COMPARISON IN SERUM PROFILE OF PEROXIDANTS (MDA) AND NON ENZYMATIC ANTI OXIDANTS (VITAMINS E AND C) AMONG PATIENTS SUFFERING FROM PLASMODIUM FALCIPARUM AND VIVAX MALARIA

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ABSTRACT

Objective: *To compare the serum profile of malondialdehyde (MDA) lipid peroxidation and major antioxidant (Vitamins E and C) in patients with Plasmodium falciparum and vivax malaria.*

Methodology: *This comparative study was conducted at Department of Biochemistry, Sardar Patel Medical College, Bikaner, India. A total of 150 patients were studied. This included 100 patients with confirmed malaria (50 with Falciparum and 50 with vivax) and 50 healthy age matched subjects as control. Serum malondialdehyde (MDA) was measured to assess the degree of lipid peroxidation. Antioxidant status was measured by estimating the levels of Vitamins E and C.*

Result: *This study suggests that serum malondialdehyde levels were significantly increased in malaria patients. The patients with Plasmodium falciparum infection showed significantly increased levels of lipid peroxides(Mean ±S D 3.22+1.09 ; p< 0.001)when compared to Plasmodium vivax malaria (2.49 + 0.86 ; p< 0.001). The antioxidant Vitamins E (p< 0.001) and C (p< 0.001) were decreased significantly in malaria patients in both the groups. Maximum decline in Vitamin C (0.20 + 0.07 ; p< 0.001) was observed in Plasmodium vivax malaria.*

Conclusion: *Lipid peroxides are significantly raised in malaria patients; more in falciparum than vivax malaria. The antioxidants (Vitamins E and C) are decreased in both types of malaria. The protective role of routine antioxidant therapy in malaria patients needs further investigation.*

Keywords: *Malaria, Lipid peroxidation, Malondialdehyse (MDA), Antioxidant, Vitamins E, Vitamin C.*

INTRODUCTION

Malaria, a disease caused by Plasmodium species, is one of the oldest and greatest health challenges affecting 40% of the World's population. It affects 300-500 million people and kills 1.5-2.7 millions people annually[1].

Bikaner District, basically an arid zone, regarded as a hypoendemic area of malaria, has recently experienced changes in ecosystem due to increased rainfall and canal irrigation[2].

During normal respiration, the human body produces oxygen free radicals which interact with polyunsaturated fatty acid (PUFA) in membranes, resulting in peroxidation and production of reactive aldehydes[3] .

The malaria parasite itself generates large quantities of reactive oxygen species and also through its interaction with phagocytes[4-6]. In malaria infection, activation of phagocytic cell population occur, giving rise to be the production of reactive oxygen species (ROS) hence increased lipid peroxidation level and increased MDA level, which is a biochemical marker for the assessment of lipid peroxidation[7]. The non specific activation of blood monocytes and tissue macrophages also

generates reactive oxygen species. The respiratory burst of stimulated phagocytes forms superoxide anion radical resulting in the most undesirable cycle of imbalance between oxidants and antioxidants[8].

Oxidative stress is also aggravated by decreased antioxidant defense system. Parasite utilizes erythrocyte proteins for its metabolic requirements, the concentration of antioxidants are decreased with parasite maturation[9].The role of antioxidants and oxidative stress in the pathogenesis of malaria in humans is unclear.

The present study was therefore undertaken to determine the extent of lipid peroxidation and to investigate the alterations in major antioxidant Vitamins E and C in plasmodium falciparum malaria and plasmodium vivax malaria.

METHODOLOGY

The present study was conducted on 100 malaria patients between the age group 20-52 years, of both sexes, enrolled in the study after informed consent, in which medication was not started. Subsequently the diagnosis was confirmed by the peripheral blood smear examination. These Patients attended OPD at Prince Bijay Singh Memorial (PBM) Hospital associated with Sardar Patel Medical College, Bikaner, with the symptoms of fever, rigor, headache, vomiting, signs include splenomegaly, hepatomegaly and anemia. The control group included 50 healthy individuals of both sexes between 20 to 55 years. Out of total 100 malaria patients, 50 patients had plasmodium falciparium malaria and 50 patients had plasmodium vivax malaria acted as study group I and II respectively. The patients in both the groups were treated with Chloroquin.

5 ml of venous blood samples were collected at the time of presentation of patients at OPD in EDTA bottles and samples were centrifuged at 3000 rpm for 10 minutes. Plasma was collected taking care to avoid hemolysis. Serum malondialdehyde (MDA) was studied by the method proposed by Buege and Aust[10], vitamin C by Roe and Kuethe's method using 2, 4 dinitrophenyl hydrazine[11] and vitamin E by colorimetric method as described by Barker and Frank using Di pyridyl reagent[12].

Statistical analysis was done by using Mann Whitney 'U' test. Correlations between the variables were estimated by Pearson's Correlation coefficients.

RESULTS

In this study, table 1 shows that the increase in plasma MDA in malaria patients was highly significant ($p<0.001$) when compared to control subjects. This indicates that lipid peroxidation is significantly increased in malaria. The patients with plasmodium falciparum infection showed significantly increased levels of lipid peroxides as compared to plasmodium vivax malaria patients. The plasma Vitamin E concentration decreased significantly ($p<0.001$) in malaria patients compared to normal subjects. A decline in Vitamin E concentration was more in plasmodium falciparum malaria as compared to plasmodium vivax malaria. Plasma Vitamin C concentration decreased in malaria patients.

According to table 2 among the controls a positive correlations were found between serum MDA ($r=0.824$) and antioxidant vitamin E and C. The values were statistically significant as evident by p-value ($p<0.001$). A strong negative correlation was obtained between MDA and vitamin E ($r= - 0.667$).A negative correlation was found between MDA and vitamin C($r= - 0.542$). The values were statistically significant as evident by p-value ($p<0.001$). (shown in table). A negative correlation was obtained between MDA and vitamin C($r= - 0.296$) that was statistically significant as evident by p-value ($p<0.05$).

Table 1: Comparison of Plasma levels of malondialdehyde (MDA) Vitamin E and Vitamin C in malaria patients and Controls (Mean ± S D)

GROUPS	MDA (nmol/ml)	VITAMIN E (mg%)	VITAMIN C (mg%)
Control (n =50)	1.16±0.49	1.09±0.25	1.02±0.31
Plasmodium falciparum (n= 50)	3.22±1.09**	0.269±0.10**	0.269±0.08**
Plasmodium vivax (n =50)	2.49±0.86**	0.33±0.13**	0.20±0.07**

Table 2: Correlation between Malondialdehyde (MDA), Vitamin E and Vitamin C

		MDA	VITAMIN E
Among control group (n=50)	VITAMIN E	r = 0.824**	–
	VITAMIN C	r = 0.817**	r = 0.852**
Among Plasmodium falciparum malaria subjects (n=50)	VITAMIN E	r = - 0.667**	–
	VITAMIN C	r = - 0.542**	r = 0.684**
Among Plasmodium vivax malaria subjects (n=50)	VITAMIN E	r = - 0.156	–
	VITAMIN C	r = - 0.296*	r = 0.234

**p < 0.001, r= correlation

DISCUSSION

The present study shows significant increase in lipid peroxides in malaria patients as compared to control subjects. The mean serum MDA level was found to be increased in the patients of falciparum malaria. The results of present series of study resembled with findings of Das et al, who analyzed significantly higher level of serum MDA in Plasmodium falciparum malaria patients than controls[13].

The patients with Plasmodium falciparum infection showed highest levels of lipid peroxides as compared to Plasmodium vivax malaria patients and control. These findings were in close collaboration with the results of Mohapatra et al who also found higher MDA level (P < .001) in patients of falciparum malaria than the controls and vivax malaria[14].

Invasion of human erythrocytes by malaria parasite brings about metabolic changes in the host cell. The host cells may then become more vulnerable to damage due to toxic metabolites derived from both the host and parasites. Reactive oxygen species generated in host-parasite interactions causes the lysis of erythrocytes and alteration in antioxidants[15].

There are many sources of ROS in malaria such as generation by intra erythrocyte parasite, production by host-phagocytes as a defense mechanism against the parasite. ROS are also generated during the consumption of hemoglobin by the malaria parasite[16].Similar increased MDA levels in our study was due to the fact, that Plasmodium falciparum trophozoite infected human red cells produce more ROS compared to vivax infections and control groups.

The present study showed significant reduction in plasma Vitamins E & C concentration in both malaria patients as compared to controls. Nmorsi et al also observed statistically significant ($p<0.001$) decreased serum vitamin E level in Plasmodium falciparum patients than control[16]. Statistically significant decrease of serum vitamin E ($p<0.001$) level was also found in both Plasmodium falciparum and Plasmodium vivax patients than control[17]. Decreased serum vitamin C level in Plasmodium falciparum malaria patients than control was also reported by Akpotuzur et al[18]. The decrease in antioxidant Vitamins E & C in the patient groups might be due to their transfer to red blood cell membrane to counteract the increase oxidative stress during acute phase of disease by inhibiting membrane lipid peroxidation or due to their increased utilization as plasma antioxidants. The impaired release of antioxidant Vitamin E may also occur during acute phase of disease. Vitamin E accounts for most of the chain breaking antioxidant activity in the erythrocyte membrane. To be functionally active, vitamin E has to be maintained in its native state for which Ascorbate contributes. Vitamin C converts tocopheroxyl radical to its native state. Therefore loss of ascorbate may interfere in Tocopherol regeneration and may lead to impaired membrane function.

Our study also showed significant decline in vitamin E in Plasmodium falciparum malaria patients as compared to Plasmodium vivax malaria patients. Similar decrease in Plasmodium falciparum malaria patients than Plasmodium vivax malaria patients were also observed by Kulkarni et al[17]. It might be possible that Plasmodium falciparum infection caused the greater degree of oxidative stress as compared to that of Plasmodium vivax infection, as such the more amount of vitamin E was consumed to provide protection against the oxidative stress resulted

greater decreased of vitamin E level was recorded in Plasmodium falciparum patients in present series of study. Significant decreased level of vitamin C was observed in Plasmodium vivax malaria patients than Plasmodium falciparum malaria patients in our study which is in agreement with the observations made by Prasanna Chandra et al.[19]

Erel et al reported that oxidative mechanism was more dominated in patients with Plasmodium vivax malaria as compared to that of Plasmodium falciparum and vitamin C also involved to spare vitamin E in Plasmodium vivax malaria so more ascorbic acid might have been consumed to minimize the oxidative stress and to protect cellular components from free radical damage and to spare vitamin E in Plasmodium vivax malaria. Hence decreased level of vitamin C was observed in Plasmodium vivax malaria as composed to that of plasmodium falciparum[20]. In the present study an inverse correlation was observed between serum MDA and vitamin E and C. Besides this the reduced antioxidant vitamin E and C content of plasma also augmented lipid peroxidation. Reduced levels of plasma vitamin E and vitamin C observed in the present study may have contributed to oxidative destruction of erythrocytes.

CONCLUSION

Lipid peroxides are significantly raised in malaria patients; more in falciparum than vivax malaria. The antioxidants (Vitamins E and C) are decreased in both types of malaria. The protective role of routine antioxidant therapy in malaria patients needs further investigation.

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