Discordant Levels of Superoxide Dismutase and Catalase Observed in ART Naïve and Experienced HIV Patients In Southeastern Nigeria

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Abstract: Background: Patients with Human Immunodeficiency Virus (HIV) present a variety of pathologic alteration that is related to oxidative stress. Since oxidative status are important in HIV infection, the activity profile of primary endogenous antioxidants may be a complementary tool in accessing the progression of HIV infection.

Summary: In this study, the activity of superoxide dismutase and catalase in 66 (33 highly active antiretroviral therapy (HAART) experienced and 33 HAART naïve subjects) HIV patients aged 40 ± 18 receiving antiretroviral therapy was evaluated. In this case-control study, endogenous enzymes and malondialdehyde were determined spectrophotometrically after confirmation of HIV statuses. Cluster of differentiation antigen no. 4 (CD4) assessment was done using cytofluorimetric analysis and Spearman’s correlation ranking was applied to generate coefficients of CD4 cells and antioxidants. The results were presented in relative percentages. Higher number of HIV positive females (66.7%), married couples (62.5%) and secondary level of education (46%) was observed to be on HAART. MDA concentration was lower in HAART experienced (2.04± 0.14) subjects than in the naive group (3.08± 0.12nmol/ml). Superoxide dismutase and catalase activities reduced significantly (P<0.05) in HAART naïve (non-HAART) subjects (catalase: 17.5± 1.01iu/ml/ SOD:2.71± 0.91 IU/ml) when compared with the HAART experienced (catalase: 32.83± 3.02/SOD: 3.73± 0.89) and the healthy control group (catalase:42.02± 2.35/SOD: 8.15± 0.78). Correlation values of CD4 and CAT/SOD were positive (r=0.551/r=0.943) for HAART and negative (r= -0.451/ r= -0.331) for non HAART subjects at P<0.01. The data obtained showed a reduced OS and a restoring primary antioxidant defense system in HIV positive patient on antiretroviral treatment. Enhanced oxidative stress contributes to HIV pathogenesis and suggests that antioxidants could reduce OS due to antiretroviral therapy.

Keywords: Human immunodeficiency Virus, Superoxide dismutase, Catalase, Antioxidant.

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) induces a variety of immunologic alterations which results in progressive development of opportunistic infection a malignancy known as Acquired Immunodeficiency syndrome (AIDS) [1]. In all the mechanisms contributing to HIV disease progression [2, 3], oxidative stress induced by the production of reactive oxygen species (ROS) plays a critical role in the stimulation of HIV replication and development of immune deficiency [4, 5]. Excessive production of ROS such as superoxide anions, hydroxyl radicals and hydrogen peroxide maybe as a result of increased activation of polymorphonuclear leukocytes during HIV infection [6]. The level of oxidative stress (OS) is determined by the balance between pro-oxidative and anti-oxidative activities which leads to increased free radical formation and possible induction of oxidative injury in cells [7]. Several studies have shown clear evidence that oxidative stress may contribute to several aspects of HIV disease, including viral replication [8,9] even in those treated with antiretroviral therapy [10,11]. This type of oxidant challenge affects the cellular system, and creates responses that may be favourable for the replication of HIV [12,13].

A balance is important in this situation because the intracellular redox environment must be more reducing than oxidative species to maintain optimal cell function [14]. Antioxidants therefore, counteracts the effect of cellular oxidants and may in patients with HIV infection and advanced immunodeficiency lead to immunological and virological effects with the possibility of having a therapeutic value [15]. However, antioxidants have been linked to have a positive influence on the CD4 cells and viral load of subjects infected with the virus [16]. These antioxidants depends first, on the integrity of an enzymatic system mostly the endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).

Superoxide dismutase (SOD: EC 1.15.1.1) allows organisms to fight against reactive oxygen species (free radicals) by destroying the free radical superoxide through its conversion to peroxides [17] that can in turn be destroyed by catalase [18]. Catalase an enzyme found in nearly all organisms when exposed to oxygen,
catalyzes hydrogen peroxide to water and oxygen [19]. SOD specifically, catalyses the dismutation of superoxide ion radical by successive oxidation and reduction of the transition metal ion at the active site in a Ping-Pong type mechanism [20]. Four classes of SOD have been identified each containing either a dinuclear Cu/Zn or mononuclear Fe/Mn/Ni cofactors [21]. In humans, three forms of SOD exist; cytosolic Cu-Zn SOD (SOD1) which is encoded by the SOD1 gene, located on chromosome 21 [22], mitochondrial Mn-SOD (SOD2) [23] and extracellular-SOD (SOD3) [24]. In HIV-1, the regulatory transactivation (Tat) protein have the ability to suppress the expression of cellular Mn-containing superoxide dismutase (Mn-SOD) [25]. Therefore, a possible direct interaction between Tat protein of HIV and Mn-SOD gene transcripts exists.

ROS as apparently known is widely used as messengers in the activation of the transcription promoter nuclear factor –kappa binding (NF-κB) for HIV-1 replication. HIV activation through NF-κB maybe a more direct consequence of ROIs [26]. The study therefore, determines the activities of the endogenous enzymes SOD and Catalase in HIV HAART experienced subjects as a possible complementary biomarker for HIV treatment response assessment.

2. RESULTS

2.1. Study Population and Sample Collection

Positive HIV Patients (120) attending HIV clinic at Federal Medical Centre Umuahia, Nigeria were recruited for this study after informed consent according to the Nigerian National Ethics and Operational Guidelines for Research on Human Subjects (NNEOGRHS). Those screened HIV positive for the first time but had not been exposed to any HAART regime and showed no clinical sign(s) of any other illness or co-infection based on laboratory and clinical criteria were grouped as non-HAART (ART naïve) subjects (n = 60). The samples (plasma) collected were screened to detect antibodies for HIV and confirmation was by enzyme immuno assay method.

Sixty (60) subjects were on first-line treatment with no other confirmed co-infection as HAART group (positive control/ART experienced). Forty (n=40) HIV-

Table 1: The Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HAART (n=120)</th>
<th>-ve Control (n=40)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40 ± 18</td>
<td>38±52</td>
<td>0.091</td>
</tr>
<tr>
<td>Education (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>secondary</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>postsecondary</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>postgraduate</td>
<td>06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (Female/Male; n (%))</td>
<td>80(66.7)/40(33.3)</td>
<td>NA</td>
<td>0.032a</td>
</tr>
<tr>
<td>Marital Status (Married/Single; n(%))</td>
<td>75(62.5)/45(37.5)</td>
<td>NA</td>
<td>0.040 a</td>
</tr>
<tr>
<td>Length of HAART (Weeks)</td>
<td>&gt;3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 Count subjects (%) n</td>
<td>1.50-200 cells/µl, (8.3), 10</td>
<td>1150±75 cells/µl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 &gt;200 cells/µl, (91.67), 110</td>
<td></td>
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</tr>
</tbody>
</table>

Comparison of data was done at P<0.05, data on gender, marital status, education and CD4 (measured as cells/µl) were presented as absolute numbers (n) and percentages (%) while age were shown as mean ± standard deviation in years.NA= indicates none applicable, Data with the superscript * indicates statistical significance.
negative healthy subjects were included as negative control group. The demographic data was recorded upon enrolment of the subjects using structured questionnaire. Venipuncture heparin anticoagulated peripheral blood samples from a total of 160 individuals of average age 40 ± 18 was thus obtained from the USAID unit of Federal Medical Centre (FMC) Umuahia from June to December 2010. Sample storage was at 4-8°C. There was no significant difference between the ages of the HAART group (40±18 years) and that of the negative control group (38±52). Subjects with secondary level of education recorded the highest incidence of HIV infection (46%). HIV positive females (66.7%) and married couples (62.5%) had a significantly increased incidence when compared with males and single subjects respectively. Over ninety-five percent (91.67%) of the HAART subjects had CD4+ cells equal or greater than 200 cells/μl while 8.3% were within the range of 50-200 cells/μl (Table 1).

2.1.1. Treatment Regimen of HAART Subjects

The subjects (n=60) received a generic fixed-dose HAART of Stavudine (40 mg) + Lamivudine (150 mg) + Nevirapine (250 mg) while 16.7% (n = 10) received Zidovudin (250 mg) + lamivudine (150 mg) + Nevirapine (250 mg) all twice daily for a minimum period of three months. The prescription of Stavudine was based on 40 mg for >60 kg and 30 mg for <60 kg of body weight.

2.2. Measurement of Lipid Peroxidation

Lipid peroxidation was measured as plasma malondialdehyde (MDA) estimation according to the spectrophotometric method of Wallin [27]. Thiobarbituric acid (TBA) reacting substances reacts with MDA to give a pinkish colour which absorbs maximally at 532nm. TBA-reacting substances were quantified as lipid peroxidation product by referring to a standard curve prepared using 1,2,3,3, tetraethoxypropane.

Figure 1. Shows level of oxidative stress measured as MDA (nmol/ml). The MDA concentration (3.08± 0.12nmol/ml) was significantly (P<0.05) increased in non-HAART when compared with the HAART experience (2.04± 0.14) and control (0.90± 0.011) groups. MDA levels of HAART experienced group was elevated significantly when compared with the healthy negative control.

2.3. CD4 Cell Expression

The CD4+ lymphocyte count was estimated by a Fluorescence Activated Cell Sorter (FACScan...
flowcytometer) count system (Becton Dickinson, Franklin Lakes, NJ, USA). Manufacturer’s instruction and guide was strictly followed and the result expressed as cells/L. The correlation of CD4⁺ expression and endogenous antioxidant (CAT and SOD) activity of non-HAART subjects was negative (r=-0.451 and r= -0.331 respectively). Conversely, the HAART group correlates positively with CAT(r=0.551) and SOD (r=0.943) activities at P<0.01 significant level (Table 2).

Table 2: Correlation Coefficients of CD4 Expression and Endogenous Antioxidant (CAT and SOD) Activity

<table>
<thead>
<tr>
<th>HAART (CD4 IU/ml)</th>
<th>Non-HAART (CD4 IU/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT 0.551</td>
<td>-0.451</td>
<td>0.01</td>
</tr>
<tr>
<td>SOD 0.943</td>
<td>-0.331</td>
<td>0.01</td>
</tr>
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</table>

2.4. Endogenous Antioxidants

The superoxide dismutase activity was determined with the direct spectrophotometric method employing KO₂ as previously described by Marklund, with certain modifications [28, 29]. Catalase activity was measured based on the method describe by Cohen et al. [30]. The activities of endogenous antioxidants, SOD and CAT (Figures 2 and 3 respectively) were similar. The results obtained from the analysis showed a significant (P>0.05) decrease of catalase (17.5 ± 1.01 IU/ml) and SOD (2.71 ± 0.91 IU/ml) activities in antiretroviral naïve subjects (non-HAART group) on comparison with antiretroviral experienced group (HAART: CAT: 32.83 ± 3.02 IU/ml and SOD: 3.73 ± 0.89 IU/ml) and the healthy control group (CAT: 42.02 ± 2.35 and SOD: 8.15 ± 0.78 respectively).

2.5. Statistical Analysis

The values were expressed as mean ± SD (Standard deviation). The statistical analysis was carried out by one way analysis of variance (ANOVA). The Pearson value was considered significant at (p<0.05) using Statistical Package for Social Sciences (SPSS) version 18. Correlations were evaluated by Spearman’s correlation significant at p<0.01.

Figure 2: SOD Activity of ART naïve (Non-HAART) and ART experienced (HAART) Subjects.
3. SUMMARY

Naturally, cells produce a variety of antioxidant enzymes that challenge the deadly effects of life with oxygen. Two important key enzymes are superoxide dismutase which converts superoxide radicals into hydrogen peroxide, and catalase which converts hydrogen peroxide into water and oxygen gas. Deliberately built, these enzymes are prevalent in the human cell patrolling the cell and counteracting the steady production of hydrogen peroxide and keeping it at a human safe level.

Several works have noted higher prevalence of female HIV infection [31,32] particularly in Africa. According to UNAIDS record [33], females constitute 58% (about 1.72 million) of persons living with HIV in Nigeria and each year 55% of AIDS deaths occur among women and girls. Result of the study had sixty-six percent (66%) of the subjects on HAART as female. It shows that HIV infection in Nigeria is gender sensitive. This could be attributed to majority of the women living in poverty and having very low economic empowerment with the consequence of adopting risky behaviours. These attitudes which include the exchange of sexual favours for food, shelter or money to support themselves and their families expose them to HIV infection. Secondary level of education (46%) and married (62.5%) subjects seem to be high in the data analyzed. It could be understood that the level of education expose individuals to the basic knowledge of HIV infection and prevention mechanism(s). Also, keep individuals abreast of current development on HIV infection and being receptive of the changes and dynamics of the disease. Studies have shown that only 23% of women in Nigeria have comprehensive knowledge of the mode of HIV transmission and prevention [34] of HIV. Several others have associated lower infection rate to higher education level [35]. The increase in HIV amongst married partners may be attributed to attitudinal change resulting from religious and cultural observation inherent in most parts or sub-Saharan Africa. Most religion do not support the use of contraceptives such as condom in marriage. For those who have extra marital affairs this attitude also extends either consciously or unconsciously to their sexual partner(s).
A higher oxidative stress (measured as MDA) condition was found in HAART naïve subjects when compared with the experienced groups, though oxidative stress is increased significantly both in naïve and experienced subjects. Humans infected with HIV are under chronic immune activation that is characterized by increased generation of reactive oxygen species (ROS) and disruption of the antioxidant defense system as was also reported by Lizette et al. [36]. In HAART naïve subjects, the high ROS production could be attributed to the activation of phagocytes and neutrophils through the virus activity. In that case, the activation of the immune system may produce a significant increase in ROS generation. Consequently, when in excess amounts, dysfunction of macromolecules such as proteins, nucleic acids, carbohydrates and lipids set in. Most cells that internalize HIV activate a ROS generating NADPH oxidase enzyme (NOX2, gp91phox) and others in turn contribute to the ROS increased production [37]. The major mechanism of HIV induced OS activation is through immune activation which mediates increase in ROS production with consequent modulation and activation of nuclear transcription factors. This ultimately leads to viral gene expression as reported by Gendron et al. [38]. He also showed that HIV-1 internal ribosome entry site (IRES) located in the 5'untranslated region is activated during viral infection. Oxidative Stress initiated by HIV infection increases the activity of the IRES. The HIV viral protein Tat, is also specifically responsible for an endogenous cellular increase of ROS. Furthermore, oxidative stress resulting from HIV infection participates in CD4+ T lymphocyte depletion through increase in the rate of Fas-induced apoptosis and facilitates nuclear factor-kappa binding (NF-kB)-dependent activation of HIV transcription. The Sp and NF-kB factor binding sites in the core promoter play important cell type-specific roles in regulating transcription and replication. Since HIV replication is reduced during HAART treatment, CD4 cell count improved and antioxidant capacity restored, the correlation results showed positive and negative correlations between CD4+ cell and SOD/CAT in HAART experienced and non-HAART respectively (Table 2). This could suggest a reduced OS environment and increased SOD/CAT in HAART experienced compared to HAART naïve subjects though both maintained higher OS and reduced endogenous antioxidants in comparison with healthy control group. The study concurs with reports provided by Pasupathi et al. [39]. It can be envisaged that increased oxidant stress is a component of the dynamic process of immune reconstitution in some subjects, rather than a more direct effect of drug.

The use of antiretroviral therapy has dramatically influenced HIV infection with significant decrease in the production of ROS and improvement in quality of life of HIV infected patients. The study findings simply raise some critical questions on the extent and implications of OS generated in HIV infection/replication process against HAART administration. Generally, it is acknowledged that antioxidant enzyme levels are sensitive to oxidative stress. Increase in SOD and CAT in HAART group could indicate progressive treatment with HAART when compared with the HAART naïve subjects (Figures 2 and 3). SOD is a key endogenous enzyme and has been linked to several disease states. Some studies have demonstrated that SOD-TAT, a fusion protein of HIV-1 Tat protein transduction domain and hCuZn-superoxide dismutase (SOD), has been proved to be effective in preventing and treating lung injury [40]. This study demonstrated SOD-TAT's radioprotective effects on lung injury in irradiated mice. The physiological importance of SODs is shown by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die few days after birth due to massive oxidative stress [41]. Several pathologies of mice deficient SOD has been observed. Deficiency of SOD1 develops hepatocellular carcinoma [42], an acceleration of age-related muscle mass loss [43], early incidence of cataracts and a reduced life span. SOD3 are more sensitive to oxidative injury [44]. Generally, knockout mice of any SOD enzyme form are more sensitive to the lethal effects of superoxide generating drugs. Also reduced SOD3 activity has been linked to lung diseases such as acute respiratory distress syndrome (ARDS) or Chronic obstructive pulmonary disease (COPD) [45].

We could presume that SOD/CAT are protective in nature as can be attested to in the correlation results. Inversely the observed decrease in CAT and SOD activities in the HAART naïve subjects could be attributed to rapid depletion of these endogenous enzymes by high level of circulating chemically reactive species associated with the HIV untreated patients [45,46]. The decrease in SOD and CAT activities in HIV untreated patients accounts for the production of high reactive oxygen species (Figure 2) in these patients. This is in line with the study of Lizette et al. [36] who suggested that increased oxidative stress might be
attributed to the deficiency of antioxidant defense system. This deficiency in HIV untreated patients maybe due to increased utilization of antioxidant enzymes such as superoxide dismutase and catalase.

From the results obtained in this study, it can be deduced that antiretroviral treatment during infection plays a major role in the reduction of reactive oxygen species associated with HIV infection. Hence restoring the antioxidant defense system in HIV-infected patients. This interpretation should not be misconstrued since OS is increased during HAART administration patients. This deficiency in AIDS untreated patients on antiretroviral therapy suggests that the patients will be better prepared for HAART compliance and possibly have a more efficient combined antiretroviral therapy if supplemented with antioxidants. These findings also support the idea that enhanced oxidative stress contributes to HIV pathogenesis and suggest that antioxidants could reduce stress due to antiretroviral therapy [47].

CONCLUSION

The study shows a reduced oxidative stress and a restoring primary antioxidant defense system in HIV positive patient on antiretroviral treatment. Improving the antioxidant defense in HIV patients serves as a better platform for HAART compliance and increased efficacy of the combine antiretroviral therapy. The study also raises the question of oxidative stress initiated by HAART versus those of HIV infection. Elaborate studies are needed to quantitatively determine the magnitude of OS generated in these two processes.

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CONFLICT OF INTEREST

There is no potential conflict of interest.

AUTHORS’ CONTRIBUTIONS

Ibeh Bartholomew O. Conceptualized and designed the research, contributed to all phases of the research and drafted the manuscript. In addition, contributed to analysis and interpretation of results, data analysis as well as preparation of the manuscript. Habu Josaih. Collected and analyzed the data for each study included in the research. Contributed in preparation of the manuscript.

S. EZE. Laboratory analysis and data generation, literature search and result analysis. All authors reviewed and approved the final version of this manuscript.

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