Serum activities of oxidative burst enzymes in HIV infected subjects

Mathias A. Emokpae, Beatrice A. Mrakpor
Department of Medical Laboratory Science, University of Benin, Benin City, Nigeria

Abstract

Introduction: Human immunodeficiency virus (HIV) infected subjects are immuno-depressed because of decreased levels of circulating CD4+ lymphocytes. It was suggested that the qualitative defects in phagocytic functions in this infection may hinder chemotaxis, lower oxidative burst enzymes, alter phagocytosis and bacterial killing processes. The objective of this study was to evaluate the activities of oxidative burst enzymes in HIV infected subjects and to determine the effect of the use of highly active antiretroviral therapy on these enzymes.

Material and methods: Serum catalase (CAT), superoxide dismutase (SOD) and myeloperoxidase (MPO) activities and CD4+ lymphocyte counts were evaluated in 176 HIV infected subjects (50 HIV naïve, 126 on antiretroviral therapy) and 40 HIV negative subjects which served as controls. The enzymes activities were assayed by the ELISA technique using reagents supplied by WKEA Med Supplies Corp (China). The CD4+ lymphocyte count was assayed using the FACScan flow cytometer technique (FACScan Becton Dickinson, USA).

Results: There were statistically significant increases in the activities of CAT ($p < 0.05$) and MPO ($p < 0.02$) while SOD ($p < 0.001$) and CD4 cell count decreased significantly in HIV infected subjects than controls. The activities of CAT ($p < 0.001$), MPO ($p < 0.01$) and CD4 cell count ($p < 0.001$) were significantly higher while SOD ($p < 0.001$) was lower in HIV infected subjects on HAART than naïve.

Conclusions: The observed increase in the activities of MPO and CAT in HIV positive subjects on HAART correlated with CD4 cell counts. Infected individuals may benefit from antioxidant supplementation in the treatment regimen.

Key words: catalase, human immunodeficiency virus, myeloperoxidase, superoxide dismutase.

Introduction

Human immunodeficiency virus (HIV) infection is a public health challenge, but the introduction of highly active antiretroviral therapy (HAART) has helped to stabilize HIV pandemicity [1, 2]. Because of the inability of HAART to completely eliminate HIV, immune activation and inflammation often result in some medical complications [3, 4]. This chronic systemic immune activation and inflammation may lead to oxidative stress, weakened immune responses, inflammation-associated complications, increased pro-inflammatory cytokine synthesis and viral replication [5].

Individuals infected with HIV are immuno-depressed due to decreased levels of a circulating cluster of differentiation (CD4) lymphocytes. In HIV infection, different abnormalities in immuno-competent B lymphocytes, monocytes,
In this study, we measured the activity of oxidative burst enzymes in HIV-1 infected African subjects. The oxidative (respiratory) burst refers to the production of reactive oxygen species (ROS) by polymorphonuclear leukocytes following a challenge by pathogens. We focused on polymorphonuclear leukocytes because they are the major phagocytes involved in host defense against microbial infection. The oxidative burst enzymes, such as myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD), and others, play a critical role in the degradation of opsonized pathogens.

**Material and methods**

**Inclusion criteria**

All adult confirmed HIV subjects who were referred to the ART clinic of the hospital and gave consent were included in the study.

**Exclusion criteria**

All HIV seropositive and seronegative individuals who had symptoms of chest infections, bacterial endocarditis or smoking that may affect oxidative burst enzymes as well as those who did not give consent were excluded from the study.

**Ethical consideration**

The research protocol was reviewed and approved by the Ethical Review Committee of the Federal Medical Centre, Yenagoa, Bayelsa state, before the commencement of the study. Informed consent was sought and obtained from all participants and utmost confidentiality of information was maintained.

**Sample size determination**

The sample size was determined by calculation using sample size determination for health studies formula [21].

**Specimen collection**

Six milliliters (6 ml) of blood sample was collected by venous puncture and 3 ml was dispensed into a bottle containing ethylene diamine tetraacetic acid (EDTA). The sample in the plain container was allowed to clot at room temperature. The clotted sample was centrifuged at 3000 revolutions per minute for 10 minutes. The serum was separated into a plain container and stored at −20°C before it was analyzed. The serum SOD, CAT and MPO were assayed by the Enzyme Linked Immunosorbent Assay (ELISA) technique using reagents supplied by WKEA Med Supplies Corp, China. The CD4+ count was estimated using the Fluorescence Activated Cell Sorter (BD FACS Flow Cytometer) count system (Becton Dickinson, USA).

**Statistical analysis**

The data (which were normally distributed) generated from this study were analyzed by the statistical software SPSS version IBM 21 (SPSS Inc., Chicago, IL, USA) for Windows. Categorical variables were expressed as mean ± standard error of mean (SEM) and compared using the analysis of variance (ANOVA) and correlation between the measured enzyme activities and CD4 cell counts was done using the Pearson correlation coefficient. A p-value < 0.05 was considered statistically significant. The data were tested for normal distribution using the Shapiro-Wilk test.

**Selection of subjects**

Human immunodeficiency infected African subjects who met the enrollment criteria were consecutively recruited for the study and they comprised 216 subjects grouped into three – 126 (26 males and 100 females with a mean age of 36.2 ± 1.4 years, range 20-52 years) confirmed HIV positive individuals receiving HAART, 50 (14 males and 36 females with a mean age of 34.2 ± 1.2 years, range 25-43 years) newly diagnosed HIV positive subjects registered in the ART clinic (ART naive) and 40 (20 males and 20 females, mean age 35.8 ± 0.6 years, range 32-40 years) HIV negative apparently healthy individuals were recruited for the study.

In this study, we observed that phagocytes from HIV infected children had changed the oxidative metabolism as a result of opsonin receptor dependent stimulation. Previous studies elsewhere on the oxidative burst in HIV infection are not consistent. An enhanced oxidative burst by neutrophils and monocytes in stage-1 HIV infection [16], a decreased oxidative burst in monocytes [17, 18], a decreased oxidative burst in AIDS which correlated with CD4 cells [19] and a normal oxidative burst in HIV [20] were reported by different authors. Studies on oxidative burst enzymes in HIV infected subjects in Nigeria are rare in the literature. The enzyme activities in the phagocytic process may be modulated in HIV infection.

The oxidative (respiratory) burst refers to the production of reactive oxygen species (ROS) by polymorphonuclear leukocytes following a challenge by pathogens. Respiratory burst enzymes have been reported to be contributory factors for HIV disease progression which are induced by the production of ROS [12, 13]. The enzymes that are involved in the phagocytic process may be modulated in HIV infection.

Macrophages, neutrophils and natural killer cells have been described [6, 7]. Because of the immuno-depression, the individuals are readily susceptible to bacterial infections [8]. Other cells such as neutrophils and macrophages (phagocytes) are also vital in host defense against microbial infection. Studies have indicated that an increased susceptibility of HIV infected subjects to bacterial infections may be due to phagocyte dysfunction [7, 9]. It has been suggested that qualitative defects in the phagocytic function in HIV infection can impair chemotaxis, alter phagocytosis, lower oxidative burst capacity and alter bacterial killing [10, 11]. The enzymes that are involved in the phagocytic process may be modulated in HIV infection.

The oxidative (respiratory) burst refers to the production of reactive oxygen species (ROS) by polymorphonuclear leukocytes following a challenge by pathogens. Respiratory burst enzymes have been reported to be contributory factors for HIV disease progression which are induced by the production of ROS [12, 13]. The enzymes that are involved in the phagocytic process may be modulated in HIV infection.
Table 1. Comparison of the activities of oxidative burst enzymes and CD4 cells count between HIV positive subjects on highly active antiretroviral therapy (HAART) and HIV positive naïve subjects (mean ± SEM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV positive on HAART</th>
<th>HIV negative HAART naïve</th>
<th>HIV negative control subjects</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>126</td>
<td>40</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>CD4+ (U/ml)</td>
<td>503.59 ± 17.80*</td>
<td>323.1 ± 25.18*</td>
<td>790.9 ± 19.6</td>
<td>0.001</td>
</tr>
<tr>
<td>CAT (µ/l)</td>
<td>18.28 ± 1.06*</td>
<td>9.50 ± 1.05*</td>
<td>11.65 ± 0.49</td>
<td>0.001</td>
</tr>
<tr>
<td>SOD (ng/ml)</td>
<td>1.49 ± 0.22*</td>
<td>1.75 ± 0.28*</td>
<td>1.78 ± 0.02</td>
<td>0.50</td>
</tr>
<tr>
<td>MPO (ng/ml)</td>
<td>9.64 ± 1.21*</td>
<td>3.63 ± 0.14*</td>
<td>6.58 ± 1.55</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CAT – catalase, SOD – superoxide dismutase, MPO – myeloperoxidase, CD4 – cluster of differentiation
*p < 0.001, †p < 0.02, ‡p < 0.05, §p > 0.05

Table 2. Correlation of oxidative burst enzyme activities with CD4+ cell counts in HIV positive subjects on highly active antiretroviral therapy (HAART)

<table>
<thead>
<tr>
<th>Measured parameters</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT/CD4 counts</td>
<td>0.179</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SOD/CD4 counts</td>
<td>0.079</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MPO/CD4 counts</td>
<td>0.176</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

CAT – catalase, SOD – superoxide dismutase, MPO – myeloperoxidase

Results

The comparison of results of the oxidative burst enzymes in HIV positive HAART naïve, HIV negative and control subjects is shown in Table 1. It shows that serum CAT (p < 0.02) and MPO (p < 0.05) activities were significantly lower in HIV positive HAART naïve subjects than HIV negative subjects. Even though SOD (p > 0.05) activity was lower, it was however not significant. The level of CD4 cell counts, activities of CAT and myeloperoxidase were significantly higher (p < 0.001) in HIV positive subjects on HAART than HIV positive HAART naïve subjects while the activity of SOD was not significantly lower (p > 0.05) in HIV positive subjects on HAART than HIV positive HAART naïve subjects. Serum CAT (r = 0.179, p < 0.05) and MPO (r = 0.176, p < 0.05) correlated positively with CD4 cell counts in HIV positive subjects on HAART (Table 2).

Discussion

The use of HAART in the management of HIV infection has improved the life expectancy of infected subjects but HIV-associated immune activation and inflammation are common. This study assesses the activities of respiratory burst enzymes in order to ascertain if HAART impacts negatively or otherwise on these enzymes in HIV positive subjects on these drugs. It was observed from this study that there were significantly higher (p < 0.001) activities of CAT and myeloperoxidase in HIV positive subjects on HAART than HIV positive HAART naïve subjects, which was consistent with previous studies [22]. It was reported that myeloperoxidase activity was significantly higher in HIV infected subjects on HAART than HAART naïve subjects [22]. But Ross et al. [23] observed elevated myeloperoxidase activity in HIV infected adults both on HAART and HAART naïve. In HIV infection, the expected and appropriate inflammatory response to an infectious process may be amplified and the risk of secondary infection increases. Apart from the microbial role through generation of ROS, myeloperoxidase has been reported to regulate the activity of polymorphonuclear leukocytes [24]. The stimulation of polymorphonuclear leukocytes results in a sudden rise of oxygen consumption with the production of ROS and the release of enzymes such as elastase and myeloperoxidase. An increase in plasma myeloperoxidase levels is a marker of neutrophil proliferation and degranulation in humans. Polymorphonuclear leukocytes are the first cell types that are activated if cells are infected by foreign microorganisms. These cells are mobilized by the chemotactic gradient to the infection site [25, 26].

The lower but insignificant SOD activity observed in HIV positive subjects on HAART could be attributed to high levels of circulating ROS associated with the HIV infection since the rate was reported to be higher in HIV infected subjects [27]. It was postulated that increased oxidative stress might be attributed to the deficiency of the antioxidant defense system in HIV positive HAART naïve subjects.

The observation of significantly lower activities of CAT (p < 0.02) and MPO (p < 0.05) in HIV positive HAART naïve subjects compared with the control subjects is consistent with previous studies [12, 27, 28]. Ibeh et al. [12] reported that superoxide dismutase (SOD) and CAT activities were significantly lower (p < 0.05) in HAART naïve subjects than the subjects on HAART and controls. The authors concluded that enhanced oxidative stress contributed to the pathogenesis of HIV infection. The combination drug therapy reduces viral replication, and improves CD4+ cell count and antioxidant capacity [12]. It could be suggested from the study that a reduced oxidative stress environment and increased oxidative burst enzyme activities in subjects on HAART compared to the HAART naïve subjects were both lower than controls. The low activity of SOD (p > 0.05) observed in our study was however not significant. Conversely, our result disagreed with other authors who reported that myeloperoxidase activity was not significantly different between HIV seropositive subjects.
on HAART and HAART naïve subjects [22]. The data from our study may be due to immune-activation, inflammation and opportunistic infection in HIV infections. Excessive immune activation, inflammation and OS are associated with the loss of immune cells, disease progression and increased risk of mortality [29, 30]. Inflammation is an important non-specific protective response of a tissue to harmful agents and may be associated with an increased production of ROS and apoptosis of CD4+ cells in HIV infection [1].

In resting cells, the respiratory burst enzyme is dormant but is activated when the cells are exposed to appropriate stimuli.

Most of the contradictory evidence of oxidative metabolism in HIV infection was reported before the introduction of HAART. The conflicting results previously reported were attributed to disease severity since the staging of the disease is critical to the interpretation of the results. It appears that either a normal or increased oxidative burst occurs in the early or stage one of HIV infection [17, 18]. As the HIV infection progresses there seems to be a decrease in the oxidative burst as the ROS and inflammatory cytokines cause the dysfunction and increase apoptosis of the polymorphonuclear cells [18, 19]. Mitochondria are a major source of ROS in the cells through electron leakage from the mitochondrial respiratory chain. The oxidative burst is believed to contribute to the overall OS which is implicated in the massive depletion of CD4 cells in early HIV infection [31].

However, with HAART intervention, the respiratory burst enzyme (CAT and MPO) activities were significantly improved even more than in HIV negative subjects.

It shows that HAART treatment has markedly increased the activities of the enzymes. It could be inferred from this study that antiretroviral drug treatment during HIV infection impacted positively by improving the oxidative burst capacity in the subjects. This may suggest that HIV infected subjects on HAART could respond faster if antioxidation supplementation is included in the treatment regimen.

In chronic infection such as in HIV HAART naïve subjects, the chronic production of ROS in polymorphonuclear neutrophils may cause the depletion of oxidative burst enzymes. Overproduction of ROS such as superoxide anions, hydroxyl radicals and hydrogen peroxide may be as a result of increased activation of polymorphonuclear neutrophils (PMNs) during HIV infection. It was suggested that myeloperoxidase may normally play a role in terminating the oxidative burst by regulating the activity of these enzymes of normal PMNs and thus appears that catalase activity is more important than that of myeloperoxidase in regulating the release of hydrogen peroxide into the extracellular medium [32]. The observed positive correlation between CAT, MPO activities and CD4 cell counts in HIV positive subjects on HAART may suggest that in virologically suppressed subjects on HAART, higher enzyme activities were associated with an increased marker of HIV disease progression.

In conclusion, the activities of MPO and CAT were lower in HIV positive HAART naïve subjects than controls. The enzyme activities were higher in those subjects who were on HAART than HAART naïve which correlated positively with CD4 cell counts. It is suggested that administration of HAART in HIV infection impacted on the activities of MPO and CAT. Therefore antioxidant supplementation may be included in the treatment regimen of infected subjects.

**Conflict of interest**

The author’s declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**References**


