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### Review Article

# Oxidative Stress Marker And Antioxidant Status In Falciparum Malaria In Relation To The Intensity Of Parasitaemia

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#### ABSTRACT

**Introduction:** Malaria is a vector-borne infectious disease caused by five protozoan parasite species of the Plasmodium, the most serious forms of the disease are caused by *P. falciparum* and is highly widespread throughout tropical and subtropical regions. Malarial infection causes significant destruction of erythrocytes. The malaria parasite itself generates large quantities of reactive oxygen species and also through its interaction with phagocytes cell system. Falciparum malarial infection causes major imbalance between oxidant and antioxidant defense system. **Objective:** The main aim of this study was to assess the lipid peroxidation and antioxidant status in patients of falciparum malaria with respect to the intensity of parasitaemia. **Methods:** Blood samples were collected in vacutainers with anticoagulant EDTA. Plasma used for estimation of MDA (Malondialdehyde), Vitamin C and Vitamin E whereas the level of parasitaemia in peripheral blood smears stained determined by Giemsa stain. The present study was conducted on 35 patients of Plasmodium falciparum malaria between the age group 4-12 year. Lipid peroxidation and antioxidants status were estimated in both study (35) and control group (40). **Results:** In mild parasitaemia MDA level was significantly increased ( $p < 0.01$ ) whereas Vitamin C and Vitamin E were non-significant as compared to control. The increase in plasma MDA level in moderate and severe parasitaemia was highly significant ( $p < 0.001$ ) and decrease in Vitamin C and Vitamin E level was highly significant ( $p < 0.001$ ) as compared to normal subjects. **Conclusions:** Oxidative stress induced by falciparum malaria leads to increased lipid peroxidation in infected children. Our study suggest that there is an imbalance between plasma oxidant and antioxidant system in falciparum malaria.

**Keywords:**

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### 1. Introduction

Malaria is among the most serious tropical infectious diseases, with Plasmodium falciparum malaria being the leading cause of death from a single pathogen [1]. According to the World Health Organization (WHO), approximately 3.3 billion people are at risk, resulting in about 250 million cases every year [2].

Plasmodium Falciparum is the most dangerous among other infections in human being the highest rates of complications and mortality have been reported [3]. The considerable mortality and morbidity in falciparum malaria is due to its protean manifestation,

multi-organ involvement and delay in diagnosis and failure of administration of treatment promptly [4, 5]. Then majority of these cases are children where the disease can exist in a severe form. The malaria parasite itself generates large quantities of reactive oxygen species and also through its interaction with phagocytes [6-8]. 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 [9, 10].

The study was designed to assess the level of MDA, which is a measure of lipid peroxidation and vitamin C and vitamin E, the non-enzymatic antioxidant in relation to parasitaemia of falciparum malaria.

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**2. Materials and Methods**

The present study was conducted on 35 patients of Plasmodium falciparum malaria, who reported ill with high grade fever, headache, vomiting, diarrhoea respiratory distress and other clinical signs and symptoms of malaria as previously documented and not taken any malarial drugs, between the age group 4-12 years and 40, age and sex matched controls without any clinical and laboratory signs and symptoms of Plasmodium falciparum malaria. The study was approved by the Institutional Ethical Committee of Rohilkhand Medical College and Hospital, Bareilly, U.P, India. Microbiological study was done at the department of Microbiology Rohilkhand Medical College and Hospital, Bareilly, U.P, India. Biochemical study was done at the Department of Biochemistry at Index Medical College, Hospital & Research Centre, Indore, M.P., India 4

Blood samples were obtained from patients and controls after taking informed consent of their parents. Blood samples were collected in vacutainers with anticoagulant EDTA. The plasma from EDTA anticoagulated blood was separated, immediately stored at -80°C and used for determination of MDA, Vitamin C and Vitamin E.

1. MDA by the method described by Ohkhwa et al [11].
2. Vitamin C estimated by the method of Roe and Kuether [12].
3. Vitamin E estimated by the method of Barker and Frank [13].

Falciparum parasitaemia was determined in peripheral blood smears stained by Giemsa stain. Level of parasitaemia was graded as

1. Low parasitaemia, with parasite density (1-999/μL) of blood
2. Moderate parasitaemia, with parasite density (1000-9999/μL) of blood.
3. Severe parasitaemia, with parasite density (>10,000/μL) of blood.

All reagents and chemicals used were of analytical grade and were purchased from Merck Chemical Co., Germany.

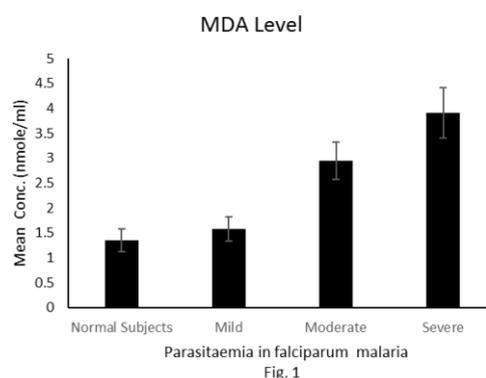
All data were expressed as mean ±SD. Unpaired student's t-test was used for between group comparisons. Differences were considered of statistical significance when the p-value was p<0.01.

**3. Results**

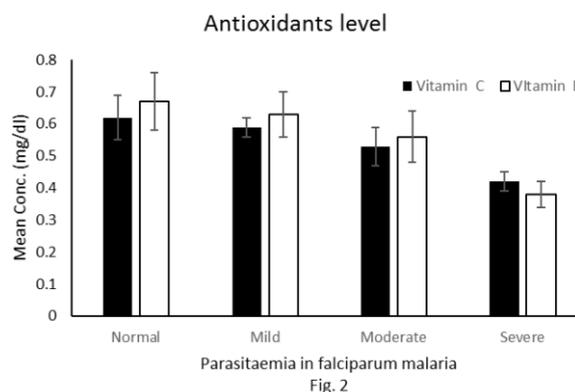
The level of MDA and antioxidant Vitamins according to the level of parasitaemia in patients of falciparum malaria and control subjects are depicted in Table 1. In case of mild parasitaemia MDA level was significantly increased (p<0.01) whereas Vitamin C and Vitamin E were non-significant as compared to control. The increase in plasma MDA level in moderate and severe parasitaemia was highly significant (p<0.001) and decrease in Vitamin C and Vitamin E level was highly significant (p<0.001) as compared to normal subjects.

Fig. 1 represents lipid peroxidation and fig. 2 antioxidant status of patients with falciparum malaria and control subjects. MDA concentration was progressively increased as shown in fig. 1 and antioxidant Vitamin C and Vitamin E were progressively decreased as shown in fig. 2 according to the intensity of parasitaemia

**Fig. 1 represents lipid peroxidation status in falciparum malaria patients and control subjects**



**Fig. 2 represents antioxidant status of patients with falciparum malaria and control subjects.**



**Table 1. Level of MDA and Antioxidant Vitamins with respect to Parasitaemia in Plasmodium falciparum infected patients and controls**

	MDA (nmol/ml)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Control (N=40)	1.35±0.23	0.62±0.07	0.67±0.09
Plasmodium falciparum (N=35)			
Parasitaemia			
Mild (650±85.23/μL) (n=9)	1.58±0.24*	0.59±0.03 <sup>NS</sup>	0.63±0.07 <sup>NS</sup>
Moderate (8750±885.89/μL) (n=11)	2.95±0.38**	0.53±0.06**	0.56±0.08**
Severe (11650±1285.98/μL) (n=15)	3.91±0.51**	0.42±0.03**	0.38±0.04**

Values are expressed as mean±S.D N= total number of subjects n=number of patients in respective groups \*P <0.01 was considered significant and \*\*P < 0.001 highly significant compared to the controls. NS= not significant

#### 4. Discussion

Malaria is caused by the plasmodium species and is a major cause of morbidity and mortality in developing countries [14]. The majority of these cases are children where the disease can exist in a severe form, often with the devastating consequences [15]. The malaria parasite itself generates large quantities of ROS and also through its interaction with phagocytic cell system [16]. The significantly higher MDA level in children with moderate and severe parasitaemia than non-parasitaemic controls suggest that enhanced plasma MDA levels is a marker of severity of malaria. Increase in MDA levels reflects an increase in peroxidation of membrane lipids in malarious patients, this is in accordance with the study of Rath et.al. 1991 [17]. Plasma ascorbate plays a pivotal role in protecting plasma lipids free radical attack. However, it is rapidly oxidized when challenged by oxidants released from activated polymorphonuclear neutrophils [18]. Once ascorbic acid has been used up, there is an initiation of lipid peroxidation [19]. We have observed significantly low levels of antioxidant Vitamin C and Vitamin E in the plasma of *P. falciparum* infected children in both moderate and severe parasitaemia. This agrees with the study of Egwunyenga et.al. 2004 [20]. and Das et.al. 1996 [21]. The decrease in Vitamin C in malarial patients following its use to regenerate Vitamin E from alpha tocopheroxyl radical at water lipid interface. Decreased Vitamin E levels may probably be due to enhanced lipid peroxidation by the *P. falciparum*.

#### 5. Conclusion

Oxidative stress induced by *falciparum* malaria leads to increased lipid peroxidation in infected children. This is confirmed by our study where we found significantly increased MDA, a lipid peroxidation marker with significant reduction in secondary antioxidant defense system. Along with antimalarial drugs some antioxidant supplements should be given to patients as preventive measure for early recovery and to restore secondary antioxidant defense system of the body.

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