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Original Research Article

Superoxide Dismutase Activity In Erythrocytes Infected With Plasmodium Falciparum and Plasmodium Vivax, A Comparison

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Abstract: Malaria, one of the most important vector borne disease of tropical and sub tropical part of globe. Among important species *Plasmodium falciparum* and *Plasmodium vivax* are more prevalent. Both the above mentioned species cause oxidative stress in the host. In this study we compared the oxidative stress in patients infected with *P. falciparum* and *P. vivax* both by estimating the activity of superoxide dismutase (SOD) and also compared the SOD activity with healthy controls. SOD activity was estimated by the method given by Joe M. McCord and Irwin Fridovich spectrophotometrically. Statistical analysis was done by using SPSS software version 22. The SOD activity in *P. falciparum* infected group was 1.08 ± 0.49 nmol/mL and that in the *P. vivax* infected group was 1.58±0.78 nmol/mL and in controls was 3.53 ± 0.06 nmol/mL. The SOD activity in the both the group of cases was significantly decreased ($P < 0.05$) as compared to the controls. The Pearson's coefficient of correlation between SOD activity and parasitemia of falciparum group was found to be -0.93 and that in vivax group was -0.98 showing strong negative relationship.
Keywords: oxidative stress, malaria, falciparum, vivax, superoxide dismutase (SOD).

INTRODUCTION

Various species of Plasmodium can cause malaria. It is one of the important causes of mortality and morbidity in tropical countries. Among various species *Plasmodium vivax* and *Plasmodium falciparum* are important species causing malaria in Indian subcontinent. *P. vivax* usually causes uncomplicated malaria, while *Plasmodium falciparum* causes complicated malaria if not diagnosed and treated timely. The burden of malaria in Asia is under appreciated, despite recent evidence suggesting that the continent contributes almost 40% of the World's malaria (Snow RW *et al.,* 2005). In sub-Saharan Africa the overwhelming majority of malaria-associated morbidity and mortality occurs with *P. falciparum* infections.

According to the World Malaria Report 2017, in the year 2016, more than half of the population (698 million) was at risk of malaria and India accounted for 6% of all malaria cases in the World, 6% of the deaths, and 51% of the global *P. vivax* cases (WHO reports, 2017). The Report estimates the total cases in India at 1.31 million (0.94-1.83 million) and deaths at 23990. With increasing global warming, it is projected that in 2050, malaria is likely to persist in Orissa, West Bengal and southern parts of Assam, bordering north of West Bengal, but may shift from the central Indian region to the south western coastal states of Maharashtra, Karnataka and Kerala. Also the northern states, including Himachal Pradesh and Arunachal Pradesh, Nagaland, Manipur and Mizoram in the northeast may become malaria prone (Bhattacharya S *et al.,* 2006).

Whatever be the cause of malaria the cells affected are continuously under oxidative stress. Which result in the production of reactive oxygen species (ROS) by the host immune system (Becker K *et al.,* 2004; Raza *et al.,* 2010; Henriques JRR *et al.,* 2012). Therefore, this study was designed to compare the oxidative stress in *P. falciparum* and *P. vivax* infected cells in the form of superoxide dismutase activity (SOD).

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MATERIALS AND METHODS

Study population

The study was conducted in confirmed patients of *P. falciparum* and *P. vivax* who attended out-patient clinics and or admitted in the wards of Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, India. The study population was comprised of 200 diagnosed cases each of *P. falciparum* and *P. vivax* with age range of 18 to 24 years old. Fifty population-based age and sex matched healthy volunteers were also included as controls. The healthy controls were free from any signs and symptoms of infection which were evidenced by the thorough physical examination and routine blood investigations. The study was approved by the Institutional Ethical Committee of Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, India.

Specimens

Venous blood was collected aseptically from the patients and controls in heparinised vials obtained from Becton Dickinson and kept in a dark environment for less than 6 h before centrifugation. RBCs were obtained after centrifugation of the specimen. The superoxide dismutase activity was estimated according to the method of McCord and Fridovich in 1969 (McCord JM *et al.*, 1969). The diagnosis of malaria was made by screening of thick and thin Giemsa-stained peripheral blood smears for the presence of Plasmodium species, Quantitative buffy coat (QBC) examination and rapid antigen detection test (RDT). The RDT cassette was obtained from SD Bioline. The parasite density (parasites/µL) was calculated by counting 200 white blood cells and the number expressed on the basis of 8 000 WBC /µL (Akanbi OM *et al.*, 2010).

Calculation of Parasitemia:

$$\text{Parasitemia per } \mu L = \frac{\text{No. of parasites seen}}{\text{No. of leucocytes seen}} \times 8000$$

Statistical analysis: Statistical analysis was done using SPSS, version 22, Statistics software. Unpaired Student's t-test was applied for the comparison of SOD activity of groups infected with vivax and falciparum and controls. Descriptive statistics including mean and SDs were calculated for each continuous variable. Pearson correlation analyses were performed to determine the degree and direction of association between two variables (parasitemia and SOD activity). $P < 0.05$ was considered as significant.

RESULTS

Compared to healthy controls the diseased cohorts showed significantly ($p<0.001$) reduced activity of SOD. The mean SOD activity of controls were 3.53 ± 0.06 nmols/mL and that of cohort 1(Falciparum malaria group) was 1.08 ± 0.48 nmols/mL and that of cohort 2 (vivax malaria group) was 1.58±0.78 nmols/mL. Pearson's coefficient of correlation between parasitemia and SOD activity of cohort 1 was – 0.93 and Pearson's coefficient of correlation between parasitemia and SOD activity of cohort 2 was -0.98. showing strong negative relationship.

Table No1. SOD activity in *P. falciparum* cases

Parasitemia per µL	SOD activity (mean ± SD) (nmol/mL)	No.of subjects
600-800	2.04 ± 0.17	18
801-1000	1.52 ± 0.29	27
1001-1200	1.33 ± 0.21	30
1201-1400	0.90 ± 0.20	28
1401-1600	0.92 ± 0.19	26
1601-1800	0.79 ± 0.15	29
1801-2000	0.55 ± 0.74	20
2001-2200	0.39 ± 0.09	22
	Mean 1.08 ± 0.49	N=200

Table no.2; SOD activity in cases of *P. vivax*

Parasitemia perµL	SOD activity (mean ±SD) (nmol/mL)	No.of subjects
600-800	2.78 ± 0.20	28
801-1000	2.36 ± 0.31	26
1001-1200	1.91 ± 0.15	22
1201-1400	1.69 ± 0.12	24
1401-1600	0.99 ± 0.16	21
1601-1800	0.93 ± 0.23	26
1801-2000	0.91 ± 0.65	25
2001-2200	0.91 ± 0.13	28
	Mean1.58 ± 0.78	N=200

SOD activity of 50 healthy controls was 3.53 ± 0.06.
$P < 0.05$

DISCUSSION

The strategies adopted by the plasmodium to live in the erythrocytes include access to host nutrients and avoidance of host immune system (Akanbi OM *et al.*, 2009), transport of macromolecules and ions across the RBC into the parasites, digestion of hemoglobin and haem detoxification. Also SODs are the important molecules of parasite to fight against the superoxide free radicals produced during the metabolism of haemoglobin. Superoxide dismutases (SODs) catalyze the dismutation of the superoxide free radical to hydrogen peroxide and oxygen. Parasite is prone to oxidative damage in the intraerythrocytic stage of their life cycle because haemoglobin degradation causes oxidation of iron from Fe2+ (ferric) to Fe3+ (ferrous) state which in turn produces reactive oxygen species (ROS) including superoxide free radical. And discussed earlier this superoxide free radical is detoxified by SOD. The SOD of parasite is used up and the SOD activity of parasite is decreased. Therefore, this study

was designed to estimate the overall SOD activity of the RBCs infected with *P. falciparum* and *P. vivax*, and compared findings with healthy controls. Studies by various researchers have also shown that the overall activity of SOD is decreased due to infection of different species of *Plasmodium* (Ifoue SHT *et al.*, 2009; Rodrigues JR *et al.*, 2009; Andrade BB *et al.*, 2010). Since we have observed significant decrease in the activity of SOD in *P. falciparum* infection and *P. vivax* infection, which could explain the oxidative stress disturbance in the erythrocyte antioxidant system encountered in malaria.

The drugs currently in use are based on the susceptibly of the malarial parasite to free radicals and oxidants. Therefore, malarial parasites are known to be vulnerable to pharmacological agents generating ROS such as primaquine (Wongtrakul J *et al.*, 2010), artimisinin (Grahame-Smith *et al.*, 2004), pyrimethamine (Legorreta-Herrera *et al.*, 2010). These agents appear to work on the principle that oxidative damage affects the parasite more than the host (Zhang S *et al.*, 2010).

To minimize the effect of ROS on host cells due to malarial parasite or antimalarials used in the treatment of malaria clinician should prescribe some antioxidant substance such as Vitamin C, Vitamin A. This may improve the probable outcome of the disease.

Because it is evident that serum levels of above mentioned vitamins are also found to be decreased in the malaria cases (Raza A *et al.*, 2009; Raza A *et al.*, 2010). Also George et al 2012 showed the improved activity of SOD, catalase, glutathione peroxidise after the administration of aqueous extract of *Aframonum sceptrum*, an antioxidant to the infected mice with *Plasmodium berghi* (George *et al.*, 2012). Therefore, the use of antioxidant supplements may constitute a far more effective regimen for the treatment of malaria that causes less damage to the host. However, further research is needed to strengthen these suggestions.

CONCLUSIONS

This study showed that more pronounced SOD activity in falciparum infected erythrocytes than vivax infected erythrocytes, so administration of some antioxidant substance may modify the pathogenesis and outcome of malaria.

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