ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 4 (6): 506-513, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.506.513

Comparative Study of the Status of a Biomarker of Lipid Peroxidation (Malondialdehyde) in Patients with *Plasmodium falciparum* and *Plasmodium vivax* Malaria Infection

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ABSTRACT

Malaria infection has been found to be associated with lipid peroxidation accompanying reduction in antioxidant capacity of the infected patients especially *Plasmodium falciparum infection*. In this study, a biomarker of lipid peroxidation, Malondialdehyde (MDA) was evaluated in adult (18-45 years) Nigerian patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria infection. The research group is comprised of fifty patients with *P. falciparum* and fifty with *P.vivax* malaria confirmed patients attending the outpatient department of University of Benin Health Services Department, University of Benin, Benin City. Their lipid peroxidation products (MDA) values estimated spectrophotometrically were compared to that of the control group who are fifty apparently healthy tested malaria negative subjects. Result showed a significant increase ($p<0.05$) in Malondialdehyde level in malaria positive patients (n = 100; 7.67±0.42 μM L^{-1}) compared to the control; malaria negative patients (n = 50; 4.43±0.32 μM L^{-1}). This increase in Malondialdehyde level was higher in *P.vivax* malaria patients (n = 50; 7.94±0.27 μM L^{-1}) than in *P. falciparum* malaria (n = 50; 7.41±0.38 μM L^{-1}) and increases as the degree of parasitaemia increases. Malondialdehyde activity in malaria was higher in males than in females and among the young adults than in the old adults. The study specified malaria to induced oxidative stress which is higher in male and in the young and as the degree of parasitaemia increases and more severe with P.*vivax* malaria infection. Conclusively, supplementation of diet with antioxidants along anti-malaria drugs during treatment of malaria patients is recommended.

Key words: Oxidative stress, malaria, *Plasmodium falciparum*, *Plasmodium vivax*, sex, age

INTRODUCTION

Earlier research workers reported about increased lipid peroxidation in malaria patients, particularly in *P. falciparum* infection (Upadhyay *et al*., 2011; Kulkarni *et al*., 2003). Instantaneous reduction in antioxidant potency in tandem with increased lipid peroxidation is also observed to be equally accountable for development of oxidative stress in malaria patients (Das and Nanda, 1999; Upadhyay *et al*., 2011; Egwunyenga *et al*., 2004). Any infection, including malaria, activates the immune system of body thereby causing release of reactive oxygen species

as an antimicrobial action (Kulkarni *et al*., 2003). In addition to host's immune system, malaria parasite also stimulates certain cells in production of reactive oxygen species thereby resulting in hemoglobin degradation (Loria *et al*., 1999; Pradines *et al*., 2005). One of the major reasons for development of malarial anemia seems to be oxidative stress (Das and Nanda, 1999; Kremsner *et al*., 2000) while changes in micronutrient metabolism alter disease progression and severity (Singotamu *et al*., 2006). Malaria is a parasitic disease caused by a unicellular protozoan plasmodium (Krotoski *et al*., 1982), leading to damage of red blood cells and vital organs of the body. It is the World most important parasitic infection (Meis *et al*., 1983) caused by *Plasmodium* species and is one of the oldest and greatest health challenges affecting 40% of the world's population (Greenwood and Mutabingwa, 2002). It affects 300-500 million people and kills 1.5-2.7 million people annually (Phillips, 2001). Four species of malaria parasites are known to infect humans and all are spread by the female Anopheles gambiae mosquitoes. The most serious forms of the disease are caused by *Plasmodium falciparum* and *Plasmodium vivax* but the other related species can also affect human (Mendis *et al*., 2001; WHO, 2005). From the epidemiological point of view, these are most important of the four parasite species pathogenic to human. It was therefore the aim of this study to evaluate the status of lipid peroxidation (Malondialdehyde) in Nigerian affected by malaria caused by *Plasmodium falciparum* and *Plasmodium vivax* species.

MATERIALS AND METHODS

Subjects: The test population consisted of one hundred and fifty three untreated confirmed malaria patients between the ages of 18 and 45 years of both sexes. However, only one hundred of them were suitable and selected for the study. The hundred selected were 50 each with *Plasmodium falciparum* and *Plasmodium vivax* malaria. They were patients attending the outpatient department of the University of Benin Health Services (UBHS), Benin City, Edo state, Nigeria between January and December 2010. The study was conducted in compliance with the Declaration on the Right of the Patient (WMA, 2000) after approval by the Ethical Committee of University of Benin Health Services (UBHS), Benin City, Edo State, Nigeria. Before enrolment for the study, the patients were informed on the significant of the study and their consents were obtained.

The control group is comprised of fifty apparently healthy Malaria negative subjects. There were twenty five (25) each of male and female and young adults (age 18-30) and old adults (age 31-45). Also, the test groups were distributed as in the age of the control.

Inclusion criteria include; Nigerian origin, It was also confirmed that the patients were not taking any anti-malarial drugs as at the time they visited the hospital.

Exclusion criteria include; pregnant women, diabetes mellitus, hypertension, obesity, smoking, alcoholism, people living with HIV, patients taking anti-malaria drugs and vitamin supplements, patients who have treated malaria in the past 2 months.

Malaria diagnosis: Malaria was diagnosed with the blood smear staining method of Warhurst and Williams (1996). The stages of malaria were confirmed by microscopic examination. Parasitaemia was determined in peripheral blood smears stained by Giemsa stain. The parasitaemia was graded as: + = low (1-999 μL^{-1}), ++ = moderate (1000-9999 μL^{-1}) and +++ = severe (>10,000 μL^{-1}).

Blood samples collection and analysis for MDA estimation: Five milliliter of venous blood sample was collected from the antecubital vein under aseptic precaution from each subject into

EDTA anticoagulant bottles. The blood was then centrifuged at 2500 rpm for 5 min and the plasma removed and stored at 4°C pending assay of product of lipid peroxidation-Malondialdehyde (MDA) estimation:

- MDA, a Thiobarbituric Acid Reactive Substance (TBARS) was estimated by the method described by Buege and Aust (1978)

Statistical analysis: The values are expressed as mean±SD. The statistical analysis of data was performed using the one way ANOVA of SPSS version 17 and value $p<0.05$ were considered significant as compared to control, values in each row and column.

RESULTS

Statistical significant increase ($p<0.05$) in plasma total lipid peroxides was noted in malaria positive patients (7.67±0.42 μM L^{-1}) as compared to controls; malaria negative patients (4.43±0.32 μM L^{-1}). The patients with *P. vivax* (7.94±0.27 μM L^{-1}) infection presented highest levels of Malondialdehyde compared to *P. falciparum* malaria patients (7.41±0.38 μM L^{-1}) (Table 1).

Malondialdehyde level in *P. falciparum* malaria patients was significant higher ($p<0.05$) in male (7.50±0.37 μM L^{-1}) compare to female (7.24±0.34 μM L^{-1}) while in *P. vivax* malaria patients there was no significant different between sexes (7.98±0.27 μM L^{-1} for male and 7.89±0.28 μM L^{-1} for female). Although not significantly different from that of the old, the young presented higher Malondialdehyde level in both *P. falciparum* and *P. vivax* malaria patients (Table 2).

On the degree of parasitemia in relation to malondialdehyde level, it was observed that malondialdehyde level increases as the degree of parasitaemia increases from mild (+) to moderate (++) and to severe (+++) parasitaemia. Furthermore, *P. vivax* malaria patients presented significant higher ($p<0.05$) malondialdehyde level compared to corresponding degree of parasitemia in *P. falciparum* malaria patients (Table 3).

Table 1: Malondialdehyde level in *P. falciparum* and *P. vivax* malaria compared to control

Parameter	Control (n = 50)	Malaria positive (n = 100)	*P. falciparum* (n = 50)	*P. vivax* (n = 50)
MDA (μM L^{-1})	4.43±0.32^a	7.67±0.42^b	7.41±0.38^c	7.94±0.27^d

Values are Mean±Standard Deviation. MDA: Malondialdehyde; Values in a row having different super scripts are significantly different ($p<0.05$)

Table 2: Malondialdehyde level in *P. falciparum* and *P. vivax* malaria in relation to sex and age

	Control		*P. falciparum*		*P. vivax*	
Characteristic	N	MDA	N	MDA	N	MDA
Sex						
Male	25	4.45±0.30^a	17	7.50±0.37^b	22	7.98±0.27^c
Female	25	4.41±0.35^a	33	7.24±0.34^d	28	7.89±0.28^c
Age						
≤30	25	4.33±0.35^a	31	7.45±0.41^b	35	7.98±0.30^c
≥31	25	4.53±0.26^a	19	7.33±0.32^b	15	7.83±0.14^c

Values are Mean±Standard Deviation. MDA: Malondialdehyde; N: Frequency; Values in a row and in each column having different super scripts are significantly different ($p<0.05$)

Table 3: Malondialdehyde level in *P. falciparum* and *P. vivax* malaria in relation to the degree of parasitaemia

		Degree of parasitaemia		
Type of malaria infection	Control	+ (n = 11)	++ (n = 13)	+++ (n = 26)
P. falciparum	4.43±0.32[a]	7.25±0.25[b]	7.37±0.44[bc]	7.49±0.38[c]
		Degree of parasitaemia		
Type of malaria infection	Control	+ (n = 9)	++ (n = 30)	+++ (n = 11)
P. vivax	4.43±0.32[a]	7.79±0.14[d]	7.95±0.28[d]	8.01±0.29[d]

Values are Mean±Standard Deviation. N: frequency. Values in a row and in each column having different super scripts are significantly different ($p<0.05$). +: Low parasitaemia, ++: Moderate parasitaemia, +++: Severe parasitaemia

DISCUSSION

The results of this study revealed oxidative stress level assessed by a biomarker of lipid peroxidation (Malondialdehyde) in patient infected with *Plasmodium falciparum* and *Plasmodium vivax* malaria to be significantly higher than the level of malaria negative subjects. Highly increased Malondialdehyde (MDA) activity found in this study in malaria positive patients is an indication of increased production of reactive oxygen species. This finding is in agreement with the findings of several previous studies where those with malaria parasite have been shown to have a higher MDA level (Hunt and Stocker, 1990; Egwunyenga *et al*., 2004; Akanbi *et al*., 2009). Interestingly, report shows malaria parasite itself to generate large quantities of ROS and also through its interaction with phagocytic cell system (Kremsner *et al*., 2000). The oxidant stress originates due to destruction of red cells which cause imbalance between the generation of reactive oxygen species and the antioxidant defense system (Bonnefont-Rousselot *et al*., 2000; Mendis *et al*., 2001). Pathogenesis of an infecting agent cause an over production of free radical species and failure of normal defense mechanism that decreases antioxidant level which leads to decreases elimination of reactive substances (Thurnhann *et al*., 1988). One of the consequences of oxidative stress is the development of malarial anaemia (Kremsner *et al*., 2000; Clark and Hunt, 1983). Plasmodium infected human erythrocytes are under increased oxidative stress exerted by the malaria parasite (Eaton *et al*., 1976; Golenser *et al*., 1991). Malaria parasite is capable of generating Reactive Oxygen Species (ROS) within the erythrocytes and the ROS resulting from immune activation can further damage the uninfected erythrocytes (Rath *et al*., 1991) which agrees with our study in which there is an increased oxidative stress in the studied population.

The present study also shows oxidative stress status to be more serious with *P. vivax* malaria infection than in *P. falciparum* malaria infection. This finding agrees with the finding of Erel *et al*. (1997) who reported that oxidative mechanism was more dominated in patients with *Plasmodium vivax* malaria as compared to that of *Plasmodium falciparum*. In contrary is the study of Rath *et al*. (1991) who reported an increased level of serum lipid peroxidation in *P. falciparum* infected patients as compared to *P. vivax* infection. Of significance also is the fact that malaria produced by *Plasmodium vivax* relapses often occurs months to years after treatment because some of the parasites can become dormant in the liver (Trampuz *et al*., 2003; Mockenhaupt *et al*., 2004). Also, significant decreased level of vitamin C (an antioxidant) has been observed in *Plasmodium vivax* malaria patients than *Plasmodium falciparum* malaria patients (Upadhyay *et al*., 2011; Prasannachandra *et al*., 2006) which support findings of the present study. On sex of the patients, malondialdehyde levels were higher in male than in female in both *P. falciparum* malaria and

P. vivax malaria patients. However, that of the *P. vivax* malaria was not significantly different from *P. falciparum* malaria between sexes. This finding agrees with the finding of Akanbi *et al.* (2010) who concludes that the cause could be due to the fact that males expose their bodies more than females when the weather is hot and thus increases their chances of being bitten by the mosquito. However, there is some information on oxidative stress in men showing a pro-oxidant effect of testosterone (Dincer *et al.*, 2001). In female decreased superoxide anion production by the endothelium in response to estrogen is considered to contribute to vascular protective properties of estrogen (Barbacanne *et al.*, 1999). In addition, the young presented non-significant higher malondialdehyde level than the old in both *P. falciparum* and *P. vivax* malaria patients. Similar result have been reported by Akanbi *et al.* (2010) who shows highest mean parasite density was found among those within the age range 18-21 years while it was least among those within the age range 26-30 years. He then concluded that this could be due to the number of exposure to mosquito bites among people within the age range of 26-30 years as it has been reported that the number of exposure to mosquito bite increases with age (Akanbi *et al.*, 2010). Thus, in agreement to the higher malondialdehyde level among the young than the old reported in this study. The number of exposure to mosquito bites by individuals had been confirmed to increase the level of immunity against malaria infection (Hommel, 1991) and this increases with age. Thus, in *Plasmodium falciparum* endemic areas, protective immunity against malaria infection is acquired slowly after a large number of infections and its maintenance requires a sustained exposure to infected mosquito (Akanbi *et al.*, 2009). The level of immunity against malaria has also been related to age of the individuals living in malaria endemic areas (Akanbi *et al.*, 2006). Some individuals have the benefits of genetically controlled protection mechanisms against malaria, such as blood group determinants, abnormal haemoglobin and red blood cell enzymes deficiency. It has been reported that severe malaria occurs more frequently in individuals with non-O blood group (Fischer and Boone, 1998) even gender can also affect the prevalence of malaria infection (Mande and White, 1984). In support of the present study is that various studies have also established that malaria infection is accompanied by increased production of Reactive Oxygen Species (ROS) which indicates the environment for oxidative stress (Akanbi *et al.*, 2009). Malaria parasite is sensitive to oxidative stress and the level of oxidative stress is influenced by the severity of malaria infection as measured from plasma parameters (Farombi *et al.*, 2003; Sibmooh *et al.*, 2004) which also agrees with present study. Though, oxidative stress destroys malaria parasites but may also render host tissues such as erythrocytes more vulnerable to oxidative damage and thereby resulting to anaemia in malaria infected individuals (Egwunyenga *et al.*, 2004). Previous study have confirmed that MDA levels may reflect the severity of a disease process (Das *et al.*, 1993) as observed in this study. In Malaria caused by *Plasmodium falciparum* and *Plasmodium vivax* the oxidative stress is due to the over load in reactive oxygen species which create an increase in the oxygen free radicals and failure of normal defense mechanism that increases the antioxidant blood serum levels (Hunt and Stocker, 1990).

CONCLUSION

Conclusively, present study showed malaria infection to induced oxidative stress which is more serious in *P. vivax* than in *P. falciparum* malaria patients, in the young than in the old, in males than in females and directly proportional to the degree of parasetaemia. It is our suggestion therefore that supplementation of diet and/or anti-malaria drugs with antioxidants is part of treatment plan for the management of malaria patients. In addition, further research in this respect cannot be overemphasized.

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