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**Increased Malondialdehyde among the Malaria- Parasitized Children in Dutse-Jigawa, Nigeria**Muhammad Y.<sup>1\*</sup>, Ahmad, M.B.<sup>2</sup>, Wali, U.<sup>3</sup>, Yeldu, M.H.<sup>3</sup> and Umar, K.<sup>4</sup>

Department of Chemical Pathology, Rasheed Shekoni Teaching Hospital Dutse<sup>1</sup>, Departments of Chemical Pathology, School of Medical Laboratory Science, Bayero University Kano<sup>2</sup>, Department of Chemical Pathology, School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto<sup>3</sup>, Department of Medical Laboratory Science, Ahmadu Bello University Zaria<sup>4</sup>.

Author for Correspondence \*: [yahyoukhan@gmail.com](mailto:yahyoukhan@gmail.com) / +234-806-473-1241

**Abstract**

Malaria infection is among the leading cause of morbidity and mortality throughout the world. Negative balance between antioxidant and free radicals in malaria parasitized children has existed and decreased packed cell volume (PCV) is a common complication. The current research enrolled one hundred and forty-two (142) participants. Subjects included ninety-three (93) malaria parasite infected children and forty-nine (49) apparently healthy non-parasitized controls. PCV was determined manually using the microhaematocrit centrifuge and reader, while Malondialdehyde (MDA) was determined using a standard chemical method. The results show a significant increase ( $p < 0.005$ ) of MDA in malaria parasitized children compared to control subjects. PCV of the malaria-infected children was significantly ( $p < 0.005$ ) lower than control subjects. Findings of this research, suggest an increase in the MDA levels among parasitized subjects. It's therefore recommended that antioxidant substances should be supplemented for protection against haemolysis and inhibition of lipid Peroxidation among malaria parasitized children.

**Key words:** *Malaria Parasites, malondialdehyde, Packed Cell Volume, Antioxidants*

**Introduction**

Malaria is one of the public health problems that afflicts more than 100 countries, also an estimated 200 million cases occur every year with over 500 thousand death annually (WHO, 2011). The highest percentage of malaria-related

death was noticed among sub Saharan African children accounting for 90% of the total death (Greenwood *et al.*, 2005; Snow *et al.*, 2005).

A blood borne parasite Plasmodium has long been recognized as a causative agents of Malaria infection. To date, four species have been identified to be responsible for malaria in humans; Plasmodium *falciparum*, Plasmodium *vivax*, Plasmodium *ovale*, and Plasmodium *malariae*. The two most common species are the *P. falciparum* and the *P. vivax* of which the most pathogenic is *P. falciparum* (Jawet *et al.*, 2010). One of the common pathophysiology presented by the malaria infection is the depression of erthropoiesis induced by interleukins due to malarial infection and the actual parasitization of red cells leading to shortened survival or death of erythrocytes (Dondorp *et al.*, 2000).

Malaria infection specifically by *P. falciparum* is one of the frequently acquired red blood cell disorders (Maier *et al.*, 2009). The infection is possible when infective female Anopheles mosquito bites an individual thereby transmitting malarial microscopic parasite in the blood (Yoshida *et al.*, 2007). During the asexual and sexual intra- erythrocytic development of *P. falciparum*, several mechanisms orchestrated the remodeling of infected and uninfected RBCs, (Maier *et al.*, 2009). One of the biochemical changes in *esquizogeny* of *P. falciparum* trophozoites is the alteration of red blood adhesiveness thereby enhancing its adhesion to the wall of capillaries thus prohibiting its passage to the spleen and consequent degradation by reticuloendothelial cells (Luse and Miller, 1971).

The expression of *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) encoded by the *var* multigene family is a key event in the escape of infected RBCs from elimination by reticuloendothelial cells (Turner *et al.*, 1994).

The hemozoin (Ferriprotoporphyrin and Methemoglobin) release resulting from destruction of red blood cells induces cytokine release (Jaramillo *et al.*, 2005). Free heme protein containing Fe<sup>2+</sup> possesses the ability to generate excessive radicals via Fenton's reaction (Keller, 2006). However, recent studies suggest that the generation of reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress, plays a crucial role in the development of systemic complications caused by malaria (Keller, 2006).

Malondialdehyde (MDA) is an end product of free radicalization on polyunsaturated fatty acid (PUFA) (Davey *et al.*, 2005). Antioxidant substances such as Vitamin A, C and E participate in scavenging the free radicals generated during malaria infection (Nmorsi *et al.*, 2007). Decrease of oxygen concentration below normal has been reported as a consequence of Ischemia and reperfusion syndrome caused by sequestration of parasitized erythrocytes (Halliwell and Gutteridge, 2007). The present study report on serum MDA among the malaria parasite infected children in Dutse Jigawa State, Nigeria.

## Materials and Methods

**Study Population** The current research included a total of one hundred and forty-two (142) participants including Ninety-three (93) were malaria parasitized children (subjects) and forty-nine (49) apparently healthy gender-matched children (controls) attending Rasheed Shekoni Specialist Hospital Dutse, Jigawa State, Nigeria.

## Blood Specimen Collection

Blood Specimen was collected aseptically by standard venipuncture from each subject and control participant and delivered into plain

container and in a heparinized capillary tube for determination of Malondialdehyde (MDA) and Packed Cell Volume (PCV) respectively. The blood sample in the plain container was allowed to clot and centrifuged to obtain serum. The separated serum was stored at -20°C until used for analysis.

## Analytical Methods

Serum MDA was determined as a marker for oxidative stress in accordance with the method of Nadigar *et al.* (1986). The method was based on the principle that acetic acid detaches the lipid and protein of the tissue. Thiobarbituric acid reacts with lipid peroxide, hydroperoxide and oxygen labile double bond to form the colour adduct with absorption maxima at 530nm, and 0.5ml of serum was mixed with 3.6ml of 10% TCA. This mixture was centrifuged at 5000 rpm for 15 minutes and the supernatant collected. Two milliliters (2.0ml) of supernatant was taken and mixed with 1.5ml of 0.67% Thiobarbituric and 1.0ml of distilled water. This was kept in a boiling water bath for 10mins. After cooling, the colour intensity was measured at 530nm wavelength spectrophotometrically. Packed Cell volume (PCV) was assayed based on the settling down of red blood cells after centrifugation of blood in heparinized capillary tube within haematocrit centrifuge. The volume occupied by the red blood cells was measured, and its ratio with the volume of the whole blood is calculated.

## Reagents

All the reagents and chemicals used in the current study were of analytical grade.

## Statistical analysis

Values were presented as mean ± standard deviation. The biochemical parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by LSD multiple comparison test using SPSS version 16.0 (SPSS Inc, Chicago, IL, USA). Differences were considered as significant when  $p < 0.05$ .

## Results

**Table 1: Age Distribution of MDA in Plasmodium Parasitized and Control Subjects**

Age group (Years)	Parasitized	Control
1-5 (n=42)	3.1±0.6	1.6±0.2
6-10 (n=51)	3.2±0.5	1.6±0.3
11-15 (n=49)	3.1±0.4	1.5±0.3

**Key:** MDA=Malondialdehyde, n=Number of subjects

**Table 2.0: Gender distribution of PCV and MDA in Parasitized and Control Subjects**

Parameters	Parasitized		Control	
	Male (n=48)	Female (n=45)	Male (n=24)	Female (n=25)
MDA (nmol/dl)	3.2±0.5	3.1±0.5	1.6±0.3	1.6±0.3
PCV (%)	32.0±4.1	31.5±3.9	38.4±2.8	37.8±2.8
p-value	0.000	0.000	0.000	0.000
Significance	HS	HS	HS	HS

**Key:** p-value=Probability value, HS=Highly Significant, MDA=Malondialdehyde, PCV=Packed cell volume.

### Discussion

The results of the current study showed a significant ( $p>0.005$ ) increase in the serum levels of MDA among the malaria parasitized children when compared to the non-parasitized controls. The increase is indicative of oxidative stress as a result of increase in antioxidant levels to counteract the effects of free radicals. This is in agreement with the previous study conducted by Metzger *et al.* (2004).

Antioxidants are very important in protecting the cell membrane from haemolysis. Nmorsi *et al.* (2007) reported a decreased in the serum levels of Alphanatocopherol in malaria infected compared to non-infected children. The decrease suggests an increased utilization of vitamin for prevention of lipid Peroxidation. Furthermore, Dattani (2010) stated that administration of curcumin obtained from *curcuma longa* prevented hepatotoxicity in rats treated with chloroquine. Likewise, the administration of glutathione promoted reduced parasitaemia and increased survival of mice infected with *P. berghei* (Gashgaenia *et al.*, 2010). Lower levels of glutathione S-transferase (GST) (Sohail *et al.*, 2007), SOD NADPH-methemoglobin reductase

(Becker *et al.*, 2004 and molecules such as vitamins A, E, C (Hassan *et al.*, 2004; Metzger *et al.*, 2004),  $\alpha$ - and  $\beta$ -carotene, lycopene, lutein and zeaxanthin (Metzger *et al.*, 2004) have long been reported in malaria parasitized individuals. Malaria infection induces the generation of hydroxyl radicals (OH) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis (Guha *et al.*, 2006). Additionally, Atamna and Ginsburg (1993) observed that erythrocytes infected with *P. falciparum* produced OH radicals and  $H_2O_2$  about twice as much compared to normal erythrocytes.

Results obtained from this study reveal a significant reduction in PCV of parasitized subjects compared to the controls. The reason could be due to destruction of red cells which occur in the spleen and this is determined by the degree of parasitaemia. Secretion of cytokine such as IL 1 and IL 5 during P-falciparum infection causes reduced erythropoietin response leading to less erthropoiesis. The present study shows no statistical difference with respect to age groups.

## Conclusion

Conclusively, the current research suggests oxidative stress in malaria infected children with low packed cell volume compared to control subjects.

## Recommendations

Having established increased oxidative stress among the malaria parasitized children, it may therefore be critical to suggest the inclusion of dietary supplementation of antioxidant substances in the management of malaria parasitized subjects.

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