CHANGE IN LIPID PEROXIDATION MARKER (MDA) AND NON ENZYMATIC ANTIOXIDANTS (VIT C & E) IN HIV SEROPOSITIVE CHILDREN IN AN URBAN COMMUNITY OF ABIA STATE, NIGERIA.

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ABSTRACT

This study was designed to evaluate the changes in antioxidant vitamins (C&E) and malondialdehyde as a marker of lipid peroxidation in HIV infected children. The study was carried out at the HIV/AIDS clinic FMC Umuahia with a total number of One hundred and twenty-six (126) subjects aged 2-12 years. They were grouped into control (group 1), HIV-infected children on therapy (group II) and HIV-infected children without therapy (group III). Each of these groups having 42 subjects with equal number of males and females. The levels of the MDA, vitamins C and E were determined spectrophotometrically after determination of the HIV-Statuses of each subject. There was an observed significant higher level of MDA (P<0.05) in HIV-infected children in group III (2.6± 0.6 nmol/L) when compared with group I (0.8± 0.1 nmol/L) and II (2.2 ± 0.5 nmol/L). The result also showed a significantly lower levels of vitamins C and E of group III children (3.7 ± 0.5 u.g/ml; 6.1 ± 0.5 u.mol/L) when compared with groups I (6.5± 0.5 u.g/ml; 10.4± 1-8 u.mol/L) and II (4.8±0.5 u.g/ml; 7.4±10.7 u.mol/L) respectively at P<0.05. A strong negative correlation was observed between MDA and the non enzymatic antioxidants. The changes were found not to be sex dependent as it suggested increased lipid peroxidation and oxidative stress.

KEYWORDS: Lipid Peroxidation Marker (MDA), Non Enzymatic Antioxidants (VIT C & E), HIV Seropositive Children
HIV infection causes deregulated production of cytokines (Monsmann, 1994) which triggers oxidative stress with consequent lipid peroxidation and loss of important antioxidants vitamins (C and E). This leads to severe decline in thymic function which in turn produces a progressive impairment of functional immunity (Correa and Murioz-fernandez, 2001) which is ROS mediated. The morbidity and mortality rate associated with HIV infection has made it a global public health challenge especially in developing countries, among which in Nigeria. There had been concerted efforts to reduce this infection in children through several initiatives that have yielded positive result especially in the Sub Saharan Africa (Wole, 2013). The intracellular sources of reactive oxygen species (ROS) include nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, xanthine oxidase, mitochondria electron transport chain, peroxosomes and the endoplasmic reticulum (Lam et al., 2010). And studies have shown the important roles they (ROS) play as signaling molecules that regulates many signal-transduction pathways and in cell survival, death and immune defenses (Huang et al., 2011). The increase in the production of ROS in the face of diseases such as HIV infection, maybe by overactive NADPH oxidase system both in phagocytic and non phagocytic cells, may set in motion a vicious cycle of radical and non radical oxidants generationin various cellular compartment which disruptsredox circuits that are normally controlled by thiol - dependant antioxidant defences and reduces a state of oxidative stress (Siham and Lisbeth, 2012). Oxidative stress is an imbalance between the system manifestation of reactive oxygen species and abiological systems ability to readily detoxify the reactive intermediate or to repair the resulting damage (Halliwell,2007). Enhanced oxidative stress has been known to favour HIV replication which is mediated by H₂O₂ - induced long terminal repeat (LTR) activation (Pyo et al., 2008) Lipid peroxidation being a free radical reaction occurs when hydroxyl radicals react with the unsaturated lipids, of biomembranes resulting in the generation of Lipid peroxide radical (ROD) lipid hydroperoxide (ROOH) and fragmentation products such as malondialdehyde (MDA) (Uchida, 2000). The peroxidation of lipid result in increased membrane rigidity, decreased activity of membrane bound enzymes, altered permeability, formation of hydrophobic centres alteration of protein structures, mutagenicity, inhibition of growth and protein synthesis (Bertholomew et al., 2011) Several studies have reported increased peroxidation products such as MDA and decreased levels of antioxidant vitamins such as C and E (Attord and Schoffer, 2006) and how increased lipid peroxidation is directly linked to HIV Progression to AIDS and HIV viral load (mRNA) (Bertholomew et al., 2011). The insight into the adverse effects of
increased ROS including the .Orchestration of the response by inducing lipid oxidation which triggers thymic stromal lymphopoietin production by epithelial cells (Siham and Lisbeth, 2012) has been known, with attendant loss of antioxidant vitamins, (C&E). This research work was aimed at ascertaining the changes associated with Malondialdehyde (MDA) following lipid peroxidation and the levels of vitamins C and E in HIV infection.

**MATERIALS AND METHODS**

One hundred and twenty six (126) subjects were recruited for the study. The test groups are HIV-positive children attending the HIV/AIDS clinic of Federal Medical Centre Umuahia. They were grouped into 42 each of control (group I) comprising of the apparently healthy children, group II are the HIV-Positive children on therapy (HAART) and group III are HIV positive children without therapy (HAART) with equal number of males and females and within the ages of 2-12 years. The subjects that reacted to any other viral infections, sicklers and other chronic diseases were excluded from the study.

**BLOOD SAMPLE COLLECTION**

About 4ml of blood was aseptically collected from each subject into a dry container. After clotting the samples were spun at 5000rpm, separated, and stored at -20°C prior to investigation.

**HIV SCREENING AND CONFIRMATION**

The samples were screened for HIV antibodies using ELIZA method and the confirmatory using westernblot method.

**ESTIMATION OF MALONDIALDEHYDE (MDA)**

MDA was determined using OXITEK TBARS assay kit which is a modification of method as described by Armstrong and Brown, (1994).

**ESTIMATION OF NON-ENZYMATIC ANTIOXIDANTS**

Vitamin C was estimated colorimetrically using Cosmo Bio assay kit which is a modification of method described by Daniel et al., (1973) and Vitamin E estimated by the method of Quaife and Dju (1949).

**STATISTICAL ANALYSIS**

The results were expressed as Mean ± SD. One way ANOVA and Pearson correlation were used for the statistical analysis at 5% level of significance using the statistical package for social science (SPSS) version 17.
Table 1: Mean ± SD of MDA and non-Enzymatic antioxidants vitamins (C & E) of the studied groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MDA (nmol/ml)</th>
<th>VITC (μg/ml)</th>
<th>VITE (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.8±0.1</td>
<td>6.5±0.8</td>
<td>10.4±1.8</td>
</tr>
<tr>
<td>II</td>
<td>2.2±0.5*</td>
<td>4.8±0.5</td>
<td>4.8±0.5</td>
</tr>
<tr>
<td>III</td>
<td>2.6±0.6**</td>
<td>3.7±0.5**</td>
<td>6.1±0.5**</td>
</tr>
</tbody>
</table>

* Significantly different from I
** Significantly different from I & II at P<0.05

Table 1: The result above shows a significantly higher (P<0.05) levels of MDA in group III (2.6 ± 0.6 nmol/ml) when compared with groups I (0.8 ± 0.1 nmol/L) and II (2.2 ± 0.5 nmol/ml) while a significant decrease (P<0.05) was observed in antioxidant vitamins (C & E) in group III (3.7 ± 0.5 μg/ml; 6.1±0.5 μmol/L) when compared with groups I (6.5 ± 0.8 μg/ml; 10.4 V 1.8 μmol/L) and II (4.8 ± 0.5 μg/ml; 7.4 1 0.7 nmol/L) respectively.
The significantly increased level of MDA is HIV infected children as observed in this study is in keeping with the generally high levels of MDA find in some other studies including that of Suresh et al. (2009). This increase was observed with decrease in the levels of antioxidant vitamins (C and E) which is indicative of oxidative stress and lipid peroxidation.

Lipid peroxidation refers to the oxidative degradation of lipid, when free radicals steal electrons from lipids in cell membranes, resulting in cell damage (Muller et al., 2007). The HIV replication secondary to ROS production by a pro-oxidant effect of inflammatory Cytokines and/or polymorphonuclear leucocyte activation causes insufficiency in the levels of both enzymatic and non-enzymatic antioxidants to circumvent the replication.

The antioxidants deficiency is probably due to depletion of antioxidant molecules when they are consume in the process of protecting cells against ROS induced oxidative damage in magnitude that is related to advancement of the disease to AIDS, and the weakened antioxidant defense system in turn lead to further enhancement in lipid peroxidation (Ogunro et al., 2005). Other researchers have shown that the low antioxidants could be from greater utilization subsequent to increase oxidative stress rather than inadequate dietary intake (Dworkin et al., 1990) or malabsorption (Kapembwa et al., 1990), inadequate nutrient release from the liver, acute infections and/or inadequate availability of carrier molecules that may influence circulating antioxidant concentration (Das et al., 1996).

Vitamin E, a potent chain breaking lipid soluble antioxidant acts by reacting with lipid peroxyl radical eventually terminating the peroxidation chain reaction and thereby reducing oxidative damage while vitamin C, that represents the major water soluble antioxidant in the human body (Stambullian et al., 2007) acts in the cytosol and plasma against the pro-oxidants. But in the face of HIV infection which causes disturbances in the normal redox state through the production of peroxides and free radicals, lead to damage to protein, lipid and DNA (Jeni, 2011) with consequent production of malondialdehyde (MDA). MDA, a three carbon, low molecular weight aldehyde, therefore is a biomarker of oxidative attacks on polyunsaturated fatty acids of biological membranes (Lipid peroxidation).

The different antioxidants have a synergistic and interdependent effects on one another that are, the action of one antioxidant may depend on the proper function of other members of the antioxidant system (pratviel, 2012). Therefore the amount of protection provided by anyone antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species, the antioxidant being considered and the status of which it interacts.

In this study there was an observed negative correlation between MDA and the non-enzymatic antioxidants (C&E) which is suggestive of increased oxidative stress and subsequent lipid peroxidation in HIV infection that must have adversely affected the normal redox state of the body cells.

CONCLUSION
The study revealed an increased levels of MDA in HIV infected children with decreased levels of non-enzymatic antioxidants (C&E) which shows an increased oxidative stress and lipid paroxidation mediated by ROS.

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