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 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

## 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.
(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 Plasmodium 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 thorough 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 (high grade fever, drowsiness, unarousable coma, seizures and sometimes psychotic behaviour[4]), 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/μL) was calculated by counting 200 white blood cells and the number expressed on the basis of 8000 WBC/μL[10].

Calculation of parasitemia:

\[
\text{Parasitemia per μL} = \frac{\text{No. of parasites seen}}{\text{No. of leukocytes seen}} \times 8000
\]

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 ± SD of SOD activity in the cases was (1.06 ± 0.51) nmol/mL (N = 200). The mean ± 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.

| Parasitemia per μL | SOD activity (mean ± SD) (nmol/mL) | No. of subjects |
|--------------------|-----------------------------------|----------------|
| 600-800            | 2.05 ± 0.17                       | 18             |
| 801-1000           | 1.52 ± 0.29                       | 27             |
| 1001-1200          | 1.33 ± 0.20                       | 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 ± 0.73                       | 20             |
| 2001-2200          | 0.38 ± 0.09                       | 22             |
|                    | 1.06 ± 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 enzymes to facilitate 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 Fe2+ 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. 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 Plasmodium 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 Plasmodium 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], artimisinin[25], pyrimethamine[26] and alternative antimalarials such as clomizamoze 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 to those of healthy subjects[29]. superoxide-dismutase and glutathione peroxidase activity, and high in the etiopathogenesis based on the finding of low levels of platelet

principle that oxidative damage affects the parasite more than the

et al. are also found to be decreased in the malaria cases[3,30]. Also George

Because it is evident that serum levels of above mentioned vitamins C, Vit A. This may improve the probable outcome of the disease.

or above mentioned antimalarials used in the treatment of malaria

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

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] World Health Organization. World Malaria Report 2013. Malaria: World Health Organization; 2013. [Online] Available from: http://www.who.int/malaria/publications/world_malaria_report_2013/en/ [Accessed on 15th March, 2015]

[2] Khan HM, Shujatullah F, Raza A, Akhtar A, Gupta S. Seasonal variations in vector borne infections-malaria and dengue. J Pure Appl Microbiol 2012; 6(4) (spl edn): 59-62.

[3] Raza A, Khan HM, Malik MA, Mahdi AA, Shahid M, Shujatullah F. Serum retinol concentration in patients with acute falciparum malaria in Aligarh, India. J Infect Dev Ctries 2009; 3(11): 865-8.

[4] Dondorp AM. Pathophysiology, clinical presentation and treatment of cerebral malaria. Neurrol Asia 2005; 10: 67-77.

[5] Becker K, Koncarevic S, Hunt NH. Oxidative stress and antioxidant defense in malarial parasites. In: Sherman IW, editor. Molecular approaches to malaria. Washington DC: ASM Press; 2005, p. 365-83.

[6] Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. Int J Parasitol 2004; 34: 163-89.

[7] Henrquies JRR, de Domnguez NG. Modulation of the oxidative stress in malaria infection by clotrimazole. Braz J Pharm Sci 2012; 48(3): 519-28.

[8] Raza A, Khan HM, Shujatullah F, Tripathi T, Malik MA, Shahid M, et al. Evaluation of serum ascorbic acid levels in acute falciparum malaria. Biomed Res 2010; 21(4): 397-400.

[9] Mc Cord JM, Fridovich I. Superoxide dismutase: An enzymic function for erythrocrupin (hemocuprin). J Bioi Chem 1969; 244: 6049-55.

[10] Akambi OM, Odaibo AB, Ademowo OG. Effect of antimalarial drugs and malaria infection on oxidative stress in pregnant women. Afr J Reprod Health 2010; 14(3): 209-12.

[11] Akambi OM, Odaibo AB, Ademowo OG. Anti-MSP-1 (19) antibody (IgG) and reactive oxygen species (ROS) response against malaria infection in pregnancy in South Western Nigeria. Asian Pac J Trop Med 2009; 2: 9-15.

[12] Bustamante LY, Crooke A, Martinez J, Diez A, Bautista JM. Dual function stem molecular beacons to assess mRNA expression in AT-rich transcripts of Plasmodium falciparum. Biotechniques 2004; 36(3): 488-92.

[13] Srivastava P, Puri SK, Dutta GP, Pandey VC. Status of oxidative stress and antioxidant defences during Plasmodium knowlesi infection and chloroquine treatment in Macaca mulatta. Int J Parasitol 1992; 22(2): 243-5.

[14] Farombi EO, Shyntum YY, Emerole GO. Influence of chloroquine treatment and Plasmodium falciparum malaria infection on some enzymatic and non-enzymatic antioxidant defense indices in humans. Drug Chem Toxicol 2003; 26(1): 59-71.

[15] Ifoue SHT, Mofor CT, Gouado I, Teto G, Asonganyi T, Zollo PHA. Evaluation of oxidative stress and antioxidant status of pregnant women suffering from malaria in Cameroon. Indian J Clin Biochem 2009; 24(3): 288-93.

[16] Rodrigues JR, Gamboa ND. Effect of dequalinium on the oxidative stress in Plasmodium berghei-infected erythrocytes. Parasitol Res 2009; 104(6): 1491-6.

[17] D’Souza B, D’Souza V, Swagata H, Vijayalaxmi K, Namratha AS. Erythrocyte antioxidant enzymes and their correlation with malondialdehyde in malaria. Biomed Res 2009; 20(1): 25-7.

[18] Andrade BB, Reis-Filho A, Souza-Neto SM, Raffaele-Neto I, Camargo LMA, Barral A, et al. Plasma superoxide dismutase-1 as a surrogate marker of vivax malaria severity. PLoS Negl Trop Dis 2010; 4(4): e650. doi:10.1371/journal.pntd.0000650.

[19] Oyewole I, Anyasor G, Ogunwemmo K, Ayodele S. Antioxidant and oxidative stress status in human Plasmodium malaria. Der Pharm Lett 2011; 3(2): 91-6.

[20] George BO, Okpogono J, Osioma E, Aina OO. Changes in oxidative indices in Plasmodium berghei infected mice treated with aqueous extract of Aframomum sceptum. Front Sci 2012; 2(1): 6-9.

[21] Boucher IW, Brzozowski AM, Brannigan JA, Schnick C, Smith DJ, Kyes SA, et al. The crystal structure of superoxide dismutase from Plasmodium falciparum. BMC Struct Bioi 2006; 6: 20.

[22] Raza A, Varshney SK, Shahid M, Khan HM, Malik MA, Mahdi AA, et al. Lipid peroxidation in cerebral malaria and role of antioxidants. IOSR J Pharm 2013; 3: 15-8.

[23] Becuwe P, Gratepanche S, Fourmaux MN, Van Beeumen J, Sawan MY, Aheke S. Antioxidant and oxidative stress status in human Plasmodium malaria. Ber Pharm Lett 2011; 3(2): 91-6.

[24] George BO, Okpogono J, Osioma E, Aina OO. Changes in oxidative indices in Plasmodium berghei infected mice treated with aqueous extract of Aframomum sceptum. Front Sci 2012; 2(1): 6-9.

[25] Doucett MM, Rose BD, Naisbitt DJ. Expression and characterization of three new glutathione transferases, an epsilon (AcGSTO1-1), omega (AcGSTO2-1), and theta (AcGSTT1-1) from Ancylostoma caninum (Diptera: Culicidae), a major Thai malaria vector. J Biol Chem 2009; 284: 243-5.

[26] Grahame-Smith DG, Aronson JK. A caninum (Diptera: Culicidae) Ancylostoma caninum (Diptera: Culicidae). Ancylostoma caninum (Diptera: Culicidae). J Med Entomol 2010; 47: 162-71.

[27] Grame-Smith DG, Aronson JK. [Treaty of clinical pharmacology and pharmacotherapy]. 3rd ed. Grame-Smith DG, Aronson JK. [Treaty of clinical pharmacology and pharmacotherapy]. 3rd ed. In: Barunabara Koogan: Rio de Janeiro: 2004.

[28] Legorreta-Herrera M, Retana-Ugalde R, Ventura-Gallegos JL, Narvaez V. Pyrimethamine induces oxidative stress in Plasmodium yoelii 17XL-infected mice: a novel immunomodulatory mechanism of action for an old antimalarial drug? Exp Parasitol 2010; 126: 310-318.

[29] Trivedi V, Chand P, Srivastava K, Puri SK, Mahdi AA, Bandyopadhyay U. Clotrimazole inhibits hemoperoxidase of Plasmodium falciparum and induces oxidative stress. J Biol Chem 2005; 280: 4129-36.

[30] Zhang S, Chen H, Gerhard GS. Heme synthesis increases artemisinin-induced radical formation and cytotoxicity that can be suppressed by superoxide scavengers. Chem Biol Interact 2010; 186: 30-5.

[31] Erel O, Ural H, Aksoy N, Aslan G, Ulukanigil M. Oxidative stress of platelets and thrombocytopenia in patients with vivax malaria. Clin Biochem 2001; 34(4): 341-4.