OXIDATIVE STRESS, CD\textsuperscript{+} CELL COUNT AND ANTIOXIDANT STATUS IN HIV/AIDS PATIENTS

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Abstract: Infection by human immunodeficiency virus (HIV) causes persistent chronic inflammation. Severe oxidative stress has been reported in HIV/AIDS patients because of malnutrition and poor immunity, the hallmark of HIV infection is cellular CD4\textsuperscript{+} immunodeficiency. The aim of this study was to investigate the lipid peroxidation products and CD4\textsuperscript{+} cell count, non-enzymatic and enzymatic antioxidant status in HIV infected patients and acquired immunodeficiency syndrome (AIDS) patients. The study population consisted of 150 subjects divided into three-groups [HIV positive patients 50 cases (HIV n=50), AIDS patients 50 cases (AIDS n=50) and an equal number of age- and sex-matched control subjects (C n=50). The level of plasma thiobarbituric acid reactive substances (TBARS) was markedly more in the AIDS patients when compared to HIV positive patients and control groups. The low CD4\textsuperscript{+} cell counts in AIDS patients (\textless 150/mm\textsuperscript{3}) when compare to the HIV positive patients (\textless 375/mm\textsuperscript{3}) and control subjects (> 750/mm\textsuperscript{3}) was statistically significant. Further the CD4 count decreased due to progression of the disease. Significantly low levels of plasma vitamin A (\(\beta\)-carotene), vitamin C, and \(\alpha\)-tocopherol were observed in AIDS patients when compared with HIV positive patients and control subjects. The activities of superoxidedismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and reduced glutathione (GSH) were significantly low in AIDS patients when compared with HIV positive patients and normal subjects. The study showed that in AIDS patient’s free radical activity is quite very high and antioxidant levels are low. The present study highlights the occurrence of lipid peroxidation and possible breakdown of antioxidant status in HIV/AIDS patients, which may subsequently increase the possibility of poor immunity. The antioxidant status was progressively depleted in HIV infected persons as the disease progressed from a symptomatic state to AIDS.

Key words: HIV, AIDS, Lipid peroxidation, CD\textsuperscript{+} cell count, Antioxidant status

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS), is a fatal illness caused by a retrovirus known as the human immunodeficiency virus that breaks down the body’s immune system, that infects CD4 cells initially and progressively leads to AIDS [1]. There are 2.47 million persons in India living with HIV, equivalent to approximately 0.36 percent of the adult population. The revised national estimate reflects the availability of improved data rather than a substantial decrease in actual HIV prevalence in India. The transmission route is still predominantly sexual (87.4 percent); other routes of transmission by order of proportion include prenatal (4.7 percent), unsafe blood and blood products (1.7 percent), infected needles and syringes.
(1.8 percent) and unspecified about 4.1 percent [2].

Oxidative stress in biological systems is caused by a relative overload of oxidants, i.e., reactive oxygen species. Sustained oxidative stress disrupts cellular structures and functions, which are maintained and mediated by critical oxidation-reduction (redox) pathways. The resulting damage to cells and tissues contributes to the pathophysiology of many diseases. Evidence has accumulated suggesting that patients infected with the human immunodeficiency virus (HIV) are under chronic oxidative stress [3].

The importance of micronutrient deficiency in determining the outcome of HIV infection is increasingly being recognized in the era of widespread antiretroviral drug treatment [4]. When the balance between free radicals and antioxidant supply is tipped, the resulting oxidative stress can cause many disease conditions and in the persons infected with the Human Immunodeficiency Virus (HIV) infection, it is reported that there is increased viral replication and a variety of biochemical and physiologic changes, which often result in metabolic impairment and cell death [5]. Antioxidants are compounds with chemical affinity for free radicals. They exist in abundance and bond with free radicals before free radicals can cause damage [6]. Compounds with antioxidant effects and replenishing mechanisms are in five classes, namely; enzymes, peptides, phenolic compounds, nitrogen compounds and carotenoids. The potential impact of reactive oxygen species (ROS) on plasma antioxidant status in HIV infection is substantial according to previous reports [7,8]. Consequently, excessive ROS production, if not countered by antioxidant molecules, can lead to oxidative stress which may play an important role in the progression of HIV infection [9]. The purpose of the present study was to determine the relationships among the levels of CD4+ cell count, antioxidant status and lipid peroxidation with predefined HIV/AIDS subjects.

MATERIALS AND METHODS

Study population: The population consisted of 150 subjects divided into three groups. HIV infected patients 50 cases (HIV n=50), AIDS patients 50 cases (AIDS n=50) and an equal number of age- and sex-matched control subjects (C; n=50) were investigated. The prospective study was carried out at the K. G. Hospital and Postgraduate Medical Institute, Coimbatore, Tamil Nadu, India from January 2006 to January 2008. For diagnosis and confirmation of HIV infection, we followed the National AIDS Control Organization (NACO) recommendations for HIV testing [10]. All the patients were subjected to detailed history taking and clinical examination. Informed consent of the patients was taken before testing.

Sample collection and erythrocyte lysate preparation: Blood samples were collected by venous puncture in heparinized tubes and the plasma was separated by centrifugation at 1000 g for 15 min. After the collection of plasma, the buffy coat was removed and the packed cells were washed thrice with cold physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate was separated by centrifugation at 2,500 g for 10 minutes at 2 °C. Biochemical estimations were carried out immediately.

Biochemical investigation: Blood glucose levels were determined using fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). Red blood cell count, total white blood cell count, total hemoglobin levels, platelet count and ESR were determined using fully automated hematology analyzer (Pentra-XL 80, USA). The CD4+ lymphocyte count was estimated by Fluorescence Activated Cell Sorter (FACS) count system (Becton Dickinson).

Estimation of lipid peroxidation: Lipid peroxides were estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Yagi [11] the pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated. The absorbance of clear supernatant was measured against reference blank at 535 nm.

Estimation of non-enzymatic and enzymatic antioxidants: Plasma β-carotene was estimated by the method of Bradle and Hombeck [12]. Plasma vitamin E was measured by the method of Baker et al. [13]. Plasma ascorbic acid was estimated by the method of Roe and Kuether [14]. Superoxide dismutase and catalase activities were estimated by the method of Kakkar et al. [15] and Sinha [16], respectively. Glutathione peroxidase was assayed
using Ellman’s reagent to quantify glutathione oxidized by the method of Rotruck et al. [17]. Reduced glutathione was determined by the method of Ellman [18]. Glutathione-S-transferase was assayed by the method of Habig et al. [19].

Statistical analysis: All the results were expressed as mean ± S.D for fifty subjects in each group. The differences between the variables assessed by unpaired T-test and Mann Whitney U-test using SPSS (version 13.0). A p value of < 0.05 was considered a significant difference between groups.

RESULTS

Information about the characteristics of investigated subjects is shown in Table I. The mean age limit was 37 ± 10 in AIDS patients, 33 ± 16 in HIV infected patients and 36 ± 9 in control subjects. Body mass index (BMI) in AIDS patients was 22 ± 4.3, kg/m², in HIV infected patients was 31 ± 5.2 kg/m² and in control 37 ± 6.2 kg/m². The body mass index was significantly low in AIDS patients as compared to HIV infected patients and control subjects. Diabetic participants were defined as those with a fasting blood glucose concentration > 120-mg /dl.

Table 2 shows the levels of total RBC count, total WBC count, platelet count, hemoglobin, hematocrite, ESR and CD⁴⁺ cell counts in control group, HIV infected patients and AIDS patients. The levels of total RBC count, total platelet, hemoglobin and hematocrite were significantly lower in AIDS patients as compared to HIV infected patients and control group. Significantly increased level of total WBC and ESR in AIDS patients when compared with HIV infected patients and control subjects was statistically significant. The decreased CD⁴⁺ cell counts in AIDS patients (p<0.001) (< 150/mm³) when compare to the HIV positive patients (< 375 /mm³) and control subjects (> 750/mm³) was statistically significant. The CD⁴ count was drastically decreased in AIDS patients as compared to HIV infected patients.

The level of plasma TBARS, vitamin A (β-carotene), vitamin C, vitamin E and GSH in control, HIV infected and AIDS patients is depicted in Table 3. Study showed that the extent of lipid peroxidation in AIDS patients was significantly increased as compared to HIV infected and control subjects. In AIDS patients, the concentration of lipid peroxidation was significantly higher than in HIV infected patients. On the other hand, the levels of non-enzymic antioxidants (β-carotene, vitamin C, vitamin E and GSH) were found to be significantly decreased in HIV/AIDS when compared to control group. The enzymatic antioxidants (SOD, Catalase, GPx and GST) in control and HIV/AIDS subjects are presented in Table 4. A significant decrease in the activities of SOD, CAT, GPx and GST in erythrocyte antioxidants was seen in AIDS patients as compared to HIV infected patients and control subjects. In AIDS patients, the non-enzymatic and enzymatic antioxidants were significantly lower than in HIV infected patients.

DISCUSSION

The present study is a comprehensive evaluation of concentrations of circulating antioxidants and markers of oxidative stress in HIV/AIDS patients. Results show lower antioxidant potential and enhanced lipid peroxidation in AIDS patients. These findings further support a role for oxidative stress in the pathogenesis of AIDS and suggest lower antioxidant capacity and higher oxidative stress in the HIV/AIDS patients than in healthy human volunteers. Reduced concentrations of β-carotene and of the anti-oxidant vitamins C and E were previously reported in patients with HIV [20-22]. We extended these findings by demonstrating significant reduction in enzymatic anti-oxidants (SOD, catalase) and non-enzymatic anti-oxidant (glutathione).

Several factors such as low food intake, nutrient mal-absorption and inadequate nutrient release from the liver, acute infections and an inadequate availability of carrier molecules may influence circulating antioxidant concentrations [23]. Increased ROS was previously reported in patients with HIV/AIDS [24]. Our finding of a significant correlation between high TBARS concentrations and low concentrations of some anti-oxidants, suggest increased utilization by ROS as an important contributing factor to the lower concentrations of anti-oxidants in HIV/AIDS patients. In fact, the combination of malnutrition leading to reduced supplementation of anti-oxidants and enhanced ROS generation leading to increased utilization of these compounds may represent a pathogenic loop that results in markedly enhanced oxidative stress during HIV infection.

The importance of vitamin A for immune function is well established. In prospective studies, a positive
association has been reported between mild exophthalmia [25], or low serum vitamin A levels and the risk of diarrhea and respiratory infection, which are important AIDS-related causes of death. Considerable evidence shows that administration of preformed vitamin A or β-carotene supplements in humans is associated with enhancement of humoral immune function [26], as well as cellular immune function, including an increase in CD4 cells and natural killer cells, a decrease in cells expressing interleukin (IL)-2 receptors, and increased delayed-type hypersensitivity [27]. Demonstration of an immunoenhancing effect of carotenoids lacking in provitamin activity suggests that an immunoenhancing effect of β-carotene may be at least partially attributable to its role as an antioxidant.

The relation of blood levels of vitamin A to progression of HIV disease has been examined in several epidemiological studies. The relationship between vitamin A deficiencies, as assessed by low plasma vitamin A levels, and mortality was examined in a nested case-control study conducted among HIV infected injection drug users [28]. In conclusion, it is well established that vitamin A is important for immune function. In observational studies, vitamin A deficiency is assessed by blood vitamin A levels has been consistently found to be associated with an increased risk of progression of HIV disease.

Vitamin E is the major lipid soluble antioxidant present in cellular membranes, which provides protection against lipid peroxidation [29]. Several studies in animals and humans suggest that vitamin E may increase immune response to antigens, improve host resistance against challenge with microorganisms, and enhance B and T cell lymphocyte functions, as well as phagocytic function [30,31]. Vitamin C is a water-soluble vitamin and can function as both an intracellular and extra cellular antioxidant [29]. Vitamin C deficiency in animal models results in depressed cell-mediated immune response, while in some human

### Table 1: Demographic characteristics of different study group. Values were expressed as means ± S.D, from fifty subjects in each group. ** P < 0.001 v/s.control; * P < 0.01 v/s.control; †P<0.001 versus HIV infected group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group (A) Control (n=50)</th>
<th>Group (B) HIV infected patients (n=50)</th>
<th>Group(C) AIDS patients (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± S.D.), years</td>
<td>36 ± 9</td>
<td>33 ± 16</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>Sex (Males)</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>10%</td>
<td>30%</td>
<td>32%</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>4%</td>
<td>25%</td>
<td>47%</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>2%</td>
<td>10%</td>
<td>18%</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>37 ± 6.2</td>
<td>31 ± 5.2 *</td>
<td>22 ± 4.3**</td>
</tr>
</tbody>
</table>

### Table 2: Blood picture and CD4+ cell count in control group, HIV infected patients and AIDS patients. Values were expressed as means ± S.D, from fifty subjects in each group. ** P < 0.001 versus control group; * P < 0.01 versus control group; †P<0.001 versus HIV infected group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group (A) Control (n=50)</th>
<th>Group (B) HIV infected patients (n=50)</th>
<th>Group(C) AIDS patients (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.73 ± 1.6</td>
<td>12.62 ± 1.8**</td>
<td>9.6 ± 2.0**†</td>
</tr>
<tr>
<td>Hematocrite (%)</td>
<td>44.1 ± 4.2</td>
<td>38.0 ± 4.0**</td>
<td>27 ± 6.1***</td>
</tr>
<tr>
<td>Erythrocyte counts (Cells/µl)</td>
<td>5.5 ± 0.65</td>
<td>5.1 ± 0.72*</td>
<td>2.82 ± 0.61**†</td>
</tr>
<tr>
<td>Leukocyte counts (Cells/µl)</td>
<td>7.8 ± 2.0</td>
<td>9.7 ± 3.2*</td>
<td>13.3 ± 3.6**†</td>
</tr>
<tr>
<td>Platelet Count (Cells/µl)</td>
<td>250 ± 60</td>
<td>95 ± 36**</td>
<td>39 ± 52**†</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>7 ± 4.5</td>
<td>75 ± 26**</td>
<td>145 ± 35**†</td>
</tr>
<tr>
<td>CD4 Count (Cells/µl)</td>
<td>850 ± 75</td>
<td>325 ± 60**</td>
<td>125 ± 30**</td>
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</tbody>
</table>

### Table 3: Levels of plasma TBARS and non-enzymic antioxidants [β-Carotene, vitamin C, vitamin E and GSH] in control group, HIV infected patients and AIDS patients. Values were expressed as means ± S.D, from fifty subjects in each group. ** P < 0.001 versus control group; * P < 0.01 versus control group; †P<0.001 versus HIV infected group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group (A) Control (n=50)</th>
<th>Group (B) HIV Infected patients (n=50)</th>
<th>Group (C) AIDS Patients (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/mL)</td>
<td>4.43 ± 0.93</td>
<td>6.12 ± 0.90**</td>
<td>9.14 ± 1.84**†</td>
</tr>
<tr>
<td>β-Carotene (mg/dL)</td>
<td>0.82 ± 0.090</td>
<td>0.51 ± 0.076**</td>
<td>0.18 ± 0.056**†</td>
</tr>
<tr>
<td>Vitamin C (mg/dL)</td>
<td>1.10 ± 0.26</td>
<td>0.65 ± 0.21**</td>
<td>0.23 ± 0.16**†</td>
</tr>
<tr>
<td>Vitamin E (mg/dL)</td>
<td>1.32 ± 0.28</td>
<td>0.98 ± 0.19**</td>
<td>0.36 ± 0.23**†</td>
</tr>
<tr>
<td>GSH (mg/dL)</td>
<td>42.52 ± 2.07</td>
<td>30.23 ± 2.75**</td>
<td>19.26 ± 4.15**†</td>
</tr>
</tbody>
</table>

### Table 4: Activities of erythrocyte SOD, CAT, GPx and GST in control, in control group, HIV infected patients and AIDS patients. Values were expressed as means ± S.D, from fifty subjects in each group. ** P < 0.001 versus control group; * p<0.01 versus control group; †P<0.001 versus HIV infected group.

<table>
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<th>Group (C) AIDS Patients (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Unit/mgHb)</td>
<td>4.91 ± 0.66</td>
<td>2.56 ± 0.17**</td>
<td>1.14 ± 0.20**†</td>
</tr>
<tr>
<td>CAT (Unit/mg Hb)</td>
<td>80.12 ± 7.46</td>
<td>50.42 ± 6.51**</td>
<td>32.34 ± 4.33**†</td>
</tr>
<tr>
<td>GPx (Unit/mgHb)</td>
<td>12.28 ± 2.59</td>
<td>8.15 ± 2.05**</td>
<td>3.74 ± 0.68**†</td>
</tr>
<tr>
<td>GST (Unit/mgHb)</td>
<td>3.91 ± 0.28</td>
<td>2.99 ± 0.30*</td>
<td>1.12 ± 0.32**†</td>
</tr>
</tbody>
</table>
studies, T and B lymphocyte proliferative responses were increased upon supplementation with vitamin C [29,31]. The latter has been shown to inhibit HIV replication in acutely and chronically infected T cells and to inhibit HIV reactivation in T cells stimulated by TNF-α [32,33].

The anti-oxidants viz., β-carotene, vitamin C, vitamin E and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione –S-transferees (GST) are integral components of a regenerating redox cycle and significantly low in HIV/AIDS patients [34-36]. Moreover, water soluble anti-oxidants such as glutathione, vitamin C and the lipid soluble vitamin E may act synergistically to protect cells from oxidative stress induced damage. Accordingly, the deficiency of total anti-oxidant status may markedly increase oxidative stress.

Altered levels and/or activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase have been observed in HIV-infected patients. For example, SOD activity was reported to be depressed in peripheral blood cells isolated from children infected with HIV. The HIV Tat protein down regulates the synthesis and overrides the induction of the manganese-dependent form of SOD [37], which is induced by oxidative stress in cultured cells. In another study, it was found that serum catalase activity increases as AIDS progresses, while serum glutathione peroxidase levels appear to remain similar to those of healthy subjects. In contrast, Favier et al. [38] reported that plasma and erythrocyte glutathione peroxidase levels were depressed in HIV-infected patients. Further work is necessary to establish the full extent of altered defense system enzymes and reconcile these conflicting observations.

Glutathione levels, glutathione peroxide and GST are depleted in plasma, lung epithelial lining fluid, [39] erythrocytes, [40,41] and lymphocytes, including T cell subsets [42] of HIV-infected individuals. It is notable, however, that even though HIV-infected patients have a consistently lower mean level of glutathione as compared to healthy subjects, the distribution of values is wide, suggesting individual variation.

Glutathione levels decrease rapidly upon infection with HIV and continue to decline as the disease progresses [39,42]. In plasma and bronchiolar lung lavage fluid, the depletion of glutathione is a consequence of a decrease in the level of its reduced form (GSH) with little change in the amount of its oxidized form (GSSG). Whether the concentrations or the ratio of the two forms vary in other compartments is not known.

The data from this study provides evidence indicating that the progression from the HIV infection to the late stage of AIDS is associated with the progressive depletion of antioxidant resources of affected individual. The depletion of the plasma antioxidant status is more apparent in AIDS patients than in the symptomatic persons with of HIV infection. The severe depletion of non-enzymatic and enzymatic antioxidant status in the AIDS stage of HIV infection apparently supports previous reports that indicate increased ROS production correlated with viral load in HIV infection [43]. Like the present study Gil et al. [44] demonstrated a significantly lower antioxidant status in HIV infected persons than a control group [45]. In this study we showed that lower antioxidant status was related to low CD4+ cell count and this too corroborated the findings of other reports [45]. Viral Tat protein is known to increase the apoptotic index by increasing intracellular ROS. It thus appears that antioxidant molecules are depleted when they are consumed in the process of protecting cells against ROS induced oxidative damage in a magnitude that is related to advancement of the disease to AIDS. In an attempt to correlate the oxidative stress with the progression of HIV, Gil et al. [44] demonstrated that increasing the plasma levels of vitamins A, C and E was associated with significant reduction of CD4+ count [4]. Serum tissue fluids and host cells are reported to possess antioxidant mechanisms including ceruloplasmin, transferring, catalase, superoxide dismutase and glutathione peroxidase among others [7]. However, this study suggests that the replenishing mechanisms for the antioxidant molecules in advanced HIV infection are ineffective as the range of values for non-enzymatic and enzymatic antioxidant status in AIDS patients is very low.

In the present study, it was observed that the higher level of free radicals, decreased the antioxidant status (enzymatic and non-enzymatic) in all AIDS cases and the CD4+ cell count decreased due to progression of the disease indicating that there is an oxidative stress, CD4 cells initially and progressively leading to AIDS. The decrease was more pronounced in the HIV group indicating that the antioxidants were
nearly completely utilized to scavenge the superoxide free radicals. In summary, oxidative stress caused by HIV infection may accelerate progression of HIV disease, which enhanced lipid peroxidation in HIV/AIDS patients with concomitant failure of antioxidant defense mechanisms.

REFERENCES


