Erythrocytic Antioxidant Enzymes, Antioxidant Vitamins And Plasma Malondiadehyde In Malaria Infected Patients In Owerri


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Abstract: Malaria is caused by Plasmodium falciparum. It is a public health problem leading to morbidity and mortality among children. This study was carried out to evaluate the changes in erythrocyte enzymatic antioxidants: superoxide dismutase (SOD), and catalase (CAT), non enzymatic antioxidants: Vitamins C and E, and Malondiadehyde (MDA) in malaria infected patients. 200 healthy subjects and 200 diagnosed malaria patients between the age of 15-25 years, attending General Hospital Owerri were involved in the study. Statistical analysis was done using student ‘t’ test. The level of antioxidants were significantly depleted in malaria infected subjects (P<0.05) while the level of MDA was significantly increased when scompared with the control healthy subjects (P<0.05). The increased level of MDA and reduced antioxidants probably implies that oxidative stress plays a role in the pathogenesis of malaria.

Key words: Antioxidants, Vitamins, MDA, malaria, Owerri

INTRODUCTION

Malaria is a public health problem caused by a parasite known as plasmodium. It is transmitted through the bites of infected anopheles mosquitoes. In the human body system, the parasites multiply in the liver and then infect the erythrocytes (Snow et al., 2005).

Fever, headache, and vomiting are major symptoms of malaria. These usually appear between 10 and 15 days after the mosquito bites. Malaria can be life threatening by disrupting the blood supply to vital organs if not treated. Different plasmodium species can infect and be transmitted by humans. Most death from malaria are caused by Plasmodium falciparum while Plasmodium vivax, p. ovale and P. malariae caused a generally milder form of malaria that is rarely fatal. Also, P knowlesi which is transmitted through animals causes malaria but can equally cause serious infections. It has been estimated that 216 million cases of malaria are documented (Muller et al., 2007). About 655,000 people died from malaria many of whom are children under the age of five. Malaria pose a major hinderance to economic development as it is associated with poverty. Malaria can be prevented by the use of medications, mosquito eradication and prevention of bites. Health education strategy promoting awareness of malaria and importance of control measure could be very helpful (Holding and Snow, 2001).

Malaria subjects are susceptible to oxidative stress which is imbalance between reactive oxygen species and the biological system ability to detoxify the reactive intermediates. In humans, oxidative stress is involved in the development of disease. It is associated with decrease in the effectiveness of antioxidants. Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide; however, it will also reduce metal ions that generate free radicals through the Fenton reaction. The relative importance of the antioxidant and pro-oxidant activities of antioxidants are an area of current research, but vitamin C, which exerts its effects as a vitamin by oxidizing polypeptides, appears to have a mostly antioxidant action in the human body. However, less data is available for these antioxidants, such as vitamins C and E as well as enzymatic antioxidants such as superoxide dismutase (SOD), and catalase (CAT) in malaria patients. Hence the need for the study.

MATERIALS AND METHODS

Subjects:

200 confirmed malaria patients (100 males and 100 females) aged 15-25 years diagnosed by Giemsa staining method were selected for the study. Patients with infections, catarrh and cough were excluded. 200 normal subjects free from malaria were served as control. Informed consent was obtained from all the subjects verbally.
Preparation of Erythrocyte Samples:
Five milliliters of blood was drawn from the cubital median vein of the subjects into plain tube and heparinized tube. The sample in plain tube was spun in a Wisperfuge (model 684) centrifuge at 1000g for 10 minutes and the serum collected into a clean dry bijou bottle.

The blood samples in heparinized tubes were centrifuged at 1000 x g for 10 min at 4°C and the upper phase was taken with a pasteur pipette into an eppendorf tube and stored at -4°C. The buffy coat on top of the erythrocyte layer was carefully removed and 10 mL isotonic NaCl solution was added.

Resuspended erythrocyte was centrifuged at 1000 x g for 10 min and the upper part removed again. Then 10 mL phosphate buffer solution (PBS) was added and the erythrocytes were centrifuged, and the upper buffer part removed by pasteur pipette. The erythrocytes were diluted 10 times with ice cold water, vortexed and stored at -40°C until used.

Measurement of Catalase Activity:
Catalase, (CAT, E. C.I.I. I. 6) enzyme converts H2O2, H2O and 1/2 O2. Catalase activity was measured by the Aebi (1974) method. The principle of this method was based on the hydrolyzation of H2O2 and decreasing absorbance at 240 nm. The conversion of H2O2 into H2O and 1/2 O2 in 1 min under standard condition was considered to be the enzyme reaction velocity.

Superoxide Dismutase (SOD) Enzyme Activity Determination:
The superoxide dismutase [SOD (E.C.1.15.1.1)] enzyme, which catalyzes the dismutation of the superoxide anion (O2 .-) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. SOD activity determination was based on SOD’s inhibition of the reaction of superoxide anion (O2 -), from xanthine by xanthine oxidase and the reduction of nitroblue tetrazolium (NBT) (Podezasy and Wei, 1998).

Determination of Hemoglobin:
The colorimetric cyanomethemoglobin procedure was used and determination was performed using spectrophotometer.

Determination of MDA:
MDA, as a marker for lipid peroxidation, was determined by the double heating method of Draper and Hadley (1990). The principle of the method was based on a spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). In brief, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test-tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer at 532 nm. The concentration of TBARS was calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 10^5 cm^-1 M^-1 and expressed in nmol/ml.

Vitamins Analysis:
Plasma vitamin C was assayed by the 2,4-nitrophenyl hydrazine method described by Tietz (1976a). The vitamin E was done by the method of Tietz (1976b) in which vitamin E caused the reduction of ferric to ferrous ion which then forms a red complex with α-α dipyridyl. Vitamin C and E were measured at 520 nm using spectrophotometer.

Statistical Analysis:
The values were expressed as mean ± standard deviation. The student t-test was used to calculate the significant differences at P < 0.05.

Results:
Parameters Malaria Subjects Control:
CAT (IU/gHb) 6.71± 1.31 7.95± 1.63*
SOD(IU/gHb) 414.75± 193 641.34± 194*
MDA(nmol/ml) 6.11 ± 1.32 1.84± 0.79*
Vitamin C(mg/dl) 0.92± 0.44 1.76 ± 0.32*
Vitamin E(mg/dl) 0.47 ±0.52 1.69 ± 0.46*
*Significantly different from control at (P<0.05)
Discussion:
Malaria is widespread in the tropics and subtropical region. The majority are mostly children in sub Sahara Africa. It is one of the most common infectious diseases and a great public health problem.

In this study, it was observed that the level of antioxidants were significantly decreased in malaria subjects. This is in line with the work of Nnodim and Nwanjo(2012). The decrease in antioxidants could be associated with free radicals induced by Plasmodium falciparum parasites. Antioxidant enzymes play important role in detoxification of oxidative damages and constitute a mutually supportive team of defense against reactive oxygen species (ROS). Reactive oxygen specie interact with antioxidants and inactivates antioxidant enzymes producing a state of oxidative stress. Specifically, superoxide dismutase is one of the important intracellular antioxidant enzyme present in aerobic cells and has antitoxic effect against superoxide radicals. While catalase protects the cells from accumulation of hydrogen peroxide by decomposing it to water and oxygen. The decrease in SOD and CAT levels in malaria patients could be linked to increased exposure to oxidant environment that destabilize erythrocyte membrane by lipid peroxidation and cause significant leakage of these intracellular enzymes(Pujar et al., 2011). Also, it could be in relation to oxidative inactivation of enzymes. Similarly, the decrease in CAT activity could be probably linked to increased in MDA observed in the study which could cross-link with amino group of protein to form intra and intermolecular crosslinks hence inactivating several membrane bound enzymes(Kiklugawa et al., 1984).

Furthermore, the level of non-antioxidant vitamins C and E were significantly decreased in malaria subjects when compared with the control(P<0.05). This is similar to the works of Bogdanska et al., (2006). Vitamin C is reduced in malaria patients following its use to regenerate vitamin E from alpha tocopheroxyl radical at water lipid interface. It is also an efficient quencher of superoxide and hydroxyl radicals. While significant reduction of vitamin E in malaria patients may probably be due to enhanced lipid peroxidation by the plasmodium falciparum. Vitamin C which is a water soluble vitamin and non-enzyme antioxidant serves directly by scavenging aqueous peroxyl radicals. Also indirectly regenerate reduced vitamin E (Nwanjo and Oze, 2007). Therefore, the reduction of these antioxidants vitamins challenges the membrane stability of erythrocytes. It is reported that vitamin C and E are chain breaking antioxidants and could stop the chain of oxidative reactions that lead to disease condition(Kutlu et al 2005). The supplementation of vitamin C and E has been described to have antioxidant effect on some pathological conditions (Ojiako and Nwanjo, 2007). Vitamin E and vitamin C can also act to overcome oxidative stress, being a part of the total antioxidant system. Vitamin E is the most important lipophilic antioxidant and resides mainly in the cell membranes and thus helps to maintain membrane stability (Baker et al,1996). Vitamin C is hydrophilic and is a very important free-radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damage(Harapanhalli et al, 1996).

Having established that the level of Vitamin C and E are depleted in malaria, it is recommended that supplementation with vitamin C and E protect the tissue from a high risk of oxidative damage which may be associated with malaria.

In conclusion, oxidative stress may be involved in malaria. There is a shift in the oxidant–antioxidant balance in favor of lipid peroxidation, which could lead to the tissue damage observed in this disease. The results of our study suggest higher oxygen free radical production, as evidenced by significant increase in malondialdehyde levels, supports the higher oxidative stress hypothesis in malaria. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The increased level of MDA and reduced antioxidants probably implies that oxidative stress plays a role in the pathogenesis of malaria.

REFERENCES


