



ORIGINAL RESEARCH

Antioxidant Status of Children Infected with *Plasmodium falciparum* Malaria in Kebbi Metropolis, Northwestern Nigeria

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Abstract

Malaria is a global life aggressive disease caused by the *Plasmodium* parasite to a host after infected *anopheles* mosquito leading to release of free radicals which have the capacity to induce oxidative stress. This study was carried out to assess the effect of malaria parasite (*Plasmodium falciparum*) on some antioxidants (vitamins A, C, E and reduced glutathione) and lipid peroxidation marker (MDA) in children attending Sir Yahaya Memorial Hospital, Birnin Kebbi, Kebbi State, Nigeria. Blood samples were collected from untreated subjects upon confirmation of *Plasmodium falciparum* parasitaemia using the Rapid test kit (SD Biotline Malaria Ag P.f) method. One hundred and twelve consenting subjects (72 positive and 40 negative subjects) comprising of both sexes were randomly selected. Vitamin A was determined using a method of Basse, et al. [1] while vitamins C and E using a method of Baker and Frank [2]. Reduced glutathione and MDA were determined using methods of Patterson and Lazarow [3] and Abubakar, et al. [4] respectively. Results were analysed using SPSS version 16.0 and significance between groups was ascertained using students' T-test. Result showed that level of antioxidant vitamins (vitamins A, C, & E) and reduced glutathione (GSH) in malarial positive subjects were significantly lower ($p < 0.05$) compared to control subjects. Similarly, lipid peroxidation marker (MDA) were significantly ($p < 0.05$) higher in children with parasitaemia than in non-parasitaemia controls. The decrease in the levels of antioxidant vitamins (A, C and E) and reduced glutathione (GSH) as observed may be due to need of antioxidants to scavenge the free radicals caused by malarial infection.

Keywords

Antioxidant markers, Children, *Plasmodium falciparum*, Lipid peroxidation

Introduction

Malaria is considered as one of the major life threatening disease in developing countries, despite numerous concerted efforts by the governments of various countries and other donor organizations including several drugs/combinations developed acting against the parasite [5]. Recent world malaria report released in 2020 by WHO showed that in 2019 alone, a global estimation of 229 million cases of infection and 409,000 deaths were recorded with children under the age of 5 years been the most vulnerable group as they accounted for 274,000 (67%) of all the malaria deaths worldwide [6]. The WHO's report also revealed that Africa has high portion of the global malaria burden because in 2019 only, the region has an estimation of 94% of all malaria cases and death. Six (6) countries were reported to be accounted for approximately half of all global malaria death with Nigeria having the highest figure of 23% followed by Democratic Republic of Congo with 11% [6]. In Nigeria, malaria is transmitted all over the country with about 76% of the populace live in high transmission areas whereas 24% of the population live in low transmission areas [7]. In southern part of Nigeria, the malaria transmission can last all over the year while in northern part is just about 3 to 5 months. Wassmer and Grau [8] revealed that malaria is an olden menace to human health and is one of the main causes of illness and death globally. There are five (5) human malarial species: *Plasmodium*

falciparum, *malariae*, *ovale*, *vivax* and *P. Knowlesi* where *Plasmodium falciparum* is the most virulent [9]. Most of the disease caused by infection with *Plasmodium spp.* is caused by *P. falciparum* [10] and it is considered as the deadliest form, it can cause acute anaemia through red blood cell destruction [9,11,12]. Most of malaria cases admitted to hospitals are due to *P. falciparum* of which children less than 5 years of age and pregnant women are most affected [13,14]. Babalola, et al. [9] reported that the mayhem triggered by *P. falciparum* malaria in Africa cannot be overemphasized.

When an infected female of anopheles mosquito bite a vertebrate host (e.g. human), the disease is triggered. In humans, an asexual multiplicative and evolutionary cycle takes place, initially in the hepatocytes and subsequently intra erythrocytes. In each intra erythrocyte cycle, merozoites are released which continuously invading other red cells in successive cycles, leading to a more or less severe anemia according to the infecting *Plasmodium* species cycle [15-17].

Frequently, in a first phase, an infected individual develops unspecific symptoms, which can be confused with any flu syndrome which includes fever, rigors, and chills [18]. Young children with severe malaria may develop a serious clinical picture with severe anaemia, extreme weakness, impaired consciousness, respiratory distress, convulsions, and hypoglycaemia [16]. Severe anemia in such cases plays a substantial part in its morbidity and mortality [19]. Malarial infection are reported to increase the production of reactive oxygen species (ROS) which may lead to erythrocyte membrane damage and contribute towards the anaemia in the host [9]. Studies by Ogbodo, et al. [20]; Atiku, et al. [21] and Babalola, et al. [9] disclosed that increased oxidative stress in patients infected with *P. falciparum* malaria infection is related to severity of disease and anaemia. Furthermore, Narsaria, et al. [22] and Anton Gotz, et al. [23] reported that malaria infection triggers the immune system of the body followed by the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radical, lipid peroxides and other related species through engaged activated monocytes and neutrophils as an antimicrobial action. Also malaria parasite activated certain cells in the provision of ROS leading haemoglobin degradation [23]. It has been reported that oxidative stress plays vital role in the progression of malarial anaemia and *Plasmodium falciparum* infected red cells produce more reactive oxygen species [24]. Despite the action of oxidative stress during malaria infection is still not elucidated, some scientists revealed a defensive role, while others confirmed a relation with malarial pathology [25].

In normal situations, there is a balance between the production of free radicals (reactive oxygen species) and defences of antioxidants production, so the interruption of this balance is responsible for the cell damage,

which is termed oxidative stress [26,27]. This happens when the excessive production of oxygen radical in the cell overwhelm the normal capacity of antioxidants [26,28]. These antioxidants molecules have the ability to carefully interact with free radicals to dismiss their harmful effect before important biological molecules are impaired. Oxidative stress is a detrimental process which occurs when excessive free radicals engulf antioxidants a condition that undesirably affect several cellular structures such as membrane lipids, proteins, lipoproteins, and deoxyribonucleic acid [29]. Francois [28] further reported that if the control of ROS failed by internal defence mechanism, this could lead to toxicity such as genotoxicity, cytotoxicity, and even when mutated cells can proliferate i.e. carcinogenesis.

Moreover, human body possesses certain mechanisms to diminish the cellular effects of reactive oxygen species perhaps through nutritional diet, which are considered as the major source of antioxidants or can be synthesized in the body through many intracellular mechanisms [30]. Also, Onyesom, et al. [30] reported that uric acid, superoxide dismutase, vitamin E restrain the damaging phase of reactive oxygen species, while glutathione peroxidase, vitamins A and C, catalase, restrain the initial production of reactive oxygen species. It has been further reported that serum antioxidant vitamins (Vitamins A, C and E) are important markers of oxidative stress and demonstrated to scavenge the radicals imposed by malaria infection [31].

An oxidative stress marker: malondialdehyde (MDA) levels in persons with malaria are shown to be higher when compared to the uninfected ones, while antioxidant enzyme catalase activity are lower in malaria infected patients compared to negative control [32]. Also, Cabrales, et al. [33] disclosed that increased production of free radicals which will lead to oxidative stress will subsequently lead to increased formation of malondialdehyde (MDA) which is an important lipid peroxidation marker.

Several studies in some part of the nation have reported that malaria infection inflicts remarkable oxidative stress on the host. However there is scarce data from Kebbi State, though there is disparity due to factors such as race, socio-economic and certain environmental or ecological factors. Hence the need to investigate the levels of serum antioxidant vitamins (Vitamins A, C and E) and endogenous antioxidants such as reduced glutathione (GSH) and lipid peroxidation marker (MDA) in malaria infected children in Kebbi State metropolis to determine if the impact of these factors may alter the already established findings as reported in a similar study by Abubakar, et al. [11]. Therefore, the aim of this study is to assess the levels of these important antioxidants (Vitamins A, C, E and GSH) and lipid peroxidation marker (MDA) in children infected with *Plasmodium falciparum* of Kebbi State metropolis.

Materials and Methods

Research location

The research was conducted in Birnin Kebbi metropolis, Kebbi State, North-western Nigeria. Samples were collected from Sir Yahaya Memorial Hospital, Kebbi State. Kebbi State has a total population of 3,238,628 based on 2006 National census and is located on the north western part of Nigeria. It has a total landmass of about 37,699 square kilometres out of which 36.46% is made up of farmland. However, about one third of the state is situated in desert prone environment thus making it one of the front-line states for the menace of drought and desertification. The state shares an extensive border with Niger Republic to the North and Benin Republic to the west with many inter-cultural and ethnic linkages.

Sample size

Simple random sampling technique was used in selecting the participants of the study. The random selection was used to guarantee that each individual has an independent and equal chance of being selected. The method is also very fair and unbiased.

Research and Ethics Committee of Usmanu Danfodiyo University have approved all the experimental protocols via a protocol number UDUS/UREC/2019/018 prior to the commencement of the study. Parents of the children enrolled were informed using a standard informed consent form.

A total of One Hundred and Twelve (112) subjects comprising seventy two (72) malarial positive subjects between the ages of 0 months to 6 years and forty (40) apparently healthy non-malaria volunteers (control) subjects (age and gender mismatched) were enrolled into the study. The research is a case control study involving *Plasmodium falciparum* malaria parasitized children (subjects) and non-malaria parasitized control subjects.

Inclusion criteria: Children that met the inclusion criteria are children aged between 0 to 6 years who are tested positive with *Plasmodium falciparum* parasitaemia and are visiting the Sir Yahaya Memorial Hospital Kebbi State, Nigeria. Furthermore, only children whose parents and guardian gave a written informed consent for their ward are included in this study.

Exclusion criteria: Children aged above 6 years and also whose parents or guardian refused to give a written informed consent for their ward to participate in this study were excluded from the study. Additionally, children reported to have transfused with blood in the last Three (3) months or are on antioxidants supplementation were also excluded.

Sample collection and sample treatments

Upon acceptance to participate in the study, a

blood sample was collected from each patient using multi sample needle, with EDTA vacutainers and plain vacutainers specimen bottle. Plain vacutainers were centrifuged for five minutes at 3000 rpm and used for biochemical analysis. The presence of *Plasmodium falciparum* malaria was determined with rapid diagnostic test (SD Bioline Malaria Ag P.f) using a blood sample in EDTA vacutainer.

Methodology

All the reagents and chemicals used for the analysis were of analytical grade. Method as described by Bassey, et al. [1] has been followed for analysis of vitamin A while a method of Baker and Frank [2] was employed for determination of vitamins C and E. Reduced glutathione was measured in blood serum using a method of Patterson and Lazarow [3] whereas lipid peroxidation marker (MDA) was measured using the thiobarbituric acid reactions (TBARS) as reported by Abubakar, et al. [4].

Statistical analysis

Result of the study are presented as Mean \pm SEM. Statistical significance between groups were assessed using students' T-test and statistical significance was established at $P < 0.05$. The SPSS package version 16 and InStat 3 was used for the analysis as described [34].

Results and Discussion

Demographic and clinical characteristic of the study subjects are presented in Table 1. For all the infected subjects enrolled into the study, male are the majority with 40 subjects representing 55.56%, while the remaining 32 (44.44%) are female. Likewise, majority of the control subjects (uninfected) enrolled are male who constituted 23 subjects (57.5%), while the remaining 17 subjects (42.5%) are female. Furthermore, majority of the malaria infected and uninfected (control) subjects included in this study were aged between 5 to 6 years, representing 47.22% and 47.5% respectively, followed by those within the age of 3 to 4 years representing 31.95% and 32.5% for study (infected) subjects and control group respectively. The remaining subjects in

Table 1: Demographic characteristics of the children with *Plasmodium falciparum* malaria.

Characteristics (n = 72)	Infected subjects (n = 40)	Control subjects
Gender		
Male	40 (55.56)	23 (57.5)
Female	32 (44.44)	17 (42.5)
Age (years)		
0 -2	15 (20.83)	08 (20.0)
3-4	23 (31.95)	13 (32.5)
5-6	34 (47.22)	19 (47.5)

N: Percentage; n: number of sample

Table 2: Effect of *Plasmodium falciparum* malaria on some antioxidants parameters of the study subjects.

Antioxidants	Infected subjects (n = 72)	Control subjects (n = 40)
Vitamin A (mg/dl)	80.384 ± 1.391*	94.609 ± 1.161*
Vitamin C (mg/dl)	81.505 ± 1.844*	97.038 ± 1.360*
Vitamin E (mg/dl)	79.847 ± 2.074*	95.892 ± 0.822*
GSH (mg/dl)	42.540 ± 1.103*	56.442 ± 1.856*
MDA (nmol/L)	68.901 ± 4.819*	43.553 ± 5.059*

n: number of sample, GSH: Reduced Glutathione, MDA: Malondialdehyde

Values are expressed as Mean ± SEM. Values with the same superscript in the same row are significantly different (P < 0.05).

both the two (2) groups were aged between 0 to 2 years.

The result of the study as presented in Table 2, showed that malarial infection significantly (P < 0.05) decreases serum levels of antioxidant vitamins: A, C and E and Reduced Glutathione (GSH) when compared with the non-infected subjects. Likewise, Malondialdehyde (MDA) levels was significantly higher (P < 0.05) in malaria infected children, in comparison with the control group.

The result presented in Table 2 showed that *Plasmodium falciparum* malarial infection significantly (P < 0.05) lower the serum levels of antioxidant vitamins A, C, and E as well as reduced glutathione (GSH) and significantly increases lipid peroxidation marker (MDA). It has been confirmed that *Plasmodium falciparum* merozoites as an antigens triggers the production of reactive oxygen species (ROS) by polymorpho nuclear and mononuclear leukocytes [35]. Malaria infection triggers the generation of hydroxyl radicals (OH•) in the liver, hence may be the foremost reason for the induction of oxidative stress and apoptosis [36]. Furthermore, it has been reported that infection of erythrocytes by *P. falciparum* produces hydroxyl group (OH•) radicals and hydrogen peroxide (H₂O₂) about doubled when compared to normal erythrocytes [37]. Nitric oxide (NO) is an innate immune mediator and another free radical species that are involved in progression of malaria infection though its role is still deliberated [33,38]. Oxidative stress in children infected with *P. falciparum* is recognised to induce anaemia via a chain of processes and the main target of oxidative stress is the red blood cell due to their principal function as O₂ carrying cells [39]. Several scientists proposed that cerebral malaria may be perhaps an ill-fated consequence of high amounts of nitric oxide (NO) production, in order to stimulate the death of the parasites [40,41] though other group of scientist believed that cerebral malaria results from a low bioavailability of nitric oxide [42]. Another possible cause of free radical in malaria infected subjects is the human haemoglobin molecule, which serves as the best source of amino acid to the malaria parasite particularly during erythrocytic stage of the infection, thereby leading to release of significant amount of

circulating heme, where Fe²⁺ is oxidized to form Fe³⁺ and the electrons produced during this process react with molecular oxygen to form ROS [43] and capable of inducing intravascular oxidative stress and resulting to changes in erythrocytes and endothelial cells, which will help the internalization of the malaria parasite in liver and brain [44]. Moreover, reactive oxygen species (ROS) are also generated in the mitochondria through various metabolic processes [22,45-48]. These free radical productions are triggered by malaria parasite which will subsequently lead to an antioxidant defence in the host cell to halt the infection [49-51]. Guha, et al. [52] and Sohail, et al. [51] reported higher levels of oxidative stress markers in malaria infected humans compared to uninfected subjects. Numerous studies have reported oxidative stress, hypoxia, increased inflammation, and hepatocyte apoptosis in malaria-infected livers [53]. Oxidative stress is considered as a main factor in the pathogenesis of malaria and contributes to the severity of malaria related complications [26].

Antioxidant molecules such as vitamins A, C, E and reduced glutathione (GSH) are very important in scavenging the free radicals released as a result of malaria infection. Reduced glutathione (GSH) molecule is reported to be the best influential protector of eukaryotic cells in the host defence against oxidative stress [37,54]. Malondialdehyde is a product of lipid peroxidation and hence recommended as significant biomarker of oxidative stress in both severe malaria and placental malarial [37,55]. Meanwhile, several documented evidences showed that malaria infection results in increased ROS as well as reduction of antioxidants, therefore oxidative stress in malaria infected patients is distinct as the disparity between the two can cause either ROS excess or antioxidant decline [9]. Increase in lipid peroxidation marker (MDA) level and decrease in antioxidants vitamins (vitamins A, C and E) have been reported to be responsible for the progress of oxidative stress in malaria patients [9,11,56]. Human biological system possesses a number of defence mechanisms against reactive oxygen species, which include the synthesis and utilization of antioxidants [57,58] as these reactive oxygen species has the ability to induce lipid peroxidation and cell damage [11]. Therefore, the effect of reactive oxygen species could lead to significant reduction of antioxidant levels as showed in this study. Furthermore, this strengthens the crucial role played by antioxidants in the defence of deleterious biological consequences induced ROS. Reduced glutathione (GSH) is an extremely important cell protectant as it directly quenches reactive hydroxyl free radicals, other oxygen centred free radicals, and radical centres on DNA and other biomolecules [54]. Vitamin C or Ascorbic acid (AH-) is a water soluble vitamin and a potent reducing agent required by the biological system to scavenge several radical species [59]. Lipid peroxidation (LPO) radical can damage the macromolecules (DNA, RNA,

and proteins) and can initiate cytotoxic, genotoxic, and inflammatory reactions. Vitamin C converts lipid peroxidation products into unreactive vitamin C-LPO products and helps to prevent the interaction of macromolecules (DNA, RNA, and proteins) with LPO• radicals [60]. Likewise, vitamin C is reported to increase iron absorption by reducing Fe³⁺ to Fe²⁺ [61]. Vitamins A and E are lipid soluble antioxidants that possess the ability to directly quench free radicals and function as a membrane stabilizer [62] and several studies have shown that vitamin A is effective antioxidant [59].

The decreased antioxidant concentrations of vitamins A, C, and E observed in this study may be due to their increased utilization by the body to solve the deleterious effects of free radicals generated by parasitaemia [37] as highlighted above. Furthermore, the decrease in the level of reduced glutathione (GSH) experienced may be due to its over utilization to directly quench the devastating hydroxyl free radicals produced by the body as a result of the parasite infection [56]. Likewise, increase in MDA observed in this study may be due to increase in peroxidation of membrane lipids caused by the parasite [63] or could be due to decreased activity of the defence system protecting tissues from free radical damage [64]. Results of this study showed an inverse relationship between antioxidant levels and lipid peroxidation. The result of the study is and are consistent with the earlier studies by Onyesom, et al. [30] and Abubakar, et al. [11] who reported a decrease in the antioxidants vitamins, increased in the levels of MDA and GSH. Guha, et al. [52]; Kulkarni, et al. [65] also reported an imbalance in the oxidants and antioxidants levels in the serum of malarial infected patients which also confirmed to the finding of this study. Similarly, the finding of our study is in conformity with the study carried out by Idonije, et al. [63] who reported decreased antioxidants capacity in malaria infected patients especially *P. falciparum* compared to uninfected subject. The finding of his study [63] directed him to conclude that there is oxidative stress in children with parasitaemia due to significantly higher levels of lipid peroxidation and reduced glutathione he observed. Studies by Egwunyenga, et al. [66] and Abubakar, et al. [11] revealed a significant decrease in the level of vitamin C which also coincided with increase in the level of MDA in *P. falciparum* infected children compared to control subjects. Additionally, studies by Sobolewski, et al. [67]; Guha, et al. [52] and Sohail, et al. [51] reported a significant increase in oxidative stress markers in malaria infected humans compared to uninfected controls. Equally, Egwunyenga, et al. [66]; Atiku, et al. [21] also reported higher level of lipid peroxidation marker (MDA) in malaria infected patients than uninfected control subjects. Moreover, a recent studies conducted by Atiku, et al. [21] and Babalola, et al. [9] showed an increase in MDA (lipid peroxidation marker) and decrease in antioxidants

Vitamin C and GSH in malaria patients compared to uninfected subjects. Likewise, another Ethiopian study carried out by Ebrahim, et al. [68] showed that the total oxidative status in malaria patients was significantly higher than that in control subjects. Consequently, the *Plasmodium falciparum* virulence appears to depend basically on the patients' antioxidant capacities and this is also determined by the concentrations of antioxidant micronutrients [20]. Nevertheless, biological system possesses several defence mechanisms to reduce the cellular effects of ROS triggered by *P. falciparum* such as intracellular synthesis of antioxidants or acquired nutritionally through diet.

Conclusion

This finding may have added more information to the sparse reports (if any) on changes in antioxidant profile of malaria infected children living in Kebbi metropolis, Kebbi State, North-western Nigeria. The finding of this study may suggest that exogenous micronutrient with antioxidant vitamins (A, C and E) may improve the management of children infected with *P. falciparum* malaria regardless of sex.

Conflict of Interest

The authors have declared no conflict of interest and no other relationship or activities that could appear to have influenced this study. Authors have not received any financial support from any organization and no financial relationship with any organization or individual that might have an interest in the previous three (3) years.

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