</tr>
<tr>
<td></td>
<td>Moderate (1000–10,000)</td>
<td>16 (26.7%)</td>
</tr>
<tr>
<td></td>
<td>High (&gt; 10,000)</td>
<td>12 (20%)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>Under weight (&lt; 18.5)</td>
<td>4 (14.8%)</td>
</tr>
<tr>
<td></td>
<td>Normal weight (18.5–24.9)</td>
<td>14 (51.9%)</td>
</tr>
<tr>
<td></td>
<td>Overweight (25–29.9)</td>
<td>6 (22.2%)</td>
</tr>
<tr>
<td></td>
<td>Obese (≥ 30)</td>
<td>3 (11.1%)</td>
</tr>
</tbody>
</table>

### TABLE 2. TOS and OSI in malaria patients and control subjects

<table>
<thead>
<tr>
<th>Serum parameter</th>
<th>Control cases (n = 40)</th>
<th>Malaria patients (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOS (µmol H₂O₂ eqv./L)</td>
<td>1.79 ± 0.26</td>
<td>3.15 ± 0.47*</td>
</tr>
<tr>
<td>OSI (TOS/TAC) × 100</td>
<td>8.10 ± 1.41</td>
<td>30.47 ± 8.57*</td>
</tr>
</tbody>
</table>

Note: Data represent mean ± SD. *, p < 0.05 vs. control.

### TABLE 3. TAC, uric acid, bilirubin, and albumin levels in malaria patients and control subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control cases (n = 40)</th>
<th>Malaria patients (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (nmol ascorbic acid eqv./L)</td>
<td>28.88 ± 1.88</td>
<td>19.40 ± 1.30*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>4.1 ± 0.07</td>
<td>4.67 ± 0.13*</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>2.6 ± 0.15</td>
<td>3.37 ± 0.18*</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.5 ± 0.13</td>
<td>2.65 ± 0.20*</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>4.9 ± 0.12</td>
<td>3.40 ± 0.13*</td>
</tr>
</tbody>
</table>

Note: Data represent mean ± SD. *, p < 0.05 vs. control.
This study assessed the connection between malaria infection and oxidative status of patients. We showed that the total oxidative status in malaria patients was significantly higher than that in control subjects, and our finding is similar to that of other reports [14, 30, 31]. The normal host resistance system recruits phagocytes (macrophages and neutrophils) that secrete large amounts of ROS and reactive nitrogen species (RNS), causing an imbalance between oxidizing species and tissue antioxidants. This disturbance is believed to trigger oxidative stress in malaria prompting the death of the parasites [17, 32] as well as aggravating host tissue injury. In addition, Plasmodium digests hemoglobin inside its acidic vacuole and discharges harmful ferriprotoporphyrin IX (FP) and ROS [33, 34]. Free FP can interact with membrane phospholipids causing structural defects due to interaction of Fe3+ with unsaturated membrane lipids. This can cause increased membrane permeability to ions resulting in cell lysis. The FP is detoxified by glutathione-dependent pathways and FP-binding proteins of both the parasite and the host [35, 36]. The host immune system secretes various cytokines which also trigger inflammatory responses prompting increment in ROS [12]. We found that during high parasitemia, malaria patients had the highest level of TOS, and this observation is consistent with that reported by others [37, 38]. This is also in line with the notion that the parasite is capable of generating ROS within erythrocytes and damaging normal erythrocytes by activation of immune cells [39].

Malaria patients had a significantly lowered TAC than the uninfected control group. This might be due to increased usage of the host’s plasma antioxidants.

<table>
<thead>
<tr>
<th>Table 4. One-way ANOVA (post-hoc) analysis of serum parameters in malaria patients with low, moderate, and high severity of parasitemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum parameter</strong></td>
</tr>
<tr>
<td>TOS (μmol H2O2 eqv./L)</td>
</tr>
<tr>
<td>TAC (nmol ascorbic acid eqv./L)</td>
</tr>
<tr>
<td>OSI (ratio of TOS/TAS) × 100</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
</tr>
</tbody>
</table>

Note: Data represent mean ± SD. a, p < 0.05 vs. low group; b, p < 0.05 vs. moderate group.

<table>
<thead>
<tr>
<th>Table 5. Correlations between TOS, TAC and OSI levels in malaria patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>TOS vs. TAC</td>
</tr>
<tr>
<td>TOS vs. OSI</td>
</tr>
</tbody>
</table>

Note: Results are expressed as Pearson correlation coefficient. *, p < 0.05.

<table>
<thead>
<tr>
<th>Table 6. Correlations of TOS, OSI, and TAC levels with parasitemia severity of malaria patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>TOS vs. OSI</td>
</tr>
<tr>
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Note: Results are expressed as Pearson correlation coefficient. *, p < 0.05.

4. DISCUSSION

This study assessed the connection between malaria infection and oxidative status of patients. We showed that the total oxidative status in malaria patients was significantly higher than that in control subjects, and our finding is similar to that of other reports [14, 30, 31]. The normal host resistance system recruits phagocytes (macrophages and neutrophils) that secrete large amounts of ROS and reactive nitrogen species (RNS), causing an imbalance between oxidizing species and tissue antioxidants. This disturbance is believed to trigger oxidative stress in malaria prompting the death of the parasites [17, 32] as well as aggravating host tissue injury. In addition, Plasmodium digests hemoglobin inside its acidic vacuole and discharges harmful ferriprotoporphyrin IX (FP) and ROS [33, 34]. Free FP can interact with membrane phospholipids causing structural defects due to interaction of Fe3+ with unsaturated membrane lipids. This can cause increased membrane permeability to ions resulting in cell lysis. The FP is detoxified by glutathione-dependent pathways and FP-binding proteins of both the parasite and the host [35, 36]. The host immune system secretes various cytokines which also trigger inflammatory responses prompting increment in ROS [12]. We found that during high parasitemia, malaria patients had the highest level of TOS, and this observation is consistent with that reported by others [37, 38]. This is also in line with the notion that the parasite is capable of generating ROS within erythrocytes and damaging normal erythrocytes by activation of immune cells [39].

Malaria patients had a significantly lowered TAC than the uninfected control group. This might be due to increased usage of the host’s plasma antioxidants.
by the malaria parasites to counteract oxidative stress. Furthermore, a negative correlation was observed between the parasite number and TAC of malaria patients, suggesting that at higher parasitemia states, there is increased utilization of total antioxidants. Patients with severe malaria had low TAC than those with mild/moderate parasitemia. This finding is similar to that in other reports [38, 40–42].

The OSI estimated in this study was significantly higher in malaria patients than in the uninfected controls. OSI is the ratio of TOS to TAC and indicates the exact degree of imbalance between oxidants and antioxidants. A compromised antioxidant defense mechanism, accompanied by increased oxidant levels and OSI values in malaria patients, might play an important role in the pathogenesis and severity of malaria. The decrease in TAC and increase in TOS were more pronounced in high parasitemia patients compared to low and moderate parasitemia, indicating that the antioxidants were extensively utilized to scavenge the ROS generated during the parasitic infection.

We showed that serum uric acid was elevated in malaria patients compared to control subjects, and a similar finding was reported by others [38, 43]. The reason might be related to an increased rate of purine catabolism [44, 45]. The increase in uric acid levels was dependent on parasitemia severity. This result also agrees with that reported by others [38, 46]. Uric acid might act as an antioxidant against ROS [46–48]. However, the exact role of uric acid in the pathophysiology of malaria remains to be elucidated.

Like what observed with uric acid, the levels of total and direct bilirubin were also significantly higher in malaria patients compared to control subjects and the elevation correlated with parasitemia severity. This may be due to intravascular hemolysis of parasitized and non-parasitized red blood cells, which is associated with increase in bilirubin biosynthesis, hepatocellular damage, biliary tract obstruction, and jaundice [38, 49]. Like uric acid, bilirubin is also a free radical scavenger [50]; however, the significance of increased bilirubin in malarial pathophysiology remains unclear.

The levels of serum albumin were significantly lower in malaria patients compared to control cases and this decrease correlated with parasitemia severity. It was reported that the plasma albumin levels were lower in both severe and mild malaria cases [43]. Akaninworo et al. also reported significant decline of albumin with increased severity of parasitemia [51]. The possible causes of decreased albumin levels could be the deficiency of nutritional factors and the presence of an acute phase response among malaria patients. Indeed, albumin is a negative acute phase protein, the level of which falls because of malaria infection [52]. Notably, albumin serves as an antioxidant in vascular compartment scavenging ROS and RNS that are generated during normal metabolism and also during inflammation [43, 53]. The antioxidant property of albumin is reported to be due to the presence of sulfhydryl groups [54]. Decreased albumin levels could thus aggravate the oxidative stress condition in malaria patients.

5. CONCLUSION

In conclusion, the serum TOS, OSI, uric acid, and total bilirubin and direct bilirubin levels were significantly higher, whereas TAC and albumin levels were significantly decreased, in malaria patients compared to control cases. As the severity of the disease (parasitemia) increases, an increased oxidative stress and decreased TAC were observed. Assessing TOS and TAC in early malaria patients may thus provide insight into disease process and help guide effective disease management.

ACKNOWLEDGMENTS

The authors thank the study participants who agreed to participate in this study as well as Addis Ababa University, Asayta and Dubti general hospitals, and Semera University for their support during the study. The authors declare no conflicts of interest.

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