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Implication of Oxidative Stress and Antioxidant Defence Systems in Symptomatic and Asymptomatic *Plasmodium falciparum* Malaria Infection among Children Aged 1 to 15 Years in the Mount Cameroon Area

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Abstract

It is known that the pathogenicity of *Plasmodium* induces the breakdown of haemoglobin, which leads to the induction of oxidative stress. This study aimed to identify the possible effects of oxidative stress and antioxidant defence systems in symptomatic and asymptomatic *Plasmodium falciparum* malaria infection in children (1 - 15 years old) in the Mount Cameroon vicinity. This cross-sectional study involved blood samples collected from 473 children and examined for malaria parasitaemia. Full blood counts were performed using an automated haemoanalyser. Serum oxidative stress status (malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and vitamin C (Vit C)) were each determined by colorimetric enzymatic assays. The prevalence of malaria parasite infection was 32.1% among the participants. Out of that, 62.5% of patients with parasitaemia were symptomatic. Anaemia prevalence increased significantly with parasite density. MDA levels were significantly higher in patients with malaria symptoms than in those without symptoms. A significant and positive correlation was detected between MDA ($r = 0.831$, $P < 0.05$), NO ($r = 0.779$, $P < 0.05$), and malaria parasite density while, a significant and negative relationship occurred between parasite density and GSH ($r = -0.763$, $P < 0.05$) and Vit C ($r = -0.826$, $P < 0.05$) levels, SOD ($r = -0.621$, $P < 0.05$) and CAT ($r = -0.817$, $P < 0.05$) activities. The SOD activity and GSH level significantly

decreased ($P < 0.05$) with an increase in the MDA levels. These findings showed that MDA and nitric oxide levels increased both in malaria participants with or without symptoms. A similar decrease in the antioxidant defence system was observed in both symptomatic and asymptomatic patients. Therefore, there is a need to develop public health policies that encourage routine diagnosis and treatment of malaria in seemingly healthy people (asymptomatic cases), and this will play an essential role in controlling malaria in tropical countries.

Keywords

Malaria, Asymptomatic, Symptomatic, Oxidative Stress, Antioxidant Defence System

1. Introduction

Globally, malaria poses an enormous threat to human life causing deaths worldwide. In 2020, an estimated 241 million cases of malaria occurred worldwide resulting in 627,000 deaths [1] [2]. *Plasmodium falciparum* is the most prevalent and fatal malaria parasite species in Cameroon, and the disease continues to be a public health concern [3]. Falciparum malaria significantly affects children, and in areas where the disease is endemic, infections can be asymptomatic, uncomplicated or severe [4].

Asymptomatic malaria has been described by WHO as the presence of asexual parasites in blood but without evidence of disease [5]. Other authors have described asymptomatic malaria as malaria parasitaemia of varying intensity, without fever or other acute symptoms, in people who have not recently received antimalarial treatment [4] [6]. This form of malaria infection is prevalent in highly endemic areas of Africa and remains a challenge for malaria prevention and control strategies [7]. A recent study in Batoke and Buea conducted by Sumbele *et al.* [8] reported 36.4% malaria prevalence and a high asymptomatic malaria prevalence of 34.0% in children 15 years and below. It is still unknown why some malaria infections manifest no symptoms. According to some researchers, it may result from genetic diversity, parasite density, nutrient sequestration, and toxin production. Others contend that host-related elements such as immune system genes, lipid peroxidation, antioxidant markers, red blood cells (RBC) polymorphisms or abnormalities, circulating immunoglobulins and chemokines could be to blame [9] [10] [11].

Prolonged and persistent malaria infections without symptoms are known to induce oxidative stress, which may result in adverse malaria outcomes and may lead to complications in the pathology of the disease [12] [13]. Phagocytes (macrophages and neutrophils) are involved in the activation of the host's natural defence mechanisms during malaria infection, which causes a significant rise in the level of reactive oxygen species and an imbalance between the production

of oxidising species and the activity of antioxidant defence mechanisms. Oxidative stress is brought on by this imbalance [14]. In normal cells, there is an appropriate pro-oxidant/antioxidant balance. Shifting this equilibrium in favour of the pro-oxidant side causes oxidative stress, which is characterised by increased numbers of free radicals and increased lipid peroxidation of cell membranes, which causes devastating cell damage. Similarly, the parasite additionally stimulates specific cells in the development of reactive oxygen species (ROS) bringing about haemoglobin corruption. These ROS could be non-specific in nature, inducing lipid peroxidation and death of even non-infected RBCs [15] [16]. More so, a decrease in antioxidant defence systems can be observed in such subjects, probably due to usage of erythrocytic antioxidants by parasites to counter the defence mechanism of patients [15] [16]. Asymptomatic malaria infection may have a large effect on the delicate balance between the induction of antioxidant defense systems, oxidative stress and the level of GSH, SOD or CAT which determines the nature of the protective or pathogenic effects subsequently induced. Molecules such as GSH, SOD, CAT and ascorbic acid (Vit C), are part of the antioxidant compounds that prevent or reduce cell membrane damages [17].

A study on the production of ROS and nitrogen species by Guha and colleagues [18] demonstrated the critical role that these compounds play in the emergence of systemic malarial sequelae [18]. Another research found that while CAT activity is lower in people with malaria than in people without the disease, oxidative stress markers such as MDA and NO levels are higher in people with malaria than in people without the disease [17] [19]. Interestingly, Vit C possesses antioxidant properties and has been shown to scavenge free radicals [20]. Another study demonstrated that the physical and clinical conditions of asymptomatic malaria infected patients are not the same as those of symptomatic patients [21]. Finding techniques to combat the oxidative stress that comes along with the development of malaria from asymptomatic to symptomatic infection could be an important tool in achieving the malaria elimination goal.

Despite this state of knowledge, there is little information available regarding the degree of oxidative stress experienced by Cameroonian children suffering from *Plasmodium falciparum* infection with or without symptoms. Evidence for the involvement of oxidative stress makers and antioxidant defence systems among symptomatic and asymptomatic patients with malaria parasitaemia in the Mount Cameroon area is lacking. This lack of information models the background against which this study was aimed at, which is identifying the possible implications of oxidative stress and antioxidant defence systems in symptomatic and asymptomatic *Plasmodium falciparum* malaria infection among children aged 1 to 15 years in the Mount Cameroon area.

2. Methods

2.1. Description of the Study Area

The research was done in the Buea Regional Hospital, Fako Division, Cameroon,

as well as in several of Buea Health District's neighbourhoods, including Mile 18, Clerks' Quarters, Bonduma, Great Soppo, Bokwango, Bova, Buea Town, Molyko, and Muea. Buea, which is situated on the south-eastern slope of Mount Cameroon is located between latitudes 3˚57'N - 4˚27'N and 8˚58'E - 9˚25'E, and is 500 - 4080 metres above sea level. Also, it has an average relative humidity of 80%, and an annual rainfall of 4000 mm. The research area experiences an equatorial climate, with temperatures ranging from 18˚C to 27˚C and two distinct seasons: a brief dry season (November to February) and a long rainy season (March to October). *Anopheles gambiae* is the primary malaria vector species, and *Plasmodium falciparum* accounts for nearly 90% of all cases of malaria that have been reported in this area [22]. The presence of Mount Cameroon in Buea makes it a popular tourist destination with a conducive economic environment. In Buea, the rainy season (March to September) is the peak period of malaria transmission [23].

2.2. Criteria for Selection of Study Population

Children aged 1 to 15 who lived in the study area and whose parents/guardians approved of their involvement in the study made up the study population. For this investigation, only febrile patients (temperature ≥ 37.5˚C), those with headache, chills (occurring every 2 - 4 days), loss of appetite, nausea, and vomiting (WHO, 2018) as well as individuals who appeared to be in good health were recruited. The study excluded patients with confirmed HIV status or more serious health conditions like diabetes or kidney disease. Additionally, those who had haematological diseases or had taken antimalarial medication two weeks before the study were not allowed to participate. Malaria was newly diagnosed in each subject.

2.3. Study Design

The study was a cross-sectional investigation carried out from December 2019 to August 2021. At presentation to the hospital and in the communities, individuals were recruited after receiving administrative and ethical clearances following education. Informed consent/assent forms were given to parents/caregivers explaining the purpose, benefits, and risks of the study. Relevant demographic characteristics, malaria history and treatment, health-seeking behaviours, and symptoms shown were recorded with the aid of a structured questionnaire. Clinical evaluation was done by a trained physician, a clinical thermometer was used to obtain axillary temperature, and fever was considered to be a temperature equal to or above 37.5˚C. Blood samples were later collected for malaria parasite detection and full blood count (FBC) analyses.

The study used a practical multistage sampling strategy where distinct urban settings within the Buea Health Area were identified and chosen at random. Then, sample neighbourhoods in the chosen health areas were randomly selected. Potential participants in the chosen neighbourhoods were informed via

the community relay agents, and on scheduled dates decided by the neighbourhood head, they were invited to a designated data collection hall. In the hospital, participants were prospectively enrolled upon presentation in the outpatient department as long as they met the study inclusion criteria.

The formula $n = z^2pq/d^2$ (Bryan *et al.*, 1996) was used to calculate the sample size for the study, where n was the necessary sample size, z was the requisite standard deviation for a 95 percent confidence interval (CI), and 33.8% represented the region's malaria prevalence [23]. q was $(1 - p)$, and d represented the 0.05 allowable error margin. 362 was the ideal number of participants. The sample size was raised to 473 to account for data loss from incorrect data entry.

2.4. Sample Collection

While ensuring all procedures were sterile, 4 mL of venous blood was collected into dry and EDTA tubes. Labeled blood samples were transported on ice to the University of Buea, Life Science Laboratory for further analyses. As soon as the blood was dispensed into EDTA tubes, thin and thick blood films were made. Standard protocols were used to fix the thin films with absolute methanol; both blood films were stained for 15 minutes with 10% Giemsa stain, and then checked for the occurrence of malaria parasite and speciation [24]. If asexual/gametocyte forms were visible, the slides were considered positive and the parasitaemia was calculated as 200 white blood cells multiplied by the patient's white blood cell (WBC) counts and stretched to 500 leukocytes if gametocytes were visible [24]. Per microliter (L) of blood, the parasite load was divided into four categories: low (1000 parasites), moderate (1000 - 4999 parasites), high (5000 - 99,999 parasites), and hyper (>100,000 parasites) [25]. Aliquots of blood were kept at −20˚C until use after being centrifuged at 3000 rpm for 5 minutes in dry tubes.

2.5. Determination of Haematological Parameters

To obtain values for haemoglobin (Hb), WBC, and platelets (Plt), a complete blood count was performed using the URIT 3300 haemoanalyser in accordance with the manufacturer's instructions. According to WHO's age-based categorization [26], anaemia was defined as Hb < 11.0 g/L for children 1 - 5 years, Hb < 11.5 g/dL for children 6 - 11 years, and Hb < 12.0 g/dL for children 12 - 15 years old.

2.6. Determination of Oxidative Stress Markers

2.6.1. Quantification of Malondialdehyde Concentration

MDA, defined as an indicator of lipid peroxidation, was evaluated in the serum of each participant. In fact, 500 µL of serum was added in a tube containing 500 µL of thiobarbituric acid solution (0.67%) and 250 µL of trichloroacetic acid solution (20%). The mixture was incubated for 1-hour at 90˚C, and later cooled with tap water and the concentration of MDA was estimated spectrophotometrically as described by Liu *et al.* [27].

2.6.2. Measurement of Reduced Glutathione Concentration

The concentration of GSH was quantified spectrophotometrically in the serum of each participant using the method described by Ellman [28]. The sera were prepared following this previous protocol with Ellman's reagent, and finally, the optical density of the mixture was determined at 412 nm. The level of GSH was expressed as µg/g tissue.

2.6.3. Determination of Superoxide Dismutase Activity

The activity of total SOD was measured as previously described by Sun *et al.* [29]. In a tube containing 134 µL of serum, a volume of 1666 µL of carbonate-bicarbonate Buffer (0.05 M; pH 10.2) and 200 µL of adrenalin (0.3 mM) were added. Thereafter, the activity of SOD was estimated as the amount of enzyme that caused 50% inhibition of the nitroblue tetrazolium reduction rate. The mixture was homogenised and then the absorbance was read twice, after 20 and 80 seconds at 480 nm, using a spectrophotometer [30].

2.6.4. Quantification of Superoxide Catalase

The protocol described previously by Yadang *et al.* [31] was used in the determination of CAT activity in the serum of each participant. Briefly, in 125 µL of serum, 125 µL of phosphate buffer (0.1 M, pH 7.4) and 0.5 mL of hydrogen peroxide (30 mM H_2O_2) were added. Thereafter, the optical density of the mixture was read twice after 30-second, 60-second, and 90-second, at 240 nm using a spectrophotometer. The specific CAT activity was expressed as U/mg tissue.

2.6.5. Measurements of Nitric Oxide Level

The total nitrite in the sample of each participant was quantified using the protocol described by [32]. The principle was the conversion of nitrate into nitrite by cadmium and followed by colour development with Griess reagent (0.1% N-naphthyl ethylene diamine and 1% sulfanilamide) in acidic medium (2.5%). In fact, Griess Reagent was used to convert nitrite into a purple-coloured azo compound in serum, which was then quantified by a spectrophotometer at 546 nm [33].

2.6.6. Determination of the Concentration of Vitamin C

The protocol described previously by Esaka *et al.* [34] was used to determine Vit C level in each sample. Vit C is reduced (ascorbic acid) and furthermore oxidised (dehydroascorbic acid). Briefly, in a 1 mL tube containing 5 µL of serum, 300 µL of chromogen reagent was added and incubated for 5 minutes at 37˚C. Thereafter, the enzyme reagent (ascorbic acid oxidase and peroxidase) was added to the mixture, vigorously shaken, and incubated at 37˚C for 5 minutes. Finally, the absorbance was read at 660 nm, twice at 30-second intervals, using a spectrophotometer and the concentration of total Vit C was expressed in mg/dL [35].

2.7. Data Analysis

The collected data from this study was expressed as mean ± Standard Error of

Mean; and analysed using Microsoft Excel 2016 and IBM-Statistical Package (SPSS, version 20). Means and standard deviations were used to describe continuous variables, whereas percentages were used to report categorical variables. The Student t-test was performed to compare the mean parasite density, the concentrations of MDA, GSH, NO, and Vit C, and the activities of CAT and SOD between symptomatic and asymptomatic malaria parasite-infected patients. Correlations were used to examine the associations between parasite density, oxidative stress, and antioxidant parameters. A model of linear regression was used to evaluate other variables that had a high and substantial correlation with parasite density. A P value < 0.05 was considered statistically significant.

2.8. Ethical Consideration

Regulatory approval was obtained from the Institutional Review Board, Faculty of Health Science, University of Buea (Ref No: 2019/811-05/UB/SG/IRB/FHS) as well as from the South West Regional Delegation of Public Health (R11/MINSANTE/SWR/RDPH/PS/295/943). The goal, dangers, and advantages of the study were thoroughly described to the parents/caregivers. Only kids whose parents agreed to their involvement in the study were enrolled. Data was managed with utmost confidentiality by assigning codes to the patients' samples.

3. Results

3.1. Baseline Characteristics of the Study Population

Table 1 indicates that a total of 473 participants were enrolled into the study, from which 195 (41.2%) were males and 278 (58.7%) were females. The mean (±SEM) age in years was 6.8 ± 4.7 among which 268 (56.7%) were ≤ 5 years, while those in the 6-10 age group were 133 (28.1%) and those in the 11 – 15 age group were 72 (15.2%). Most (324, 68.5%) of the participants enrolled at presentation to the hospital had at least a secondary level of education 218 (46.1%) while 149 (31.5%) were enrolled in various neighbourhoods (Molyko, Government Reserve Area, Buea Town, Bokwango, Bokova, Great Soppo, Mile 18, Clerks quarters, Federal quarters and Bondum,) in the Buea community. Overall malaria prevalence in the study population was 32.1% (152) while symptomatic and asymptomatic malaria prevalence in the study population were respectively 62.5% (95) and 37.5% (57). More males were positive for malaria 59.9% (91) than females 40.1% (61). The highest malaria prevalence was seen in the 1 - 5 age group 47.4% (72) in which 45.6% (26) were asymptomatic followed by the 6 - 10 age group 28.3% (43) with 7.0% (4) being asymptomatic as shown in Table 1.

3.2. Clinical Characteristics of Study Participants

It can be depicted from Table 2 that 136 (28.8%) febrile cases were recorded with a mean temperature of 37.9˚C ± 2.6˚C. The prevalence of anaemia in the study population was 62.1% (294) whereas the various prevalence of oxidative

Table 1. Baseline characteristics of the study participants.

	Gender		Age group (years)			Total
	Female	Male	1 - 5	6 - 10	11 - 15	
Participant % (n)	58.7 (278)	41.2 (195)	56.7 (268)	28.1 (133)	15.2 (72)	100 (473)
Mean age ± SEM (years)	7.3 ± 3.7	8.9 ± 4.2	2.7 ± 1.5	8.3 ± 1.5	13.3 ± 1.6	6.8 ± 4.7
Mean ± SEM Hb (g/dL)	10.6 ± 1.5	10.9 ± 1.4	10.6 ± 1.5	10.5 ± 1.8	11.2 ± 1.2	10.8 ± 1.4
Socio-demographic factors						
Point of presentation						
Community (n)	64.4 (96)	35.6 (53)	49 (73)	28.2 (42)	22.8 (34)	31.5 (149)
Hospital (n)	56.2 (182)	43.8 (142)	60.2 (195)	28.1 (91)	11.7 (38)	68.5 (324)
Educational level of parent						
No formal/Primary (n)	63.2 (79)	36.8 (46)	57.6 (72)	32.0 (40)	10.4 (13)	26.4 (125)
Secondary (n)	51.4 (112)	48.6 (106)	65.1 (142)	23.4 (51)	11.5 (25)	46.1 (218)
Tertiary (n)	62.3 (81)	37.7 (49)	53.1 (69)	29.2 (38)	17.7 (23)	27.5 (130)
Malaria infection status						
Negative % (n)	64.8 (208)	35.2 (113)	58.9 (189)	18.7 (60)	22.4 (72)	67.9 (321)
Positive % (n)	40.1 (61)	59.9 (91)	47.4 (72)	28.3 (43)	24.3 (37)	32.1 (152)
Symptom status						
Asymptomatic % (n)	43.9 (25)	56.1 (32)	45.6 (26)	7.0 (4)	47.4 (27)	37.5 (57)
Symptomatic % (n)	37.9 (36)	62.1 (59)	48.4 (46)	41.1 (39)	10.5 (10)	62.5 (95)

Hb: haemoglobin; SEM: Standard Error of Mean; n: number of participants.

stress parameters were as follows: MDA 83.5% (395), GSH 54.1% (256), CAT 45% (213), SOD 41.6 % (197), Vit C 35.3% (167) and NO 79.3% (375), respectively. Mean WBC and platelet count (SD) × 10^3/μL were 69.0 (1007.4) and 223.0 (94.3) respectively while the geometric mean parasite density (SD) in parasites/μL of blood was 577 (3240.9) as shown in Table 2.

3.3. Relationship between Malaria Parasite Infection and Mean Haemoglobin Level among the Study Participants

As shown in Table 3, study participants who tested positive for malaria parasitaemia had significantly lower mean Hb levels with respondents in the age group 1 - 5 years recording the lowest mean Hb levels (7.4 ± 1.3 g/dL) when compared with their counterparts who tested negative for malaria parasite infection (10.3 ± 1.1 g/dL) (P = 0.001). Interestingly, the mean Hb level was significantly lower (P = 0.001) in female participants who tested positive for the malaria parasite infection (7.5 ± 1.4 g/dL) when compared with those who tested negative (12.1 ± 1.6 g/dL). Equally, a significantly lower (P = 0.001) mean haemoglobin level was observed in males positive for malaria parasite infection (7.6 ± 1.5 g/dL) when compared with their contemporaries who were negative for infection (12.4 ± 1.2 g/dL).

Table 2. Clinical characteristics of the study population.

Parameter	Category	% (n/N)
Fever status	Febrile	28.8 (136/473)
	Afebrile	72.2 (337/473)
Anaemic status	Anaemic	62.1 (294/473)
	Non-anaemic	37.8 (179/473)
Malaria parasite status	Positive	32.1 (152/473)
	Negative	67.9 (321/473)
Oxidative stress status	Malondialdehyde	83.5 (395/473)
	Reduced glutathione	54.1 (256/473
	Catalase	45.0 (213/473)
	Super oxide dismutase	41.6 (197473)
	Vitamin C	35.3 (167/473)
Nitrergic stress status	Nitric oxide	79.3 (375/473)
Mean temperature (SEM) in degree Celsius (˚C)	37.9 (2.6)	
Mean Hb (SEM) in g/dL	10.4 (1.6)	
Mean WBC (SEM) × 10^3/μL	69.0 (1007.4)	
Mean Platelets (SEM) ×10^3/μL	223.0 (94.3)	
Geometric mean parasite density (SEM) in parasites /μL of blood	577 (3240.9)	

Hb: haemoglobin; SEM: Standard Error of Mean; n: number of participants; N: total number of participants; WBC: white blood cells.

Table 3. Relationship between malaria parasite infection and mean haemoglobin level.

	Haemoglobin, mean ± SEM (g/dL)				
	Gender		Age group (years)		
	Female	Male	1 - 5	6 - 10	11 - 15
Malaria infection status					
Negative	12.1 ± 1.6	12.4 ± 1.2	10.3 ± 1.1	10.4 ± 1.2	10.6 ± 1.3
Positive	7.5 ± 1.4	7.6 ± 1.5	7.4 ± 1.3	7.6 ± 1.2	7.8 ± 1.4
t values	1.542	2.152	2.825	2.954	2.603
P values	0.001	0.001	0.001	0.001	0.001
Malaria symptom status					
Asymptomatic	9.1 ± 1.5	9.0 ± 1.1	8.2 ± 1.1	8.4 ± 1.2	8.7 ± 1.3
Symptomatic	7.4 ± 1.3	7.7 ± 1.1	7.2 ± 1.3	7.5 ± 1.2	7.7 ± 1.4
t values	−1.138	−1.273	−1.814	−1.143	−1.034
P values	0.212	0.103	0.192	0.152	0.251

SEM: Standard Error of Mean.

3.4. Effects of Malaria Parasite Infection on the Occurrence of Anaemia among the Study Participants

Student's t-test analysis showed that there was a statistically significant difference among anaemic cases in age groups (Table 4). Children with malaria who were 1 to 5 years old had the highest anaemia prevalence (39.9%, P = 0.001) followed by those aged 6 - 10 years, where the prevalence was 30.8% (P = 0.001), and 11 to 15 years, where it was 38% (P = 0.001), respectively. From Table 4, it can be depicted that the prevalence of anaemia varied significantly (P < 0.05) with the different age groups (Table 4).

3.5. Relationship between Oxidative Stress Markers and *Plasmodium falciparum* Infection

The serum oxidant and antioxidant levels in children with malaria parasites are shown in Table 5. The concentrations of MDA and NO were significantly elevated (P = 0.001) in patients who tested positive for malaria parasite (0.52 ± 0.02 µmol/L and 0.11 ± 0.02 mM) when compared with those who tested negative (0.37 ± 0.01 µmol/L and 0.04 ± 0.01 mmol, respectively).

Meanwhile, the enzymatic activities of SOD and CAT were significantly lower (P < 0.05) in malaria positive participants (0.78 ± 0.02 µmol/mL/min and 1.59 ± 0.24 µmol/mL/min respectively) when compared with those who were negative

Table 4. Effect of malaria parasite infection on the prevalence of anaemia among the study participants.

	Anaemia status						Total
	Anaemic			Non anaemic			
	Age group						
	1 - 5 (142)	6 - 10 (104)	11 - 15 (93)	1 - 5 (59)	6 - 10 (40)	11 - 15 (35)	
Malaria infection status							
Negative % (n)	9.2 (14)	3.3 (5)	2.0 (3)	38.2 (58)	25.0 (38)	22.4 (34)	152
Positive % (n)	39.9 (128)	30.8 (99)	28.0 (90)	0.3 (1)	0.6 (2)	0.3 (1)	321
Total							473
t values	2.521	2.519	3.062	2.931	2.263	2.712	
P values	0.001	0.001	0.001	0.001	0.001	0.001	
Malaria parasite density							
Low (n)	52	40	38	1	1	1	133
Moderate (n)	44	37	27	0	1	0	109
High (n)	32	22	25	0	0	0	79
Total							321
t values	1.063	1.193	1.074	1.632	1.032	1.021	
P values	0.012	0.027	0.042	0.027	0.043	0.027	

n: number of participants.

Table 5. Relationship between oxidative stress markers and Plasmodium falciparum infection.

Oxidative Stress marker	Malaria Infection Status	Mean ± SEM	t values	P values
MDA (µmol/L)	Negative	0.37 ± 0.01	−6.173	0.001
	Positive	0.52 ± 0.02		
GSH (µg/mL)	Negative	0.12 ± 0.01	−5.570	0.002
	Positive	0.08 ± 0.01		
SOD (µmol/mL/min)	Negative	6.12 ± 0.21	0.041	0.021
	Positive	0.78 ± 0.02		
CAT (µmol/mL/min)	Negative	3.18 ± 1.02	2.809	0.029
	Positive	1.59 ± 0.24		
Vit C (µg/mL)	Negative	0.95 ± 0.07	−4.187	0.037
	Positive	0.41 ± 0.02		
NO (mmol)	Negative	0.04 ± 0.01	−3.048	0.001
	Positive	0.11 ± 0.02		

MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; CAT; catalase; Vit C: vitamin C; NO: nitric oxide.

(6.12 ± 0.21 µmol/mL/min and 3.18 ± 1.02 µmol/mL/min respectively) as seen in Table 5.

Furthermore, the mean serum level of Vit C was significantly lower (P = 0.001) in patients who tested positive for the malaria parasite (0.04 ± 0.01 mmol) when compared with those who tested negative (0.11 ± 0.02 mmol). The mean serum GSH level was significantly lower in patients with malaria parasite (0.08 ± 0.01 µg/mL), whereas GSH was significantly higher (P < 0.01) in malaria negative participants (0.12 ± 0.01 µg/mL) (Table 5).

3.6. Relationship between Oxidative Stress Parameters and Malaria Symptoms in Infected Participants

The mean concentration of MDA was raised significantly (P = 0.001) in participants who showed symptoms of malaria (0.97 ± 0.02 µmol/L) when compared with those who were asymptomatic (0.61 ± 0.03 µmol/L). Likewise, the average concentration of nitric oxide was also significantly higher among symptomatic patients (0.10 ± 0.01 mmol) when compared with asymptomatic participants (0.08 ± 0.01 mmol). The activities of the respective antioxidant parameters; SOD (0.59 ± 0.03 µmol/mL/min; P = 0.291) and CAT (1.18 ± 1.02 µmol/mL/min; P = 0.249) were reduced but not significantly in malaria parasite infected and symptomatic participants when compared with those who tested positive for malaria parasite and who were asymptomatic (SOD; 0.70 ± 0.02 µmol/mL/min; P = 0.291), (CAT; 1.45 ± 0.11 µmol/mL/min; P = 0.249). Furthermore, there was no

significant difference (P = 0.227) in the mean concentration of Vit C among those with symptomatic (0.27 ± 0.02 µg/mL) and asymptomatic (0.38 ± 0.03 µg/mL) malaria (Table 6).

3.7. Pearson Correlation Coefficient between Parasite Density, Lipid Peroxidation, Antioxidants, Nitric Oxide, and Vitamin C in Malaria Parasite-Infected Patients

Using the Pearson correlation coefficient, the relationship between parasite density, lipid peroxidation, antioxidants, NO, and Vit C in malaria parasite infected-patients were examined. A strong, positive and significant relationship existed between MDA (r = 0.831, P < 0.05) and density of infection as well as NO (r = 0.779, P < 0.05) and density of infection (Table 7). In addition, a negative and significant correlation existed between density of infection, SOD (r = −0.621, P < 0.05), GSH (r = −0.763, P < 0.05), CAT (r = −0.817, P < 0.05) and Vit C (r = −0.826, P < 0.05), in malaria parasite infected participants. However, the levels of SOD, GSH, CAT, and Vit C also lessened significantly (P < 0.05) with an increase in MDA levels in malaria positive participants (Table 7).

3.8. Relationship between the Mean Parasite Density versus Concentrations of Malondialdehyde, Nitric Oxide, Reduced Glutathione, Vitamin C, and the Activities of Catalase and Super Oxide Dismutase

There was a positive and significant correlation between mean parasite density

Table 6. Relationship between oxidative stress markers and malaria symptoms in infected patients.

Oxidative marker	Infection status	Mean ± SEM	t value	P value
MDA (µmol/L)	Symptomatic	0.97 ± 0.02	2.741	0.001
	Asymptomatic	0.61 ± 0.03		
GSH (µg/mL)	Symptomatic	0.06 ± 0.01	−2.019	0.105
	Asymptomatic	0.07 ± 0.01		
SOD (µmol/mL/min)	Symptomatic	0.59 ± 0.03	−1.964	0.291
	Asymptomatic	0.70 ± 0.02		
CAT (µmol/mL/min)	Symptomatic	1.18 ± 1.02	−0.593	0.249
	Asymptomatic	1.45 ± 0.11		
Vit C (µg/mL)	Symptomatic	0.27 ± 0.02	−4.187	0.227
	Asymptomatic	0.38 ± 0.03		
NO (mmol)	Symptomatic	0.10 ± 0.01	5.931	0.000
	Asymptomatic	0.08 ± 0.01		

Symptomatic patients were those with signs such as headache, fever, stiffness, chills, loss of appetite, nausea, and vomiting according to WHO, 2013. MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; CAT; catalase; Vit C: vitamin C; NO: nitric oxide.

and the concentration of MDA ($P < 0.05$) and the mean parasite density and the level of NO ($P < 0.05$), respectively (Figure 1(a) and Figure 1(b)). The regression equation showed that MDA equal to 7E−05 (density) + 0.2921 ($R^2 = 0.7967$) accounting for 79.67% of the variations observed in MDA with respect to parasite density. In addition, the regression equation for NO was 2E−0.5 (density) +

Table 7. Pearson's correlation coefficient between parasite density, lipid peroxidation, antioxidants, nitric oxide, and vitamin C in malaria parasite infected-patients.

	Density	MDA	GSH	SOD	CAT	Vit C	NO
Density	1						
MDA	0.831*	1					
GSH	−0.763*	−0.418*	1				
SOD	−0.621*	−0.472	0.594	1			
CAT	−0.817*	−0.305	0.201	0.528	1		
Vit C	−0.826*	−0.169	0.283	0.339	0.624	1	
NO	0.779*	0.402*	−0.427	−0.701	−0.202	−0.521	1

MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; CAT; catalase; Vit C: vitamin C; NO: nitric oxide. *P < 0.05 statistically significant.

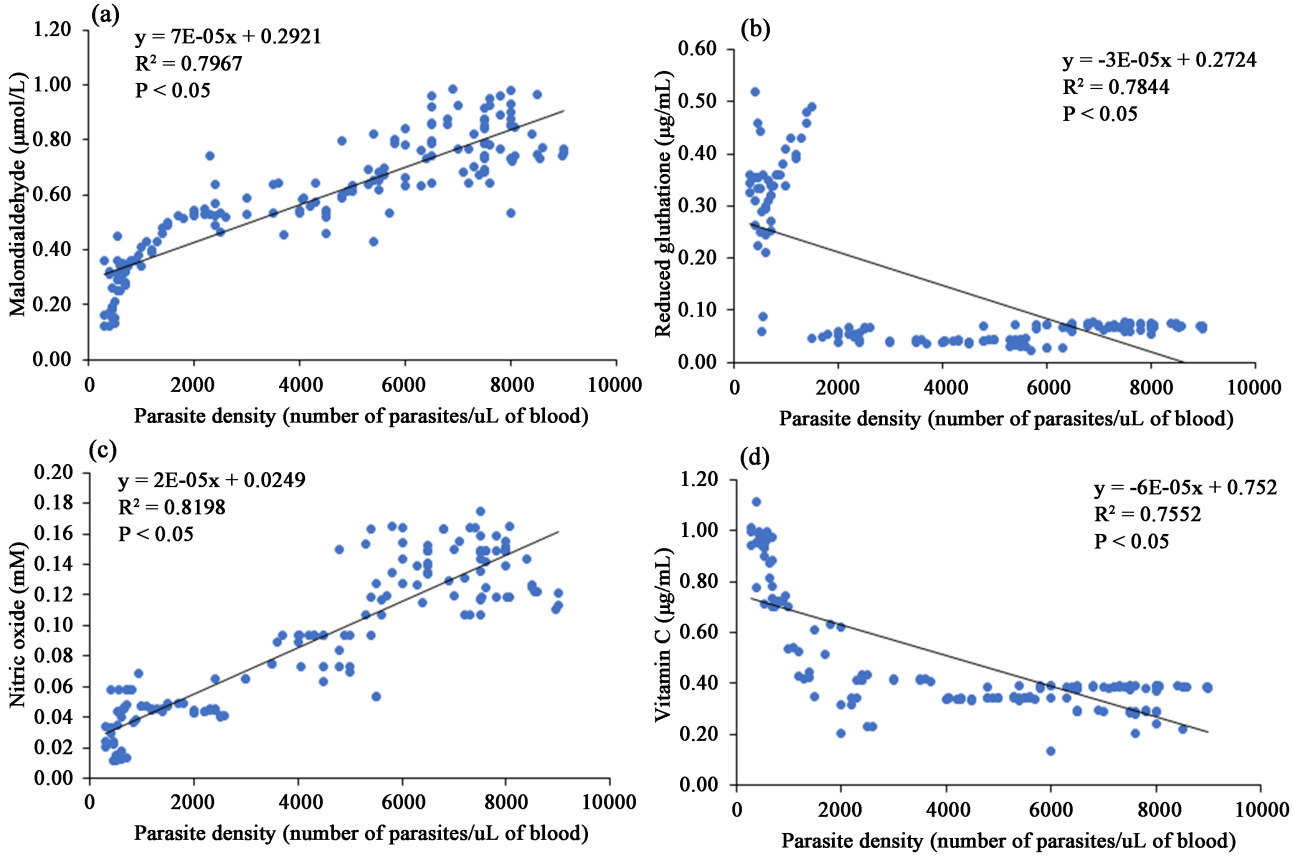

Figure 1. Relationship between the mean parasite density and MDA levels (a), reduced glutathione level (b), nitric oxide level (c), and vitamin C level (d) in malaria parasite infected-patients.

0.0249 (R^2 = 0.8198) accounting for 81.98% of the variations obtained in NO with respect to parasite density. Furthermore, a significant and inverse relationship existed between parasite density, GSH ($P < 0.05$) and Vit C ($P < 0.05$). The regression equation showed that GSH equal to −3E−05 (density) + 0.2724 (R^2 = 0.7844) accounting for 78.44% of the variations observed in GSH with respect to parasite density. It also showed in Figure 1(d) that Vit C equal to −6E−05 (density) + 0.752 (R^2 = 0.7552) accounting for 78% of the variations observed in Vit C values (Figure 1).

Furthermore, a significant, inverse, and strong relationship existed between malaria parasite density versus the activities of SOD ($P < 0.05$) and CAT ($P < 0.05$), respectively among the infected participant (Figure 2). The regression equation showed that SOD activity equal to −5E−05 (density) + 0.8812 (R^2 = 0.6763) accounting for 66.93% of the variations recorded in the activity of SOD with respect to parasite density. On the other hand, the regression equation for CAT activity was −7E−05 (density) + 1.5452 (R^2 = 0.6145) accounting for 75.52% of the variations observed in CAT activity with respect to parasite density (Figure 2).

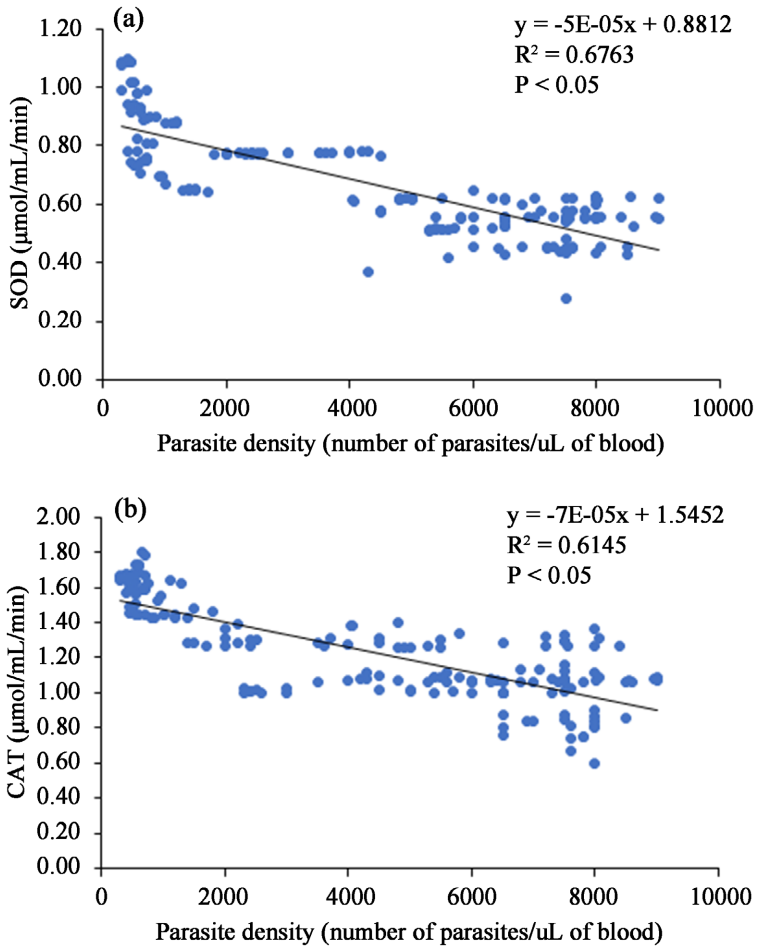

Figure 2. Relationship between the mean parasite density and the activities of super oxide dismutase (a) and catalase (b) in malaria parasite infected-patients.

4. Discussion

Oxidative stress contributes significantly in the pathogenesis of malaria and it can be demonstrated that *Plasmodium falciparum* infected red blood cells (RBC) produce ROS in infected patients. The increase in lipid peroxides as an indicator of oxidative stress in the organism has been documented as an ultimate toxic effect of raised reactive oxygen species production by the immune system of the body, as well as a synchronised release of oxygen radicals during haemoglobin degradation by malaria parasites [36] [37]. This study investigated the implication of oxidative stress and antioxidant defence systems in symptomatic and asymptomatic *Plasmodium falciparum* malaria infection in children (1 to 15 years old) at presentation to the Regional Hospital, Buea and in some communities of the Mount Cameroon vicinity. The microscopically observed overall prevalence of malaria parasites (32.1%) is higher than the 27.7% reported by Sama *et al.* [38]. This indicates that malaria is still a threat in the Mount Cameroon vicinity. Despite increased control efforts, there is still a high incidence of malaria. This may be attributed to some factors like heavy rainfall, persistent humidity, and the neglect of asymptomatic cases of malaria, which serve as reservoirs for the parasite in this region.

Although malaria parasite-driven haemolysis contributes to a decrease in the concentration of haemoglobin in childhood malaria, one of the first pathways inducing the reduction of haemoglobin concentrations in children with malaria infection and anaemia, is impaired and/or ineffective erythropoiesis [39], as well as overlapping features, including lysis of infected and uninfected RBC [40], splenic sequestration of RBC [41], dyserythropoiesis and bone marrow suppression [42]. The study findings showed that the prevalence of anaemia in the study population was 62.1% with the highest anaemia prevalence occurring in malaria positive (39.9%, $P = 0.001$) participants (1 - 5 years old). Out of that, these results could be justified by the elevated prevalence of malaria parasite in the Mount Cameroon vicinity. Malaria parasite infection has been reported severally as a risk factor of anaemia due to the degradation of haemoglobin during malaria infection [38]. In addition, some of the participants in this study were positive for malaria parasite infection but asymptomatic. Therefore, it can be suggested that *Plasmodium* malaria can be asymptomatic in positive participants as part of adaptation to survival. Being asymptomatic may result in infected participants not seeking treatment against malaria parasite infection [9].

It can be depicted from the results of this study that anaemia was associated with malaria parasite infection of the peripheral blood in some of the participants. In fact, malaria can induce anaemia through the malaria parasitic damage of RBC and autoimmune reaction [43] [44]. Additionally, there was an understandable increase in the prevalence anaemia with an increase in malaria parasite density followed by the occurrence of nitrosative and oxidative stresses. It has been previously well-known that in *Plasmodium falciparum* malaria infection, there is

a significant relationship between an individual's asexual erythrocytic-stage parasite density with the latency of presentation to a health care provider and the severity of clinical symptoms of the disease [45]. Oxidative stress is well known to induce anaemia through a sequence of processes. The principal target of oxidative stress is RBC due to their primary role as O_2-carrying cells [46]. Oxidative stress-altered erythrocytes induce molecular signals that lead to the activation of the calcium ion-permeable cation channels, which enable calcium ions entry into the cells. This finally activates calcium ions-sensitive potassium ions channels thereby leading to cell shrinkage and scrambling of the erythrocyte membrane. Afterwards, phosphatidylserine gets exposed at the surface of the erythrocyte, distinctive signs of eryptosis (RBC impairment) leading to severe anaemia [46] [47]. Surprisingly, there was no significant modification in the mean concentration of haemoglobin between symptomatic and asymptomatic malaria-infected patients. This finding further buttresses and justifies how dangerous asymptomatic malaria could be; although there are no apparent signs of malaria parasite infection, a lot of impairments would have been induced by the "hidden" parasite. Therefore, it is essential to understand the host-related factors that are responsible for this major setback.

As expected, the mean concentrations of MDA and nitric oxide were significantly higher among malaria parasite-infected patients compared to non-infected participants. This is a consequence that malaria patients had significantly reduced antioxidant defence system such as the mean concentration of GSH, and the activities of CAT and SOD than the non-infected participants. Oxidative stress is triggered by, respectively, the increase of reactive oxygen and/or nitrogen atoms that have unpaired electrons in their outer shell [48]. The oxidative stress markers (MDA and NO) recorded in this study were expansively higher in malaria parasite-infected patients than in the uninfected participants. This pathological development increases the oxidative stress index, as indicated in the results obtained, which is the ratio of total oxidative stress to total antioxidant activity and demonstrates the precise level of oxidative and antioxidant imbalance in infected patients [49].

A compromised antioxidant defence system, alongside increased oxidative stress markers and eventually oxidative stress index values in malaria parasite infected-participants, might play an essential involvement in the induction of pathology, establishment and severity of malaria disease [49]. Studies have revealed that oxidative stress is frequent in malaria patients [47] [50] as a result of the stimulation of the immune responses by the malaria parasite, in that way causing, respectively, the production of nitrogen atoms and/or reactive oxygen species [49] [51]. An imbalance between free radicals and antioxidants in the system can lead to oxidative stress [52]. When free radicals are produced in amounts greater than the scavenging capacity of the endogenous antioxidant system, it brings about oxidative stress [53]. Malarial parasite infection is related to an increased production of reactive oxygen species by phagocytic cells. During

Plasmodium blood stage infection, a relation between oxidative stress and inflammation is evidenced by reactive oxygen species-induced stimulation of macrophages. Alterations due to toxic metabolites of the host and parasite may render erythrocytes more exposed to impairment [54] [55].

Antioxidants are molecules which can combine with free radicals safely to stop the chain response before essential molecules are impaired. Excessive quantities of free radicals and oxidants can result in oxidative stress, a detrimental process that can dramatically affect wellbeing; and successively a variety of tissues, cellular elements, or components such as membrane lipids, proteins, lipoproteins, and deoxyribonucleic acid are all impacted [54]. Antioxidants such as GSH, SOD, and CAT are known to play a major function in the preservation and control of ROS levels during malaria parasite infection [56]. Hence, this may explain the variations in the level of oxidative stress and antioxidants in infected and uninfected respondents in this study. On the other hand, the antioxidant defence system and serum Vit C levels were significantly reduced among malaria parasite-infected patients compared to non-infected participants. This study shows that patients with malaria parasite infections had a significantly higher overall oxidative status than did healthy controls [49]. The fact that a significant and direct relationship occurred between parasite density and MDA, as well as NO, and an inverse relationship between parasite density and GSH supports the fact that malaria parasites are capable of inducing oxidative stress, and antioxidant levels (GSH) are equally reduced while combating the effects of oxidative stress [17]. This oxidised form of GSH reacts with free radicals and prevents the generation of most toxic hydroxyl radicals [17]. The decrease in GSH in malaria parasite-infected patients indicated that during infection, there was excessive oxidative stress and, as a consequence, glutathione concentrations were depleted while combating oxidative stress. The inverse relationship between parasite density and super oxide dismutase as well as CAT and Vit C may be suggesting the degradation of these antioxidants in fighting malaria-related oxidative stress [17] [35] [49].

MDA (a biomarker of lipid peroxidation and a measure of oxidative stress) was increased among symptomatic malaria parasite infected patients when compared with those who were not symptomatic. Malaria pathogenesis and the severity of malaria-related complications could be significantly influenced by some factors such as anaemia and oxidative stress [57] [58]. Malaria stimulates the immune system of the body triggering the release of redox active by-products like superoxide, hydrogen peroxide, free heme hydroxyl radicals, lipid peroxides, and other related species [58]. A probable source of free radicals in malaria infection is the host's haemoglobin degradation since the parasite utilises haemoglobin for its own nutrition, releasing a lot of circulating heme. These heme groups can provoke intravascular oxidative stress, inducing modifications in erythrocytes and endothelial cells and eventually favouring the entry of the parasite in tissues like the liver, spleen, heart, intestine and cerebrum [15].

It can be depicted from the results obtained that there was no significant modification in the antioxidant defence system between symptomatic and asymptomatic malaria parasite infected-patients, suggesting that there is an equal amount of antioxidant responses to the parasite in both symptomatic and asymptomatic malaria parasite infected hosts. These results may explain in part why a patient will be symptomatic or asymptomatic, and concomitantly may suggest that the occurrence of oxidative stress as a result of malaria parasite infection may justify whether a patient will exhibit symptoms of malaria or not. However, there is a necessity for additional studies on factors that are responsible for malaria-related oxidative stress as well as asymptomatic malaria if the burden of malaria is to be controlled in Africa and other affected regions.

5. Conclusion

The estimated malaria parasite infection prevalence was 32.1% with the majority of the patients exhibiting parasitaemia being symptomatic. Malaria parasite infection of the peripheral blood was associated with anaemia and oxidative stress. Oxidative stress was associated with parasite density. Mean concentrations of MDA and NO were higher among malaria parasite-infected participants compared with non-infected group. A significant and direct relationship existed between parasite density and MDA as well as NO. In contrast, the antioxidant markers (GSH, SOD, CAT, and Vit C) did not change with whether a patient exhibited symptoms or not. However, oxidative stress parameters remained significantly linked to malaria with malaria symptoms in malaria parasite infected-participants. These findings imply that asymptomatic malaria is a serious obstacle to the malaria eradication strategy. Accordingly, there is a need for mechanistic studies, to explore the implication of oxidative stress in the pathogenesis of asymptomatic malaria and its complications, and to inform health policies that instigate routine diagnosis and therapy of *Plasmodium* malaria in supposedly healthy people if the malaria eradication goal is to be achieved in Africa.

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Authors' Contributions

OAT, DDSF, GST, and HKK conceived and designed the study. OAT and VTJ conducted the research including data collection. OAT, DDSF, VTJ, and GST were responsible for data management and analysis. OAT interpreted the results and wrote the first draft of the manuscript. DDSF, GST, and HKK supervised and critically reviewed the manuscript for important and intellectual content. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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