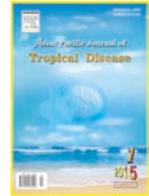




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Superoxide dismutase activity in patients of cerebral malaria

Adil Raza^{1*}, Sumit Kumar Varshney², Haris Manzoor Khan¹, Mohammad Ashraf Malik³, Abbas Ali Mehdi⁴, Indu Shukla⁵¹Section of Parasitology, Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, UP, India, 202002.²Section of Mycobacteriology, Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, UP, India, 202002.³Department of Paediatrics, Jawaharlal Nehru Medical College, AMU, Aligarh, UP, India, 202002.⁴Department of Biochemistry, CSMMU, Lucknow, UP, India.⁵Section of bacteriology, Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, India, 202002.

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ABSTRACT

Objective: To estimate superoxide dismutase (SOD) activity in erythrocytes infected with *Plasmodium falciparum* in the cases of cerebral malaria.**Methods:** The diagnosis of cerebral malaria was made clinically and by Giemsa stained peripheral blood smear examination, quantitative buffy coat (QBC) examination and rapid antigen detection test (RDT). Parasitemia per micro litre of blood was evaluated by counting 200 white blood corpuscles and used to calculate parasite density considering 8000 white blood corpuscles per micro litre. SOD activity was estimated by the method given by Joe M. McCord and Irwin Fridovich spectrophotometrically. Statistical analysis was performed by using SPSS software version 17.**Results:** The SOD activity in the cases was found to be (1.06 ± 0.50) nmol/mL and that in the controls was (3.55 ± 0.07) nmol/mL. The SOD activity in the cases was significantly decreased ($P < 0.05$) as compared to the controls. The Pearson's coefficient of correlation between SOD activity and parasitemia was found to be -0.93 showing strong negative relationship.**Conclusions:** There is severe oxidative stress in falciparum malaria due to reactive oxygen species and supplementation of antioxidants may modify the course and outcome of the disease.

1. Introduction

Malaria is one of the most important vector borne disease, and it accounts for nearly 500 million infections worldwide and more than 1 million deaths per year, mostly in Sub-Saharan Africa. According to the latest estimates, released in December 2013, there were about 207 million cases of malaria in 2012 and estimated 627 000 deaths. Malaria mortality rates have fallen by 42% globally since 2000, and by 49% in the World Health Organization African Region[1].

In India, there were 1.49 million cases and 767 casualties due to malaria in 2010[2]. Malaria is associated with seasonally warm semi-

arid areas. Most cases of malaria in India occur in Orissa. Orissa has a population of 36.7 million (3.5% of India), and surprisingly it contributes to 25% of a total of 1.5-2.0 million reported malaria cases annually, 39.5% of *Plasmodium falciparum* (*P. falciparum*) malaria, and 30% of deaths caused by malaria in India. Uttar Pradesh (UP), India's largest state, contributes to only 5% of total cases[3].

The unicellular protozoan *P. falciparum* causes the most malignant form of the disease including cerebral malaria. Cerebral malaria is one of the complications of the malaria caused by *P. falciparum* with clinical signs and symptoms of high grade fever, drowsiness, unarousable coma, seizures and sometimes psychotic behaviour[4]. *Plasmodium* is continuously exposed to reactive oxygen species (ROS)[5]. This is due to their lifestyle in different environment of intra- and extracellular, the high metabolic rate of the rapidly multiplying parasite, the intraparasitic haemoglobin digestion, and the ROS produced by the host's immune system[6]. Therefore, falciparum infected human RBCs are under constant oxidative stress, because *P. falciparum* generates reactive oxygen species

*Corresponding author: Dr. Adil Raza, Assistant professor, Section of Parasitology, Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, UP, India, 202002.

Tel: +919760596119

E-mail: dradil786_786@yahoo.co.in

(ROS) within erythrocytes infected and from immune activation[7,8]. Thus, this study was designed to estimate SOD activity of RBCs in the confirmed cases of cerebral malaria.

2. Materials and methods

2.1. Study population

The study was conducted in confirmed patients of *P. falciparum* infection who attended out-patient clinics or those admitted in the wards of Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, India. The study population was comprised of 200 patients 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 investigations of controls. The study was approved by the Institutional Ethical Committee of Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, India.

2.2. 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[9]. In addition to clinical findings (high grade fever, drowsiness, unarousable coma, seizures and sometimes psychotic behaviour[4]), the diagnosis of cerebral 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. Other causes of unarousable coma were ruled out on the basis of detailed history (*viz.* alcohol intake, hypertension, head trauma, history of convulsions) and investigations (severe anemia, hypoxic encephalopathy as in pregnancy, diabetic ketoacidosis, hyperthyroidism, electrolyte imbalance). In addition to above mentioned aetiology bacterial causes were ruled out on the basis of negative blood and CSF culture, and also untreated severe anemia, mixed infection with *Plasmodium vivax* and coinfection with any bacterial agent (positive blood culture) were excluded from the study. The parasite density (parasites/ μ L) was calculated by counting 200 white blood cells and the number expressed on the basis of 8000 WBC / μ L[10].

Calculation of parasitemia:

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

Statistical analysis: Statistical analysis was done using SPSS, version 17, Statistics software. Unpaired Student's *t*-test was applied for the comparison of SOD activity of cases 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.

3. Results

As observed in the Table 1, mean \pm SD of SOD activity in the cases was (1.06 ± 0.51) nmol/mL ($N = 200$). The mean \pm SD of the fifty healthy controls was (3.55 ± 0.07) nmol/mL, which was significantly higher ($P < 0.05$) than the mean of cases [(1.06 ± 0.51)

nmol/mL]. Due to attrition no follow up study could be pursued. From Table 1, it was obvious that SOD level showed downward trend as the parasitemia increases. The coefficient of correlation between parasitemia and SOD activity was found to be -0.93 showing strong negative relationship.

Table 1

SOD activity versus parasitemia.

Parasitemia per μ L	SOD activity (mean \pm SD) (nmol/mL)	No. of subjects
600-800	2.05 \pm 0.17	18
801-1000	1.52 \pm 0.29	27
1001-1200	1.33 \pm 0.20	30
1201-1400	0.90 \pm 0.20	28
1401-1600	0.92 \pm 0.19	26
1601-1800	0.79 \pm 0.15	29
1801-2000	0.55 \pm 0.73	20
2001-2200	0.38 \pm 0.09	22
	1.06 \pm 0.51	$N = 200$

*SOD activity of 50 healthy controls was 3.55 ± 0.07 . $P < 0.05$

4. Discussion

The strategies adopted by the malarial parasite to survive inside the RBCs include access to host nutrients and avoidance of host immune system[11], transport of macromolecules and ions across the RBC into the parasites, haemoglobin digestion and haem detoxification, novel metabolic pathways, immune evasion strategies and multiple drug resistance. Also SODs are the important ammunition in the armoury of malarial parasite to fight against the superoxide free radicals produced during the metabolism of haemoglobin. Superoxide dismutases (SODs) are the important enzymes to catalyse the dismutation of the superoxide 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 Fe^{2+} (ferrous) to Fe^{3+} (ferric) 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. The production of superoxide is controlled by the *PfFeSOD* gene which is expressed highest during intraerythrocytic stages of life cycle[12]. Therefore, this study was designed to estimate the overall SOD activity of the RBCs infected with *P. falciparum*. Studies by various researchers have also shown that the overall activity of SOD is decreased due to infection of different species of *Plasmodium*[13-20]. Our results were in accordance with the studies cited previously, since we have observed significant decrease in the activity of SOD in *P. falciparum* infection, which could explain the oxidative stress disturbance in the erythrocyte antioxidant system encountered in the cerebral malaria.

The superoxide radical is toxic to all living cells[21]. Superoxide radical oxidises and degrades biological molecules such as lipid and proteins resulting disturbed normal cell biology[22]. Previously it was thought that *plasmodium* had no requirement for an endogenous superoxide dismutase (SOD) and only used the activity of the host enzymes in the erythrocytes. However, in 1996 a *Plasmodium falciparum* iron-dependent SOD (*PfFeSOD*) was identified in parasites isolated from infected blood cells[23]. The importance of reactive oxygen species (ROS) in host defense against various parasitic infections is well known. ROS are toxic to plasmodia and also are the important components of the host's defenses against malaria. The antimalarial agents' current use is based on the susceptibility of the malarial parasite to free radicals and oxidants. Therefore, malarial parasites are known to be vulnerable to

pharmacological agents which generate ROS such as primaquine[24], artemisinin[25], pyrimethamine[26] and alternative antimalarials such as clotrimazole an antifungal[27]. These agents appear to work on the principle that oxidative damage affects the parasite more than the host[28].

Oxidative stress damage of thrombocytes has also been implicated in the etiopathogenesis based on the finding of low levels of platelet superoxide-dismutase and glutathioneperoxidase activity, and high platelet lipid peroxidation levels in malaria patients, when compared to those of healthy subjects[29].

To minimize the effect of ROS on host cells due to malarial parasite or above mentioned antimalarials used in the treatment of malaria clinician should prescribe some antioxidant substance such as Vit C, Vit 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[3,30]. Also George et al 2012 showed the improved activity of SOD, catalase, glutathione peroxidase after the administration of aqueous extract of *Aframomum sceptrum*, an antioxidant to the infected mice with *Plasmodium berghei*[20]. 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 confirm these suggestions.

Conflict of interest statement

We declare that we have no conflict of interest.

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