

## **Erythrocyte antioxidant enzymes and their correlation with malondialdehyde in malaria.**

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### **Abstract**

**Invasion of human erythrocytes by malaria parasites causes alterations in antioxidant potential of the red cells. The present study was undertaken to investigate the erythrocyte antioxidant enzymes like superoxide dismutase(SOD), catalase(CAT) in malaria patients. Oxidative stress was estimated by measuring malondialdehyde(MDA) which is a marker for lipid peroxidation. 30 malaria patients were enrolled in this study. They were divided into 2 groups of 15 each with Plasmodium vivax malaria and Plasmodium falciparum malaria. Results were compared with 20 healthy control subjects. Significant decrease( $p<0.001$ ) in SOD and CAT and increase in MDA ( $p<0.001$ ) indicates that there is reduction in antioxidant enzymes and increased vulnerability to free radical damage in erythrocytes. This study therefore emphasizes the need for early treatment of malaria patients to reduce the red cell damage.**

**Key words:** Malaria, MDA, Superoxide dismutase, catalase

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### **Introduction**

Plasmodium infected human erythrocytes are under increased oxidative stress exerted by the malarial parasite [1,2]. Malarial 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 [3]. It is also known that erythrocytes are equipped with antioxidant enzymes that could protect them against damage [4]. In the present study attempt has been made to assess the changes in antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in human erythrocytes infected with Plasmodium falciparum and Plasmodium vivax. The correlation between antioxidant enzymes and malondialdehyde (MDA) is also included under this study.

### **Materials and Methods**

The study group consisted of 30 untreated malaria patients between the age group of 18 to 60 years of both sexes. These patients attended "Malaria Clinic" OPD at Wenlock District Hospital, Mangalore, with the symptoms of fever and rigor, headache, vomiting. Signs include splenomegaly, hepatomegaly and anemia. The con-

trol group included 20 healthy individuals of both sexes of the same age group.

A finger prick blood sample was taken to prepare thick and thin blood films to determine the presence or absence of malaria parasites. Patients with malaria were enrolled in the study after informed consent was obtained from the patients. This study was approved by Institutional Ethical Committee of Kasturba Medical College, Mangalore. Of the total 30 malaria patients, 15 patients had Plasmodium vivax and 15 patients had Plasmodium falciparum malaria.

### **Sample Collection**

5ml of venous blood samples were collected randomly in EDTA bottles from malaria patients and normal healthy subjects. Blood samples were centrifuged at 3000g for 10 minutes. Plasma was discarded. The cells were washed three times in cold saline. The RBC's were then suspended in an equal volume of 0.9% saline and used for the estimation of malondialdehyde(MDA), superoxide dismutase(SOD) and catalase(CAT).

Lipid peroxidation( MDA) : The method of Stocks et al was followed [5]. Malondialdehyde a secondary product

of lipid peroxidation, reacts with thiobarbituric acid(TBA) in acidic medium to give a pink coloured pigment. The pink colour is extracted with butanol and the absorbance read at 535 nm. Values were expressed as nanomoles per deciliter.

SOD activity was measured using the method of Beauchamp and Fridovich [6]. The principle of SOD activity was based on the inhibition of nitroblue tetrazolium(NBT) reduction. The blue coloured formazan was measured at 560nm.

CAT activity was estimated by the method of Brunnan et al [7]. The assay is based on the disappearance of H<sub>2</sub>O<sub>2</sub> in the presence of enzyme.

Values were expressed as Units/gm Hb. The hemoglobin content of the erythrocytes was determined by the cyanmethemoglobin method [8].

**Results**

Lipid peroxidation was highly increased in both P.vivax and P. falciparum malaria patients(p<0.001) when compared to control subjects. Increase in MDA in P. falciparum malaria patients was much more when compared to P.vivax malaria patients.

SOD activity was significantly decreased in both P. vivax and P.falciparum patients .Maximum decline in SOD activity was found in P.falciparum patients.(p<0.001).

The catalase activity was also significantly decreased in P.vivax and P.falciparum patients when compared to normal subjects(p<0.001). Maximum decline in CAT activity was seen in P.vivax patients (Table 1).

A negative correlation was obtained between MDA and antioxidant enzymes SOD and CAT (Table 2 ).

**Table 1: Erythrocyte MDA, SOD and CAT levels in malaria patients and control subjects.**

Parameters	Control	P. vivax	P. falciparum	H	p-value
MDA nmoles/dl Mean± SD	334.415±100.832	566.420±153.091	645.352±234.238	19.99	0.001**
SOD U/gm Hb Mean± SD	7197.942±1063.374	5215.039±249.783	4461.130±649.203	34.46	0.001**
CAT U/gm Hb Mean±SD	5439.750±3445.730	657.160±272.833	1692.966±1737.208	35.79	0.001**

H=Kruskal- Wallis test

SD = Standard Deviation

p-value = Probability of chance being the cause for the differences in the mean of the two groups.

\*\* = very highly significant

**Table 2: Correlations**

	SOD	CAT
MDA r	- 0.570	-0.393

r = correlation coefficient

**Statistical Analysis**

Statistical analysis was done by using Kruskal-Wallis test. The p-value <0.05 was taken as significant. Correlations between the variables were estimated by Pearson's correlation coefficients.

**Discussion**

Highly increased MDA found in our study in malaria patients indicates that there is increased production of reactive oxygen species(ROS) in these patients. The malaria parasite itself generates large quantities of ROS and also through its interaction with phagocytic cell system[9]. Some of these radicals attack the plasma membranes and hemoglobin. Erythrocytes are rich in polyunsaturated fatty acids which makes them very vulnerable towards oxidative stress. The ROS generated in host-parasite interactions can cause several biochemical changes like lysis of erythrocytes and alteration in major antioxidants of erythrocytes[10,11,12 ]. In line with these authors we have found in our study that there is highly significant

increase in lipid peroxides in malaria patients and the increase in MDA is much more in *P. falciparum* patients compared to

*P. vivax* patients which indicates the severity of *falciparum* malaria.

During their intra erythrocytic development the parasites are able to increase in some cases and decrease in others the levels of some antioxidant defenses of the host erythrocytes. Pabon A. et al have reported increase in SOD and glutathione peroxidase activity in patients with non-complicated malaria [13]. Higher concentration of antioxidants may be involved in clearance of malaria parasite. Amy M et al have reported increase in  $\alpha$ -carotene in acute malaria in children [4,10]. In contrast, we have found in our study highly significant decrease in erythrocyte antioxidants SOD and CAT activities in both *P. falciparum* and *P. vivax* patients. Since the malaria parasite itself utilizes erythrocyte proteins for its metabolic requirements, the concentration of enzymic antioxidants are decreased with parasite maturation.

[12,14]. There is a decreased utilization of the reduction potential in detoxification of reactive oxygen species. Oxidative stress is therefore aggravated by reduced effectiveness of the antioxidant defence system. The negative correlation between MDA and SOD and CAT further support this finding.

The increased vulnerability of erythrocytes to damage and decreased antioxidant system emphasizes the need for early treatment of *P. falciparum* and *P. vivax* malaria patients to minimize the red cell destruction and resulting anemia.

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