

Changes in Serum Manganese Concentration in Adult Patients with Acute, Uncomplicated *Falciparum* Malaria Infection: Potential Implications for Mitochondrial Manganese Superoxide Dismutase (MnSOD) Activity and Mitochondrial Function

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ABSTRACT

Serum manganese concentration was measured in 100 adults (age range, 18-40 years) comprising of 40 males and females each presenting with acute, uncomplicated falciparum malaria infection and a control group of 20 age-matched, healthy individuals. Patient selection and pre-qualification was done by simple random sampling of individuals presenting at the Bauchi State Specialist Hospital Outpatient Department with a history of fever and malaise, and who were confirmed to be infected with the *Plasmodium falciparum* malaria parasite by microscopic examination of Giemsa-stained thin blood slides (parasite density in the range of 1,000-10,000 asexual forms/ml of blood). Mean serum manganese concentration in both patients and healthy individuals were compared using one way Analysis of Variance (ANOVA). The method of Least Significance Difference (LSD) was used to assess the source of difference where the ANOVA returned a P value of < 0.05. A significant decrease was found in the mean serum concentration of manganese in both the infected males and females. Mean serum manganese concentration was $198.0 \pm 16.45 \mu\text{g/dl}$ and $134.0 \pm 16.30 \mu\text{g/dl}$ in the infected males and females, respectively. These values represent a significant decrease in serum manganese (Mn) relative to their concentration in their healthy counterparts of $404.0 \pm 50.33 \mu\text{g/dl}$, where $P < 0.01$. Mitochondria are the major intracellular sites of reactive oxygen species (ROS) production due to the activity of the mitochondrial electron transport chain. Normal mitochondrial ROS homeostasis is predominantly maintained by a Mn-dependent mitochondrial superoxide dismutase (SOD). Decreased serum Mn can lead to a compromised ROS scavenging activity by the mitochondrial SOD. In consequence, this can lead to defective mitochondrial function via ROS-induced peroxidation of mitochondrial membrane lipids and an accompanying drop in patient energy status. This usually translates symptomatically into a state of lethargy, general body weakness and malaise at great economic cost to falciparum malaria patients and malaria endemic countries.

Keywords: blood, disease, health, minerals, parasites

INTRODUCTION

Trace elements have been found to play a role in a number of essential metabolic functions, and their importance in biomedical research has grown accordingly (Russel 2000). Human trace element status has been known to influence growth and reproduction, immune function, lean body mass, bone density, cognitive functions, insulin sensitivity and oxidative stress (Leach and Harris 1997). In addition, anti-oxidant trace elements like zinc, selenium and copper are involved in cellular anti-oxidant defenses and protect against accelerated aging process (Bray and Brettger 1990; Lavender and Burk 1996; Jones *et al.* 1997). Among these trace elements, manganese (Mn) has been reported to be essential in both animal and plant nutrition and physiology (Miller *et al.* 2000). This metal is necessary for the formation of connective tissue and bone growth, amino acid, carbohydrate and lipid metabolism, embryonic development of the inner ear and reproductive physiology (Nielsen 1999). In addition, Mn is an important component of a number of metal-requiring enzymes known as metalloenzymes (Keen and Zindenberg 1996). Mn metalloenzymes include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and Mn-superoxide dismutase (Williams *et al.* 1998; McMillan-Crow and Cruthirds 2001). This metal is also important in proteoglycan synthesis and bone formation, since

changes in Mn status in animals have been reported to affect the activity of glycosyltransferases and xylosyltransferases, both of which are key enzyme groups required in bone formation. The major manifestations of Mn deficiency observed in animal studies include impaired growth, skeletal abnormalities, depressed reproductive function, lack of muscular coordination in newborns and defects in lipid and carbohydrate metabolism (Freeland-Graves and Llanes 1994; Finley and Davis 1999; IEH 2004). In this study, we assessed the changes in serum levels of Mn in adults with acute *Plasmodium falciparum* malaria infection considering the crucial role of this metal in mitochondrial energy metabolism and the observed decrease in energy status of acute falciparum malaria patients manifesting in lethargy, muscular weakness and general malaise (Buyse *et al.* 1996).

SUBJECTS AND METHODS

Study design

Patient selection and pre-qualification was done by simple random sampling of individuals presenting themselves at the Bauchi Specialist Hospital Outpatient Department with a history of fever and malaise within a period of 1-8 days, and who were confirmed to be infected with the falciparum malaria parasite by microscopic examination of Giemsa-stained thin blood slides. All the patients were

found to present moderate parasitaemia, with a parasite density in range of 1000-10,000 asexual forms/ml of blood. Patients presenting concomitantly with any of the following illnesses: liver diseases, anemia, alcoholism, metabolic bone disease, protein energy malnutrition, and acute *falciparum* malaria infection greater than eight days were excluded in this study. Similarly, patients with a history of self-medication within the prescribed period of acute infection (day 1-8) were also not enrolled in this study. Based on these criteria a total of 80 patients comprising 40 males and 40 females were enrolled in the study. The control group was made up of 20 healthy male and female adults. The age range for both the patients and the control group was 18-40 years.

Collection and preparation of serum samples

Blood samples were collected between 9.00 a.m. and 11.00 a.m. by venepuncture of the antecubital vein into clean, sterile, plastic centrifuge tubes. The samples were centrifuged at 3000 x g for 10 min after clotting. Sera was collected by aspiration using a Pasteur pipette and assayed within 24 h.

Assay for serum Mn concentration

The concentration of Mn in serum was determined using a Buck Scientific Atomic Absorption Spectrophotometer (AAS) VPG System, Model 210 (Buck Scientific Corporation, California, U.S.A.). Serum samples were digested using a mixture of nitric and perchloric acids. Serum (0.5 ml) was mixed with 5 ml nitric acid and 2 ml perchloric acid. The mixture was heated for 2 h at 100°C. The resulting clear, colourless sample at the end of the digestion was indicative that all carbonaceous materials had been combusted via the digestion process. After cooling, the digest was made up to 25 ml with double-distilled, deionised water and used for the Mn determination as follows. Nitric acid (0.1 M) was aspirated for 30 seconds and then the absorbance of the instrument was zeroed. After that, the absorbance for both standard and serum sample solutions were measured in triplicate readings, with aspiration of 0.1 M nitric acid for 10 sec after each reading to clean the burner.

Statistical analysis

Data analyses were effected using MINITAB-10 Statistical Software. Comparison of mean serum Mn concentration between the control group and patients were done using One-Way Analysis of Variance (ANOVA). Where P values were < 0.05, the Least Significant Difference (LSD) was used to test for the difference between pair of means. P values < 0.05 were considered significant.

Ethics

This work was conducted in accordance with the following ethical declarations: World Medical Association's Declaration of Helsinki (1996), World Medical Association's Declaration of Lisbon on the Rights of the Patient (1995), CIOMS/WHO International Guidelines for the Conduct of Research Involving Human Subjects (1993).

RESULTS AND DISCUSSION

The results are shown in **Table 1**. The mean serum Mn concentration in both male and female patients were all lower than the control serum value of $404.0 \pm 50.33 \mu\text{g/dl}$, $P < 0.01$. Among the male patients, the mean serum Mn concentration decreased by 66.60%, while it decreased by 50.99% in the female patients, $P < 0.01$.

Several studies have reported the role of reactive oxygen species (ROS) in the pathogenesis of diseases (Frey *et al.* 1994; de Zwart *et al.* 1999; Fosberg *et al.* 2001). Specifically, oxygen-derived free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals are generated during normal metabolism, with their concentration increasing following ethanol consumption, cigarette smoking and exposure to ionizing radiation (Millikan *et al.* 2004). Because of their unstable electronic configuration, free radi-

Table 1 Serum manganese concentration in adult male and female *falciparum* malaria patients and control.

Subjects	n	Mean serum manganese ($\mu\text{g/dl}$)
Control	20	404.0 ± 50 *a
Male patients	40	135.0 ± 16 *b
Female patients	40	198.0 ± 16 *b

* Significant at $p < 0.01$ (ANOVA)

a, b Significant at $p = 0.01$ (LSD)

b, b Not significant.

cals either take or donate an electron to an adjacent compound in order to restore its own orbital stability. The ensuing electron transfer leads to the disruption of the structure of adjacent compounds and impairment in the biological function of such compounds (Cavallini *et al.* 1984; Zhang *et al.* 1994). Among the major biochemical consequences of free radical activity are the mutation of DNA and initiation of lipid peroxidation, which in turn leads to the destruction of cell membranes (Williams *et al.* 1998). Several enzyme systems counteract free radical-induced oxidative damage; including catalase, glutathione peroxidases and the superoxide dismutase family. Superoxide dismutase (SOD) converts superoxide to peroxide and finally to molecular oxygen. Three superoxide dismutases are known to exist: cytosolic SOD, mitochondrial SOD and extra-cellular SOD (Keen *et al.* 1999). Among these, mitochondrial SOD is dependent on Mn in carrying out its free-radical scavenging activity. Several reports have shown that *falciparum* malaria infection is associated with the increased production of ROS (Rath *et al.* 1991; Nanda *et al.* 2004). ROS production has been linked to the failure of some key anti-oxidant defenses such as vitamin E, serum iron, and glutathione peroxidase and superoxide dismutase during *falciparum* malaria infection (Kulkarni *et al.* 2003). Since the results obtained in this study showed a decrease in serum concentration of Mn, it could have negative implications on the ability of the mitochondrial SOD (MnSOD) to effectively scavenge ROS in the mitochondria. This is because under normal metabolic conditions the mitochondria contain high concentrations of ROS arising from the activity of the mitochondrial electron transport chain (St. Pierre *et al.* 2002; Pudim and Kunz 2005; Vercesi 2005). Under this condition, the excess ROS produced is removed by the action of MnSOD, a Mn-containing enzyme located exclusively in the mitochondria (Kinnula and Crapo 2003). Decreased serum Mn concentration will lead to a low MnSOD activity and consequent reduction in ROS scavenging capacity in the mitochondria. The resulting oxidative stress arising due to a compromised MnSOD activity can lead to a disruption of mitochondrial respiratory chain function via ROS-induced mitochondrial membrane lipid peroxidation (Shusen 2005) and a decrease in mitochondrial energy production. The decreased energy status following ineffective mitochondrial MnSOD activity can account for the general weakness and malaise associated with this endemic tropical disease. From an economic point of view, low patient energy status has made *falciparum* malaria infection a costly disease. An estimated US\$1.80 billion has been reported as a direct cost in terms of lost productivity, time cost in hospital/clinic visits and other direct costs and losses due to *falciparum* malaria infection. This amount is approximately equal to the entire GDP of some countries such as Malawi, Benin or Togo (Foster 1998).

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