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Antioxidant status of children with *Plasmodium falciparum* malaria in Owerri municipal council of Imo state

***Nwosu,D.C.[1], Obeagu,E.I.[2], Ezenwuba,C.[3], Agu,G.C.[4], Amah,H.[5], Ozims,S.J.[5], Nwanjo,H.U.[1], Edward,Amaka[5], Izuchukwu,I.F.[4], Amadike,J.N.[4], Nwagwu,A.J.[3] and Emesowum,A.C.[3]**

[1.] Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri.
[2.]Diagnostic Laboratory Unit, Department of Health Services, Michael OKpara University of Agriculture, Umudike, Abia State, Nigeria.
[3.] Department of Nursing Science, Faculty of Health Sciences, Imo State University, Owerri.
[4.] Department of Optometry, Faculty of Health Sciences, Imo State University, Owerri.
[5.] Department of Public Health, Faculty of Health Sciences, Imo State University, Owerri.
*Corresponding Author: emmanuelobeagu@yahoo.com

Abstract

This study was carried out to assess the antioxidant status of children with *Plasmodium falciparum* malaria in Owerri. A total of 50 children aged between 1-10 years were recruited for this study. They were grouped into two, 30 *Plasmodium falciparum* infected children (test) and 20 apparently healthy children (control). The test group were children attending Federal Medical Centre, Owerri who were infected with malaria as confound by caumsa reduces. Antioxidant vitamin C and E were evaluated using standard colorimetric procedures, while antioxidant enzyme Superoxide Dismutase (SOD) was evaluated using enzyme assay Kit. The mean antioxidant concentrations for vitamin C and E among plasmodium parasitized subjects were 0.4±0.08 mg/dl and 0.60±0.09 mg/dl respectively. The mean concentrations of vitamin C and E among the non-malaria parasitized controls were 1.49±0.15 mg/dl and 1.70±0.11mg/dl respectively. The antioxidant enzyme, SOD level of the test and control group was 4.52±0.64m/mol and 7.31±0.55m/mol respectively. I observed that the mean antioxidant concentration of vitamin C and vitamin E and SOD were lower among the test subjects compared with the control subject ($p<0.05$). These antioxidants are also lower in younger children than older children, and in males than in females. In conclusion, my study has shown that the antioxidant levels in plasmodium parasitized children are lower than non-plasmodium parasitized children.

Keywords: Antioxidant status, Children, *Plasmodium falciparum* malaria, Owerri Municipal Council.

Introduction

Malaria is a life threatening parasitic diseases caused by many species of an infected female anopheles mosquito. Malaria causes symptoms that typically include fever and headache which in severe cases can progress to coma or death.

According to the world Health Organisation in 2011 (WHO), malaria is a significant public health problem in more than 100 countries and causes an estimated 200 million infections each year, with more than 500 thousand deaths annually. Over 90% of these deaths occur in sub-sahara Africa, where the disease is

estimated to kill one child every 30 second. In other areas of the world malaria causes substantial morbidity especially in the mid area of some countries in Asia and South America. In contrast, despite previous estimation in regions like the United States and Western Europe, the phenomenon of 'imported malaria' introduced by immigrants and traders still contributes with specific cases in these regions.

In Brazil 2011 a country in which malaria is endemic the situation is equally alarming. Even with a cutback in the number of reports on malaria cases in recent years, the high risk of malaria incidence and transmission in the Amazon region persists. According to the ministry of health 99.7% of malaria cases are concentrated in the Amazon region. Strengthening of the national malaria control programme in 2000 has resulted in a steady decrease since 2005, according to the external parasitic incidence in the Amazon area. Although the malaria rate has decreased, resistance to drug therapy has increased, especially in patients infected with *Plasmodium falciparum,* responsible for about 20% of the cases in this region.

In fact, current drugs such as chloroquine and artemisinin already present resistant strains of *Plasmodium falciparum* . However, factors leading to this resistance are still not well known owning to a lack of through understanding on the physiopathogenic mechanism of the disease. Several authors have diseased the implications of free radicals through oxidative stress in the physiopathogenesis of malaria (Pablon et al., 2002). Mechanism involvement may be reacted to the pathogenic mechanisms triggered by the parasite, as well as free radical production and antioxidant defences in host cells to abate the infection. The generation of reactive oxygen and nitrogen species associated with oxidative stress (Dondorp et al., 2003) plays a crucial role in the development of systemic complications caused by malaria. Malaria infection induces the generation of hydotyl radical (OH) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis. A potential source of free radical production in this disease in the host's hemoglobin molecule, since the parasite uses this molecule as a source of amino acids for its own nutrition daring the erythrocytic stage of the disease, resulting in the liberation of large amounts of circulating heme. By having Fe2+ associated groups, these heme groups are able to induce intravascular oxidative stress, causing change in erythrocytes and endothelial cells and facilitating the internalization of the parasite in tissues such as the liver and brain. A free radical species which appears to be involved in this disease in Nitric oxide (No) (Cabrales et al., 2011). However, its role is controversial. Some researchers claim that cerebral malaria is probably an infortune in consequence of high amount of nitric oxide production to promote the death of the parasites, white others support the idea that celebral malaria results from a low bioavailability of this compound (Gramaglian et al., 2006).

Additionally, host-parasite interactions are quite complex and promote constant changes in the delicate balance between pro-oxidant and antioxidant molecules since the host and parasite are capable of producing both. Periodic vitamin A supplementation could reduce the increase of febrile episodes and parasitemia due to *Plasmodium falciparium.*

Vitamin A is essential for normal immune function and has been shown to influence both antibody response and cell-mediated immunity. Vitamin C concentration correlates inversely with white cell count alpha-1-acid glycoprotein and interleukin-6, all of which are markers of inflammation vitamin C can rejuvenate vitamin E making it an indirect contributor to fighting free radical damage in membrane lipids. These free radicals are products of oxidative stress that aggravated in malaria infection to decrease the antioxidant defense system. One of the implications of oxidative stress is the development of malaria anamia. Antioxidant such as carotenoid vitamin C and E, suporoxide dismutase, and Nitric oxide produces protection against oxidative stress induced by malaria. Nevertheless, even anti-malaria drug therapy consititutes a source of oxidation, as many drug such as chloroquine, primaquine and derivatives of artemisinin are inducers of free radical production.

Objectives of study

To determine the level of vitamin C among the children with *Plasmodium falciparum* malaria and apparently healthy children.
To determine the level of Vitamin E
To determine the level of SOD among the children with *Plasmodium falciparum* malaria in Owerri.
To compare the antioxidant level of male and female malaria children.
To know the effect of age on antioxidant level of malaria parasitized children.

Study area

This study was conducted in Owerri Imo state Nigeria between January and March 2015. Imo state lies on Latitude 4° 45N and 7° 15°N, and longitude 6°-50°E and 7°,25°E with an area of around 5,100 sq km and is located in the rainforest belt of Imo state, endemic for *P. falciparum* malaria parasite which is transmitted by the female Anopheles mosquito. It has a rainy period of April to November which is also the mosquito bites are more rampant. The rainforest belt where the state is located is also very good habit for mosquito.

Occupation: Education is given priority in the state. The state is also rich in Agriculture and natural resources including crude oil, Natural gas, lead and zinc.

Study population

A total of 50 male and female children aged between 1-10 years were recruited for this study. They were grouped into two, 30 plasmodium falciparum infected children (Test) and 20 healthy children (control). These study population were recruited at the outpatient clinic of the Department of paediatrics of Federal Medical Centre Owerri in Imo state Nigeria.

Advocacy, mobilization and pre-survey contacts

An introduction letter from the HOD of medical Laboratory Science of Imo State University containing the protocol of this study was presented to the head of the department of Paediatrics ward. Ethical clearance was obtained from the management of the hospital and informed consent was obtained from the parent/guardian of each child after thoroughly explaining the scope, nature and objective of the study.

Selection/exclusion criteria

Subjects recruited for this study were those who gave their consent. Test subject included where those with signs and symptoms suggestive of simple/uncomplicated malaria. Fever auxiliary temperature > 37.5°C, headache, vomiting, diarrhoea, prostration, pallor, jaundice, respiratory distress and other clinical signs and symptoms. Demographic data including age, weight and height were measured and recorded. Those excluded from the study were with anaemia, sickle cell, HIV infection, those on antimalarial drugs.

Sample collection and processing

The study population in this case comprised 30 malaria affected children both male and female who attended the padiatric clinic department of Federal Medical Center in Owerri. Twenty healthy age and gender matched non-parasitized children were included as controls. The number of control participants is adequate for the power of the five milliliters of the blood was collected into EDTA (ethylenediamine tetra acetic acid) bottle for the determination of parcked cell volume, confirmation of malaria parasitemia, speciation and parasite count. Blood smears (thin and thick films) were prepared for all malaria positive subjects and non-parasitized controls. Thick and thins films were skin with field skin (for confirmation, speciation and parasite load determination) and were road for 200 fields. Parasite counts were reported per 500 white blood cells and for counts above 1 000 parasites. Malaria parasitemia was defined by the presence asexual forms of *Plasmodium falciparum*, confirmed by microscopic examination of the peripheral blood. The parasitemia was graded as low (<500 parasites/ul), moderate (3000 parasites/ul) and severe (> 10000 parasites/ul). Three milliliter of venous blood was collected into a clean plain tube without anticoagulant and allowed to clot at room temperature. The serum was obtained by centrifugation for 10 minutes at 3000 rpm.

Laboratory procedure

All reagents used were prepared, and standard operating procedures were strictly followed except Superoxide dismutase (SOD).

a. Determination of Vitamin C

Principle: The ascorbic acid (vitamin C) is converted to dehydro ascorbic and by shaking with cupric sulphate solution and this is then coupled with 2, 4, -dindtrophynyl hydrazine (DNPH) in the presents of thiourea as a mild reducing agent. Sulphuric acid then converts the dini trophenyl hydrazine into red compound which is assayed spectrometrically.

Procedure: Assay method from serum vitamin C. the quantities, 1.0ml fresh serum, 1.0ml of 10% trichloroacetic acids, 0.5ml chloroform were added to the test tube, they were stoppered shaken vigorously for 15 seconds and centrifuge for l0 minutes.
After centrifuging tubes: Test (T), standards(s) and blank (B) were set up. To the test 1.0ml of test clear supernatant was added and nothing was, added to standard and blank than 0.4ml of colour reagent was added in all the tubes. These were stoppered, mixed and placed in 56° C water both for 1 hour. They were cooled in an ice bath for 5mins. After cooling pipette into each tube and left at room temperature for 30mins and later remained.
It was transferred to cuvet with a maximum capacity not exceeding 3ml and absorbance measured against the blank at 520nm.
Calculation: optical density of sample X 2 in Mg/lOml
Optical density of standard Reference Value = 0.3 - 2.0 mg/dl

b. Determination of plasma vitamin E

Principle: this method is based on the reduction of ferric to ferrous ions by vitamin E which then forms a red complex with diphyridyl. Vitamin E and carotene v/ere first extracted into zylene and the extinction read at 460nm to measure the carotene. A correction is made for this after adding ferric chloride and read at 540nm,

Procedure: Assay method for plasma vitamin E

The quantity 1.5ml of serum was added to test tube (T) and none was added to
Standard(S) and blank (B).
5ml of standard (D-12 tocopherol (10mg/l) was added to standard tube (S) and
1.5ml of water (Blank) added to blank.

Absolute ethanol 1.5ml was added in all the tubes. It was then mixed properly and centrifuged for l0 mins.
1.0ml of xylene layer was added in all the tubes and 1.0ml of 2, 2 dipridyl (1.201m) added in the tube.
It was stopped and mixed, then 1.5ml of the mixture was pipette into cuvette and the extinction of test and standard were read against the blank at 460 nm. Then in the tubes, beginning with the blank, 0.33ml of ferric chloride solution was added, mixed and after exactly 15mins, the test and standard were read against the blank at 520nm.

Calculation: Plasma vitamin E –

(Reading of unknown 520nm Reading at 460nm) X 0.29X1 OOg Reading of standard at 520nm.

c.Determination of SOD Antioxidant

Kit Name: Abcams SOD activity assay kit (Catalog No 9665354).
The principle of superoxide dismutase (SOD)

Abcams SOD activity assay kit (colorimetric) is a sensitive kit using WST-1 that produces water soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the Xanthenes oxidase (xo) activity, and is inhibited by SOD. Therefore the inhibition activity of SOD can be determined by a colorimetric method.

Statistical analysis

All values were expressed as mean ± standard derivation. The results were analysed for statistical significance using the independent student t-test. P-values < 0.05 were considered statistically significant.

Result and analysis

Table 1: vitamin c, vitamin e and sod levels of malaria children (test) and healthy children (control)

	TEST	CONTROL	
PARAMETER	TEST (n=30)	CONTROL (n=20)	P-VALUE
Vitamin C (mα/dl Vitamin E (mg/dl) SOD (mg/dl)	0.48±0.08 0.60±0.09 4.52±0.64	1.49±0.15 1.70±0.11 7.31±0.55	0.0001* 0.0001* 0.001 *

Data are Mean ± standard Deviation
*: statistical significant (P<0.05)
SOD: Super Oxide dismutase.

The label 1 above indicates the antioxidant (Vitamin C, Vitamin E and SOD) levels of the test and control groups.
The vitamin C level of the Test was 0.048±0.15mg/dl. Comparison of test with control shows that the test group is significantly (P < 0.05) lower than the control group.

The group means of antioxidant vitamin E as indicated in table 1 shows a lower concentration in test group (0.60±0.09 mg/dl) than the control (1.70±0.11 mg/dl); and this difference is statistically significant (P<0.05). This indicates that malaria has effect on antioxidant level.

Superoxide dismutase (SOD) concentration is lower in the test group (4.52±0.64 mg/dl) than the control group (7.31 ± 0.55 mg/dl). Statistical comparison of both indicates significant difference (P < 0.05) among their mean.

Table 2: Effect of age on antioxidants in malaria infected children.

PARAMETER	1-5years (n=29)	6-10years (n=10)	P-value
Vitamin C (mg/dl)	0.44 ±0.06	0.55 ±0.07	P<0.05
Vitamin E (mg/dl)	0.59 ±0.08	0.62 ±0.10	P > 0.05
SOD (ng/dl)	4.43 ±0.66	4.69 ±0.59	P > 0.05

Values are Mean ± standard deviation.

Table 2 above shows the antioxidant levels of malaria children according to age variation. Vitamin C concentrations of those between 1-5 years are 0.44 ± 0.06mg/dl and that of those between 6-10 years are 0.55 ± 0.07mg/dl. Comparison of both shows that lesser age group vitamin C level is significantly (P < 0.05) lowers than the higher age group.

Vitamin E level of the lower age group is lower than the higher age group but the difference among their means is not significant (P>0.05).

SOD antioxidant level of subject between the age group of 1-5 years (4.43 ± 0.66mg/mol) is not significantly lower than those between 6-10 years (4.69 ± 0.59mg/mol).

Table 3: Antioxidant status in children with plasmodium falciparum malaria in relation to sex.

PARAMETER	Male (n=17)	Female (n=13)	P-value
Vitamin C (mg/dl)	0.43 ±0.05	0.54 ±0.07	P < 0.05
Vitamin E (mg/dl)	0.58 ±0.07	0.62 ±0.10	P > 0.05
SOD (ng/dl)	4.42 ±0.67	4.64 ±0.60	P > 0.05

Values are Mean ± standard deviation, n = Sample size.

Table 3 above indicate the antioxidant level of malaria children in relation to sex from the table it is shown that vitamin C level of male children (0.43 ± 0.05mg/dl) are significantly lower that of the female children (0.54 ± 0.07).

Vitamin E level of male is 0.58 ± 0.07mg/dl and that of female group is 0.62 ± 0.l0mg/dl. Comparison of both shows that the male vitamin E level is no significant (P > 0.05) lowers than that of the female group.

Comparison of the SOD of the male (4.42 ± 0.67u.g/mol) and female group (4.64 ± 0.60u,g/mol) shows a non-significant (P > 0.05) among their mean.

Discussion

Malaria is widespread in the tropics and subtropical region. The majority are mostly children in Sub-Sahara Africa. It is one of the most common infectious diseases and great public health problem.

In this study, it was observed that the levels of antioxidants were significantly decreased in malaria subjects jus as seen in Nwosu et al.(2016). This is in line with the work of Nnodim and Nwanjo (2012). The decrease in antioxidant could be associated with free radicals induced by plasmodium falciparum parasites.

In this study, the level of antioxidant vitamin C and E were significantly decreased in malaria subjects compared with the control (P<0.05). This is similar to the work of Boganska et al. (2006). Vitamin C is reduced in malaria patients following its use to regenerate vitamin E from alpha tecopherolescly

radical at water liquid interface. It is also an efficient quencher of superoxide and hydroxyl radicals. The significant lower concentration of vitamin C among the children with these without malaria suggests that these micronutrients may be utilized in the face of increased malaria parasitaemia. This observation is in agreement with a previous investigation performed in patients, indicating that the lower concentration of vitamin C observed among 1-10 year old children corrected with malaria parasitaemia (Akpotuzor et al., 2007).

While significant reduction of vitamin E in malaria patients may probably be due to entranced lipid peroxidation by the *Plasmodium falciparum*. Vitamin C which is a water soluble vitamin and non-enzyme antioxidant serves directly by scavenging aqueous peroxyl radicals. Also indirectly regenerate reduced vitamin E (Nwanjo and Oze, 2007). Therefore the reduction of these antioxidants vitamins challenges the membrane stability of erythrocytes. It is reported that vitamin C and E has been described to have antioxidant effect on some pathological conditions (Ojiako and Nwanjo, 2007). Vitamin E and Vitamin C can also act to overcome oxidative stress, being a part of the total antioxidant system. Vitamin E is the most important lipophilic antioxidant and resides mainly in the membrane stability (Baker et al., 1996). Vitamin C is hydrophic and is a very important free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damage (Karapanhalli et al., 1996).

In this study, superoxide dismutase (SOD) activity was measure as a guage of the level of reactive oxygen species generated during malaria infection. The level of SOD was found to be significantly lower in malaria children than those without malaria. This may be due to the utilization of SOD in the mapping up of high reactive oxygen species generated during malaria infection. This implied a higher oxidative stress status in parasitaemic patients, confirming the findings in a similar study by Stocker et al. (2009). The findings in this study are also consistent with the suggestion in a study conducted by Kharazmi et al. (2011), that reactive oxygen intermediates (Rol) generated during malaria infection contribute to parasite death.

Furthermore, there was a lower antioxidant level in children between 1-5 years as compared with those of 6-10 years old. In this age bracket, it could be seen that children had much reduced antioxidant level, which may be the consequence of frequent malaria infection or of seventy of malaria.

On sex of the patients, antioxidants are lower in males than in females.

Conclusion

Conclusively, present study showed malaria infection to induced oxidative stress which is more serious in the younger than in the older children, in males than in females.

The decrease in antioxidants probably implies that oxidative stress plays a role in the pathogenesis of malaria.

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