ORIGINAL ARTICLE

Pro-oxidants in pathogenesis of severe malaria in children

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Citation

Narsaria N, Singh AK, Mishra P, Luthra M. Pro-oxidants in pathogenesis of severe malaria in children. Indian J Comm Health. 2015; 27, 3: 346-350.

Source of Funding : Nil **Conflict of Interest:** None declared

Article Cycle

Submission: 16/07/2015; **Revision:** 30/07/2015; **Acceptance:** 20/08/2015; **Publication:** 30/09/2015

Abstract

Introduction: Malaria is a major cause of morbidity and mortality in developing countries. The role of oxidants in the pathogenesis of malaria in children are lacking. **Objective**: This study was done to assess the plasma oxidant level of children with severe malaria. **Material and Methods**: This prospective study was done in Mayo Institute of Medical Sciences, a tertiary care hospital. 40 patients of confirmed malaria in the age group of 0–16 years and other 40 age- and gender-matched healthy children (controls) were included in our study. Plasma malonyldialdehyde (MDA) was estimated by thiobarbituric acid test. Protein carbonyl level was analyzed by the methods of Reznick and Packer. **Results:** The average plasma level of oxidants was significantly higher in cases as compared to control group ($p < 0.05$). The plasma MDA level in the cases was significantly higher ($p= 0.03$) than in control group. The plasma level of protein carbonyl in cases was significantly higher ($p = 0.038$) as compared to control group. The plasma level of Copper (Cu) and Nitrite were also found significantly higher ($p < 0.05$) in cases. **Conclusion**: The plasma MDA, protein carbonyl, copper and nitrites were significantly raised in cases with severe malaria as compared to controls ($p <0.05$) reflecting the increased oxidative stress in cases as compared to controls.

Key Words

Oxidants; Nitrite; MDA; Malaria

Introduction

Malaria transmission and consequently, the disease burden may vary widely, even within a small geographical area (1, 2, 3). In the last few decades, research has been able to define new tools and strategies for malaria control (4,5). Malaria is a major cause of morbidity and mortality in developing countries, accounting for an estimated 300 to 500 millions morbid episodes and 2 to 3 millions deaths every year worldwide (6,7). Malaria is a mosquito borne infectious disease of humans caused by parasitic protozoans belonging to the genus Plasmodium. It is transmitted by the bite of female anopheles mosquito and manifest symptomatically 10 to 15 days after the bite. Most deaths are caused by Plasmodium falciparum which is also the commonest cause of severe malaria (8). Severe malaria comprises of a set of clinical and laboratory parameters associated with an increased risk of death. In young children, these criteria are predominantly altered consciousness, severe anemia, and respiratory distress (9,10). Any infection including malaria activates the immune system of the body thereby causing release of reactive oxygen species as an antimicrobial action through the massively recruited and activated monocytes and

neutrophils. In addition to host's immune system, malarial parasite also stimulates certain cells in production of ROS (reactive oxygen species) thereby resulting in hemoglobin degradation (11).

Several authors have discussed the implications of these ROS through oxidative stress in the physio-pathogenesis of malaria (12, 13, 14). This involvement may be related to the pathogenic mechanisms triggered by the parasite as well as free radical production and antioxidant defenses in host cells to abate the infection (15,16). These free radicals are short-lived and cannot be measured directly, but their activity can be measured by estimating the by products involved in defense against the oxidant injury. Markers of oxidant stress can be malondialdehyde (MDA), a byproduct of lipid peroxidation; protein carbonyl; nitrite; and trace metals such as copper. The role of free radicals in the pathogenesis of severe malaria has been mainly shown in experimental and animal studies, but studies in humans, especially in children, are lacking. Some authors suggest a protective role, whereas others claim a relation to the physiopathology of the disease (17).

Aims & Objectives

To assessing the plasma oxidant status of children with severe malaria by estimating the levels of MDA, protein carbonyl, nitrite and copper.

Material and Methods

Study design: This was a prospective, hospital-based study that included 40 patients with severe malaria of both genders in the age group 0–16 years admitted to pediatric ward of a tertiary care hospital between September 2012 to September 2014. Children aged up to 16 years having one or more of the clinical features in the presence of asexual parasitemia and/or positive serology (Rapid Antigen Detection Test) were taken in the study (18). The clinical features included cerebral malaria, severe anemia, renal failure, metabolic (Lactic) acidosis/acidosis, pulmonary edema or acute respiratory distress syndrome (ARDS), hypoglycemia, hypotension and shock (algid malaria), abnormal bleeding and/or disseminated intravascular coagulation, repeated generalized convulsions, hemoglobinuria and added World Health Organization (WHO) criteria from 2000 like impaired consciousness, prostration, hyperparasitemia, hyperpyrexia and jaundice (hyperbilirubinemia). The clinical history, examination and relevant investigations (haematological, renal function test, liver function tests, coagulation screening and cerebrospinal fluid – cytology and biochemistry as per the need to establish diagnosis were recorded. Another 40 age and gender-matched normal children attending the outpatient department of general pediatrics for routine check-up were included to serve as controls. They had normal nutritional status and no abnormality was seen on general physical and systemic examinations. Informed consent regarding inclusion in the study was given by the parents in each case, and the study protocol was approved by the Institute Ethics Committee.

Blood sample collection and estimation: Taking all aseptic precautions, about 10 ml of blood was drawn by venipuncture from a peripheral vein, with a heparinized disposable syringe and transferred to sterile, heparinized, de-ionized poly-ethylene vials. Plasma was separated from the blood samples immediately by centrifugation at 2000 rpm for 5 minutes and was stored in separate de-ionized vials in a deep freezer. Plasma MDA was estimated by thio-barbituric acid test. Protein carbonyl and nitrite were analyzed by the methods of Reznick and Packer *et al*. and Moshage *et al*. respectively. Plasma copper concentration was estimated by atomic absorption spectrophotometry.

Statistical analysis: Data were analyzed using SPSS 16.0 software. Student's t test was applied to the parameters with normal (Gaussian) distribution, and Mann–Whitney U test was used to the parameters with distribution different from normal. χ^2 tests were used to find the association of age group with study variables. Statistical significance was taken as 0.05 ($p < 0.05$).

Results

The mean age of presentation of children with severe malaria was 5.8 ± 3.9 yrs which was comparable to control values. Among the cases, the mean age of presentation was higher for males but the difference was not statistically significant. Maximum number of cases of severe malaria was encountered in the 0-6 year's age group. The mean hemoglobin, total leucocyte count, absolute platelet count, blood urea and serum creatinine values in cases were 6.8 ± 2.5 (g/dl), 9.8 ± 5.9 ($\times 10^3$ / µl), 1.0 ± 0.52 ($x10^5$/ mm^3), 75.2 ± 42.7 (mg/dl) and 1.6 ± 1.5 (mg/dl), respectively.

The plasma MDA, protein carbonyl, nitrite and Cu levels in children with severe malaria are shown in Table 1. The mean plasma levels of MDA, protein carbonyl, Nitrite and Cu were significantly higher in patients with severe malaria as compared to control values ($p<0.05$ each). The mean values of all the parameters were comparable in male and female cases. Out of 40 cases, 29 were caused by Plasmodium falciparum and 11 were caused by species other than Plasmodium falciparum.

Discussion

Oxidative stress is a situation in which there is an imbalance between the production of reactive oxygen-species (ROS) that can damage cell structures and the body's ability to detoxify these molecules or repair the resulting damage (19) and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant system in intact cells. Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include oxidizing compounds such as superoxide radicals, hydrogen peroxide hydroxyl radical, lipid peroxides and other related species (20). During a malaria infection, both host and parasite are under oxidative stress. Increased production levels of reactive oxygen species (ROS, e.g. superoxide anion and the hydroxyl radical) are produced by activated neutrophils in the host and during degradation of hemoglobin in the parasite (21). Inordinate or aberrant generation of ROS is widely incriminated in the pathogenesis of tissue injury and play a role in the pathology of malaria (22). However, oxidative stress during malaria is even beneficial to the patient in the combat against its intra-erythrocytic parasite and few studies have been described in which induction of oxidative stress by treatment with pro-oxidants proved to be effective against malarial infection. During a malaria infection, both host and parasite are under oxidative stress. Increased production levels of reactive oxygen species (ROS, e.g. superoxide anion and the hydroxyl radical) are produced by activated neutrophils in the host and during degradation of hemoglobin in the parasite (22). The effects of ROS in malaria can be both beneficial and pathological, depending on the amount and place of production. Enhanced ROS production after the administration of pro-oxidants, which is directed against the intra-erythrocytic parasite, inhibits the infection both in vitro and in vivo. However, ROS are also involved in pathological changes in host tissue like damage of the vascular endothelial lining during a malaria infection (cerebral malaria).

The increased lipid peroxidation as depicted by the high concentration of MDA and protein carbonyl, may arise from a variety of factors such as enhanced generation of free radicals, reduced level of antioxidants available, enhanced consumption, leakage or destruction of antioxidants, decreased protective capacity including antioxidants enzymes, leakage of electrons from the disrupted mitochondrial electron transport chain and phagocyte recruitment and activation (23). Oxidative stress plays an important role in the development of malarial anemia and P. falciparum trophozoite infected human red cells produce more ROS compared to vivax infections (21) thus making anemia a reliable predictor of the severity of disease. In this study the results indicates that serum concentrations of MDA and protein carbonyl in the severe malaria patients was significantly higher than ($p<0.05$) that of the control group. Protein carbonyl is a by-product of protein oxidation and as far as it could be reviewed, no related studies have been done on it in severe malaria in children till now. Thus this study can bring out its role as an oxidant in severe malaria. High blood levels of NO have been associated with protection against severe malarial disease, but may also contribute to the pathophysiology of cerebral malaria and severe anemia. Increased plasma Cu concentrations in patients with malaria might be the result of the inflammatory response of the host against parasites. The plasma MDA, protein carbonyl, copper and nitrites were significantly raised in cases with severe malaria as compared to controls ($p <0.05$) reflecting the increased oxidative stress in cases as compared to controls. The increased levels of nitrite and copper reflect that these are bifunctional and the levels will be high or low depending upon the role they assume in severe malaria. Therefore, it appears that these biochemical alterations are indicative of oxidative damage during severe malaria. However, further studies are needed to determine the cause-and-effect relationship and its prognostic value in patients with severe malaria.

The role of oxidative stress in the pathophysiology of malaria is a multifactorial phenomenon and represents an important aspect of the intricate and complex host-parasite relationship. Much efforts have been put in the research of new antimalarial

drugs and development of vaccine against Plasmodium falciparum and understanding of the role and mechanism of oxidative stress may eventually help in this and in evolution of new concept of treatment. The parasite's ability to express antioxidant proteins is one of the resistance mechanisms to anti-malarials, since early transcriptional response of genes involved in antioxidant protein expression confers the adaptive capacity to certain antimalarial drugs (24). Other pro-oxidant treatment strategies include alternative therapies with antifungal agents such as clotrimazole, based on their ability to inhibit hemo-peroxidase with consequent oxidative stress induction (25).

Conclusion

The use of antioxidant supplements of synthetic or natural origin may constitute a far more effective adjuvant antimalarial strategy that causes less damage to the host.

Recommendation

Further research is needed to confirm these suggestions.

Authors Contribution

NN: Concept designing, data collection; AKS: Compilation and analysis of data, manuscript writing, PKM & ML: Critical analysis of data mad finalization of manuscript.

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Tables

TABLE 1 SERUM MDA, PROTEIN CARBONYL , NITRITE AND CU LEVELS IN CASES OF SEVERE MALARIA (N=40) AND CONTROL (N=40

Parameters	Mean ± 1S.D.		p-Value
	Cases	Control	
MDA (µmol/l)	0.68 ± 0.18	0.42 ± 0.08	0.03
Protein carbonyl (nmol/mg)	38.8 ± 15.5	21.8 ± 0.69	0.038
Nitrite (µmol/l)	78.2 ± 14.9	30.4 ± 2.9	0.01
Cu (mg/dl)	1.21 ± 0.12	0.82 ± 0.18	0.024