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RESEARCH ARTICLE

BIOCHEMISTRY

COMPARATIVE STUDY OF FREE RADICAL ACTIVITY IN PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX MALARIA PATIENTS

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ABSTRACT

The present study was undertaken to study the free radical activity in malaria patients suffering from plasmodium falciparum (n=14) and plasmodium vivax (n=21) against the healthy control subjects (n=25). Lipid peroxidation was assessed by measuring serum lipid peroxide in both groups of malaria patients by using Malondialdehyde as standard. The serum Ceruloplasmin is also studied along with serum Lipid peroxidation. The level of serum Lipid peroxidation was significantly high ($p<0.001$) in patients with Plasmodium falciparum malaria as compared to Plasmodium vivax infected patients and healthy controls. Increase in serum Lipid peroxidation may be due to over activity of free radical, which corresponds with severity of tissue damage while increase in serum Ceruloplasmin could be due to acute phase response in malaria.

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KEYWORDS

Malaria, Oxidative stress, Lipid Peroxidation, Ceruloplasmin, Erythrocyte

INTRODUCTION

Malaria is a major health problem in developing countries accounting for 2-3 million deaths per year. Malaria a tropical protozoan disease transmitted through anopheline mosquitoes. It is mainly caused by various species of plasmodium parasite[1].

The disease in human is attributable to the direct effect on red cell invasion and destruction. In response to malaria infection, phagocytic cell such as; polymorphonuclear leucocytes and macrophages releases highly reactive oxygen radical during the onset of respiratory burst. The reactive oxygen radical has been shown to kill intra-erythrocytic parasite by eliciting oxidative stress[2].

Superoxide radical is generated during acute phase of malaria. This radical particularly attacks on polyunsaturated fatty acid causing damage to erythrocyte cell membrane. Peripheral blood phagocytes are stimulated by plasmodium components in vitro to generate Reactive Oxygen Species (ROS). The circulating plasma lipids are therefore exposed to the oxidant stress and become vulnerable to ensuing lipid peroxidation. The role of antioxidants and oxidative stress in the pathogenesis of malaria in humans is unclear[3]. Therefore, the present study was undertaken to determine the extent of lipid peroxidation and role of Ceruloplasmin in plasmodium falciparum and plasmodium vivax infected malaria patients as acute phase protein.

MATERIAL AND METHODS

The present study was undertaken in following groups;

Group-A: 21 individuals infected with Plasmodium Vivax

Group-B: 14 subjects infected with Plasmodium Falciparum

Group-C: 25 normal controls.

All patients and controls were in the age group 15-60 years. The subjects infected with malaria parasite were diagnosed by physician on the basis of clinical findings and microscopic examination of thick and thin blood smear films. Patients having evidence of ischemic heart disease, rheumatoid arthritis or any malignancy, which could cause an increase in ROS, were excluded from study group.

All the healthy normal controls were symptomless. They maintained normal Hemoglobin level and show no abnormality in clinical examination particularly in the context of the metabolic and nutritional disorders.

Random blood samples of patients with different types of malarial parasite were collected in plain bulb. After an hour, clear serum was separated by centrifuging at 3000 rpm for 5 minute and collected in polythene tube with cork. Precautions were taken to avoid any hemolysis.

Estimation of serum samples are analyzed for lipid peroxides: By method of Kei Satoh[4] and serum Ceruloplasmin: By Schosinsky Karl H et al method[5].

The results were expressed as mean ± SD and analyzed by student t-test.

RESULTS

Study includes 60 subjects out of which 25 controls and 35 patients having malaria.

Table No. 1 show mean ± SD levels of biochemical indices for free radical metabolism.

Mean values of serum Lipid peroxidation was significantly higher ($p<0.001$) in the plasmodium falciparum infected patients as compared to plasmodium vivax infected patients and the healthy controls. Though Ceruloplasmin level in serum was marginally higher in malaria patients when compared with the normal controls, they remain non significant.

Table No – 1
Biochemical indices of free radical activity

Groups	Number of patients (n)	LPO (nmol/ml)	CPL (units /L)
		Mean ± SD	Mean ± SD
Group A: Plasmodium Vivax	21	5.3 ± 0.1*	111.7 ± 29.54 NS
Group B: Plasmodium Falciparum	14	6.7 ± 0.01*	109.33 ± 17.99 NS
Group C: Normal controls	25	3.47 ± 0.7	94.73 ± 33.73

**P< 0.001 highly significant compared to the controls. NS= Not Significant.*

DISCUSSION

Malaria is one of the most important parasitic diseases in the world. Prevalence of malaria is found not only in tropical countries like India but more than half of the world population is under the shadow of this disease[1]. Malaria is caused by protozoan parasite of the genus plasmodium and transmitted by the infected mosquitoes. Parasites are capable of generating ROS within the erythrocytes and further damage to the uninfected erythrocytes is the effect of immune reaction by host. Erythrocytes are equipped with antioxidant enzymes that could protect them against damage[6].

In our study, we found increased level of LPO in malaria patients which indicates that there is increased production of ROS in these patients. The malaria parasite itself generates large quantities of ROS and also through its interaction with phagocytic cell system[7]. Erythrocytes are rich in polyunsaturated fatty acids which makes them very vulnerable towards oxidative stress. The ROS generated in host-parasite interaction can cause several biochemical changes like lysis of erythrocytes and alteration in major antioxidants of erythrocytes[8,9,10].

Ceruloplasmin is a copper containing antioxidant which inhibits iron and copper dependent lipid peroxidation. We found increased Ceruloplasmin activity in malaria patients as compared to controls but it remained non significant. Das BS, Thurnham DJ 1993 and Das BS, Patnaik JK 1997 also found an increase in serum Ceruloplasmin level in both symptomatic and asymptomatic malaria[11,12]. Excessive free iron generated during hemolysis and copper participate in Fenton reaction, leads to generation of ROS, which may be the added factor inducing LPO.

The increase in activity of Ceruloplasmin could be one of the protective mechanisms to trap ROS[13] against lipid peroxidation and could serve as the marker for acute phase response in malaria.

Our results correlate well with the existing scientific knowledge of increase lipid peroxidation and oxidative damage to erythrocyte membrane in malaria. Mechanism of such damage required to be explored. Role of increase level of Ceruloplasmin in protecting erythrocyte membrane require to be studied further. Also it will be interesting to study the effect of oxidative stress on glycoprotein of cell membrane as result of malarial parasite.

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