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Alterations in superoxide dismutiase, vitamins C and E in HIV infected children in Umuahia, Abia state.

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Abstract

Alterations in superoxide dismutase (SOD), vitamins C and E status of HIV-infected children in Umuahia were investigated at the HIV/AIDS clinic Federal Medical Centre Umuahia. A total number of one hundred and twenty six (126) subjects aged 2 -12 years were recruited and grouped into three (3) of forty-two (42) each of control (group 1), HIV-infected children on therapy (HAART) (group II) and HIV-infected children without therapy (HAART) {group III) with equal number on males and females. The levels of the parameters were evaluated spectrophotometrically after determination of the HV statuses of the subjects. HIV-infected children in group III showed significantly lower values of SOD (2.8±0.9 u/ml), vitamin C (3.7±0.5 ng/ml) and vitamin E (6.1±0.5u,moL/L) (L) when compared with group II (3.4±1.Ou/ml; 4.8±0.5u.glmL and 7.4±0.7u.ml/L) and group I (7.8±1.5ulml; 6.5±0.8|ag/ml and 10.4±1.8u>mol/L) respectively at $p<0.05$. A significant strong positive correlation was found between SOD, vitamins C and E and the alterations observed were not gender dependent at $P<0.05$. The results showed an increase oxidative stress following HIV-infection with loss of antioxidants which accelerates the disease progression.

Keywords: Alterations,Superoxide Dismutiase, Vitamins C and E, HIV Infected Children, Umuahia.

Introduction

Human immunodeficiency Virus (HIV) infection, the forerunner of acquired immune deficiency syndrome (AIDS) induces a variety of alterations in immunologic mechanisms due to chronic activation of immune cells with increase in the production of reactive oxygen species {ROS).

The increases morbidity and mortality rates of HIV infection have made it a global health problem (Lizet.te et al., 2003). Globally there were 3.3 million children living with HIV infection in 2011, 330,000 new infections, 230,000 deaths from AIDS and approximately 17.3 million AIDS orphans and 88% of whom live in sub-Saharan African (UNAIDS, 2012) among which is Nigeria. Though there have been an evidence based progressive fight against HIV infection as the number of deaths from this infection declined by 32% from 1.8 to 1.2 million between 2009-2011 and the number of newly infected children fell by 24% in Nigeria (Wole, 2013), more is needed to be done to create more awareness and to reduce it to the bearest minimum especially among children.

Researches have shown the decreased levels of major antioxidant molecules and increased oxidative stress

following high level of inflammatory cytokines and the production of reactive of reaction oxygen species (ROS) in seropositive patients (Lizette et al., 2003).
Oxidative stress occurs when the production of oxidants exceed that of the antioxidants. And as studies have shown the roles oxidative stress plays in HIV disease progression including viral replication even in the face of antiretroviral drugs (Drain et al., 2007), there is therefore need for a balance in the intracellular redox environment which should be more reducing than oxidative species to maintain optimal cell function. This is of greater importance because the study carried out by Nkengfack et al. (2012) shows that the antioxidants depend, firstly on the integrity of an enzymatic system mostly the endogenous antioxidant enzyme such as superoxide dismutase and their effective interactions with biological membranes and other co-antioxidant molecules like vitamins C and E (Young and Lowe, 2001).

Superoxide dismutase acts by catalyzing the breakdown of the superoxide anion produced during the activation of polymorphonuclear leucocyte (Adler et al., 1999) into oxygen and hydrogen peroxide (Johnson and Guilivi, 2005). In human, three forms of SOD exists; cystolic SOD (SODI Cu-zn) (Milani et a!., 2011), mitochondrial SOD (SOD2 Mn-SOD) and extracellular SOD (SOD3) (Antonyuk et al., 2009}. The mitochondrial isozyme seem to be the most biologically important of these three (Johnson and Guilivi, 2015) and there seem to be a possible direct interaction between Tat protein of HIV-1 and SOD2 transcripts gene as it has the ability to suppress it (SOD2).

Vitamins C acts by reducing the prooxidants in the cytosol and blood plasma (Benzie, 2003). Antioxidant protects the cell membrane against tipid *peroxidation. The neutralizing capabilities of these antioxidants reside on their ability to donate electron to ward off the deleterious effects of the highly reactive radical or by converting them into more harmless molecules (Bartz and piantadosi, 2010). Several studies have reported the reduced levels of SOD, vitamins C and E (Attord and Schoffer, 2006) in HIV-infection as a result of immunological response to eliminate the virus, and the possible health benefit to the immune system in their adequate supplementation with antiretroviral drugs.

As a result of the call for an urgent and result driven approach in order to reduce the mortality and morbility rates of HIV in children and the effective management of the already infected ones, the research was designed to evaluate the levels of SOD, vitamins C and E in children infected with this virus.

Materials and Methods

Eighty four (42 male and 42 female) HIV positive children aged 2-12 years attending HIV/AIDS Clinic at Federal Medical Centre Umuahia, were recruited for the study after receiving both written and oral informed consents. Controls were forty-two (21 male and 21 female) apparently healthy subjects still within 2-12 years of age.

Those screened HIV positive for the first time but had not been exposed to any antiretroviral drug and showed no AIDS indicator conditions based on laboratory and clinical evaluations were taken as group III while those on antiretroviral drug were in group II then the controls as group I. Subjects thereby neutralizing them into less harmful substances while Vitamin E as a Lipid soluble showing any underlying chronic illnesses and reactive to any other viral infection other than HIV were excluded from the study.

BLOOD SAMPLE COLLECTION

In all subject 4ml of blood was collected into a dry plain tube and allowed to clot. The clotted samples were centralized at 3000rpm for 10 minutes to express out the plasma. The sera were stored at -20°c prior to use.

HIV TESTING AND CONFIRMATION

The samples were screened for HIV antibodies using Enzyme linked immunosorbent assay method while the confirmation was done using Western blot immuno assay method.

ESTIMATION OF SUPEROXIDE DISMUTASE

Superoxide dismutase was estimated colorimetrically using Caymann Assay Kit which is a modification of method described by Malstrom et al. (1975).

ESTIMATION OF NON-ENZYMATIC ANTIOXIDANTS

Vitamin E was estimated using the method as described by Quaife and Dju, (1949) while the vitamin C was estimated colorimetrically using cosmo Bio Assay Kit, a modification of method described by Daniel et al. (1973).

STATISTICAL ANALYSIS

The values of the results were expressed as mean ± SD (standard deviation). The statistical analysis was done using one way ANOVA and pearson correlation with statistical package for social sciences (SPSS) version 17 the level of significant were at $p<0.05$ and $p<0.01$ respectively.

Results

TABLES 1 MEAN ± SD OF SUPEROXIDE DISMUTASE AND NONENZYMAT1C ANTIOXIDANT VITAMINS (C AND E) OF THE STUDIED GROUPS

GROUPS	SOD(u/mL)	VITC (ng/ML)	VITE (nmol/L)
1 II	7.81 1.5	6.510.8	10.411.8
	3.4+1.0*	4.810.5*	7.410.7*
III	2.8±0.9**	3.710.5**	6.110.5**

*- significantly different from I (control)

** = significantly different from groups 1 and II at P<0.05

Table 1: Shows the results of SOD, vitamin C and E of the studied population. There was a significant (P<0.05) decrease in the levels of SOD, Vitamins C & E {3.4± 1.0(U/ml), 4.81.0.5(u,g/ml) and 7.4 ± 0.7 (nmol/L) of group II when compared with control (groupl) (7.8 ±1.5(U/ml); 6.5 ± 0.8 (Ng/ml); and 10.4 ± 1.8 (Nmol/L). And the group III showed a more significant decrease (P<0.05) when compared with the group I (control) (and group II.

Discussion

This study shows an increase in oxidative stress measured by SOD, vitamins C and E in the HIV infected children. Stress as described by Dimitrios et ai, (2003) is a process of altered biochemical homeostasis produced by psychological, physiological or environmental stressors .

The oxidative stress occurs when there is increased production of reactive oxygen species (ROS). And ROS are produced within cells by redox reaction associated with normal physiological process such as in mitochondria electron transport chain perosomes, endoplasmic reticulum and by the phagocytic cells (Neutrophils and macropheges) (Lam et al., 2010). These phagocytic cells use the emzymes nicotinamide adenine dinucleotide phosphate (NADPH)- oxidase and myeLoperoxidase which they contain to produce hydrogen peroxide and hypochlorous acid respectively, needed to destroy engulfed pathogens. On the other hand NADPH oxidase catalyzes the production of superoxide anion from NADPH from where hydrogen peroxide is produced by the action of superoxide dismutase.

Under normal physiologic condition the ROS produced in the course of metabolism are contended by the antioxidant system thereby protecting the functional and structural moleculesagainst ROS mediated tissue damage (Aquaro et al., 2008). But in pathologic condition as observed in HIV infection, there is increased production of these ROS especially the hydroxyl radical, formed from the reaction between hydrogen peroxide and metal ions (Fe 2+or Fe 3+) which reacts spontaneously with protein and DNA leading to emzyme inhibition and denaturation .

The decline in vitamin C and E, including SOD in the study stems from their involvement in the termination of the chain rections instituted by ROS by removing the free radical intermediates and inhibition of further oxidation reaction by being oxidized themselves, hence consumed in the process. This is in agreement with the finding of Farumbi et al. (2000) that the cellular level of vitamins C and E and other enzymatic antioxidants are closely interlinked to each other.

The improvement in antioxidant status of HIV-infected children on HAART supports the finding of Marta et al. (2011) which posits that antiretroviral therapy leads to a decrease in viral replication, a reduction in the inflammatory environment and a reco nstitution of immunity. Hence the need for the supplementation of these antioxidants since they have been observed to down regulate Tissue necrosis factor activity which enhances oxidative stress.

Conclusion

This study shows a reduced levels of SOD vitamin C and E in Hiv infection. Hence there are increase loss of other micronutrients including zinc and copper which are components of SOD whose deficiency may affect the enzyme activity there is therefore need to extend a comprehensive research on the levels of these micronutrients for the restoration of the immune system and effective management.

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