

## **Assessment of Oxidative Stress Level in HIV/AIDS Patients on HAART Treatments**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors FOE, OI,>NNL designed the study and wrote the protocol. Authors FOE, SOO, KA and MKE performed the laboratory study. Authors FOE, MKE and HT performed the statistical analysis, managed the literature searches, analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Human Immunodeficiency Virus (HIV) infection is associated with the production of reactive oxygen species (ROS) and secondarily cellular damage of varied severity. Highly active antiretroviral therapy (HAART) are currently the therapy of optimal considerations for HIV-infected patients.

**Aims:** The present study was designed to assess the oxidative stress levels in people living with HIV/AIDS on HAART.

**Methods:** A total of one hundred and twenty (120) patients attending the Jos University Teaching Hospital APIN Centre were recruited for the study following the inclusion criteria. Superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) were evaluated in blood samples of recruited patients.

**Results:** Superoxide dismutase (SOD) activities increased in control patients with no significant difference ( $p < 0.05$ ) compared with HAART. However, significant increases ( $p > 0.05$ ) were observed in reduced glutathione (GSH) concentration of control patients compared with HAART and HAART

Naïve patients. More so, significant increase ( $p > 0.05$ ) was observed in malondialdehyde (MDA) of HAART Naïve patients compared with HAART treatment and control. No significant differences ( $p > 0.05$ ) was observed in alanine aminotransferase and creatinine in HAART, HAART Naïve compared with the control patients.

**Conclusion:** The study indicated that HIV/AIDS positive patients express reduced levels of SOD, and GSH with an increase level of MDA. This could further lead to oxidative stress complications and thus HAART-naïve patients are encouraged to use HAART to prevent further complication that could arise from oxidative stress.

**Keywords:** HAART; oxidative stress; markers; HIV/AIDS; treatment.

## ABBREVIATIONS

AIDS : Acquired Immunodeficiency Syndrome  
 APIN : AIDS Prevention Initiative Nigeria  
 CD<sub>4</sub> : Cluster of Differentiation 4  
 EDTA : Ethylenediaminetetraacetic Acid  
 GSH : Reduced Glutathione  
 HAART : Highly Active Antiretroviral Therapy  
 HIV : Human Immunodeficiency Virus  
 MDA : Malondialdehyde  
 PEPFAR: President's Emergency Plan for AIDS Relief  
 SOD : Superoxide Dismutase

## 1. INTRODUCTION

Oxidative stress results from an imbalance in which the manifestation of reactive oxygen species (ROS) overwhelms the antioxidant capacity of the cells [1]. It is a condition portrayed as an imbalance between the production of Reactive Oxygen Species (ROS) and their counteraction by the antioxidant defences, leading to the build-up of Reactive Oxygen Species (ROS) and their derived metabolites, with changes in the redox status of the cell [2]. The occurrence of both local and systemic oxidative stress in HIV-1 infection has been implicated in the progressive loss of CD<sub>4</sub><sup>+</sup>T lymphocytes, and disease progression, as well as in the development of other secondary complications associated with HIV infection [3].

Reactive oxygen species (ROS) is a general term for oxygen intermediates with high reactive capacity towards various biological molecules. They include hydroxyl radical (-OH), singlet oxygen, superoxide anion, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and other reactive species [4]. Reactive oxygen species (ROS) are produced in various cellular processes and organelles" [5], electron leakage from the mitochondrial electron transport chain, degradation of lipids, amino acids, and biogenic polyamines, and protein folding in the

lumen of endoplasmic reticulum (ER) [6]. The most reactive type of ROS is the hydroxyl radical [7]. It is produced from hydrogen peroxide that oxidizes divalent iron cations via the Fenton reaction or as a result of the Haber-Weiss cycle that involves a reduction of ferric ions by superoxide anions into ferrous ions followed by the Fenton reaction" [8]. To prevent further complications by ROS in diseases, antioxidants are key mediators, which chelate the circulating free radicals.

Highly active antiretroviral therapy (HAART) is presently the therapy of optimal considerations for HIV-infected patients [9]. However, despite the amazing viral replication suppression and immune response restoration, long-term HAART appears of limited use in many patients because of additional adverse effects and/or regimen adherence difficulties [10]. HAART may increase chemically reactive species in circulation, possibly by producing more oxidized metabolites deriving from the interaction between ROS and infected-cell biomolecules [11]. Furthermore, poor adherence to HAART can have serious consequences, including loss of serum HIV suppression, development of drug-resistant HIV strains, and increased probability of illness progression [12]. It is generally accepted that oxidative stress is involved in HIV infection. However, the role in oxidative balance of HAART is still debated [13]. The present study was therefore designed to assess the oxidative stress levels in people living with HIV/AIDS on HAART treatment.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in Jos University Teaching Hospital APIN Centre, located in Jos North Local Government Area of Plateau State, Nigeria. The State lies between latitude 8°24" north and longitude 8°32" and 10°38" east.

## 2.2 Inclusion Criteria

HIV positive individuals on HAART, and HIV negative apparently healthy controls were included in this study. Individuals with AIDS or any other chronic disease condition such as hepatitis B virus, hepatitis C virus, tuberculosis, diabetes among others were excluded from the study (Fig. 1).

## 2.3 Sample Size

This was a comparative cross-sectional study involving HIV positive individual on HAART, and HIV negative individual. A total of 120 subjects of both sexes (Table 1) aged between 20 to 60 years (Table 2) were studied prospectively and classified as HIV positive patients on HAART (Group 1, n=40), and apparently healthy HIV negative individuals as control (Group 2, n=40). The minimum sample size of 120 was arrived at by using the standard (sample size) formula  $n = \frac{z^2 p q}{d^2}$  [14] and putting into consideration the HIV prevalence in Plateau State of 5.3%. Where n=desired sample size, z=standard normal deviation at 1.96 which is corresponded to 95% confidence limit, p=proportion of

population with HIV, d= precision in proportion and  $q = (1-p)$ .

## 2.4 Sample Collection

Seven millilitres (7 mL) of venous blood was drawn from each subject. The blood sample was dispensed into a 5 mL plain vacutainer, allowed to clot and centrifuged (4000 rpm for 15 minutes) to separate serum from cells. The serum was transferred, using sterile pasteur pipette, into 2 mL cryovial containers, labelled, and stored at  $-80^{\circ}\text{C}$  (Ultralow NUAIRE, Germany) until required for analysis of some indices of oxidative stress which included reduced glutathione [15], malondialdehyde [16], and superoxide dismutase [17].

## 2.5 Statistical Analysis

The various results obtained were subjected to statistical analyses using One-way analysis (ANOVA), and student's T-test. Differences at  $p < 0.05$  was considered significant. The results are presented as mean  $\pm$  standard deviation (SD) and graphs generated using GraphPad Prism 6 software (GraphPad Software, California, USA).

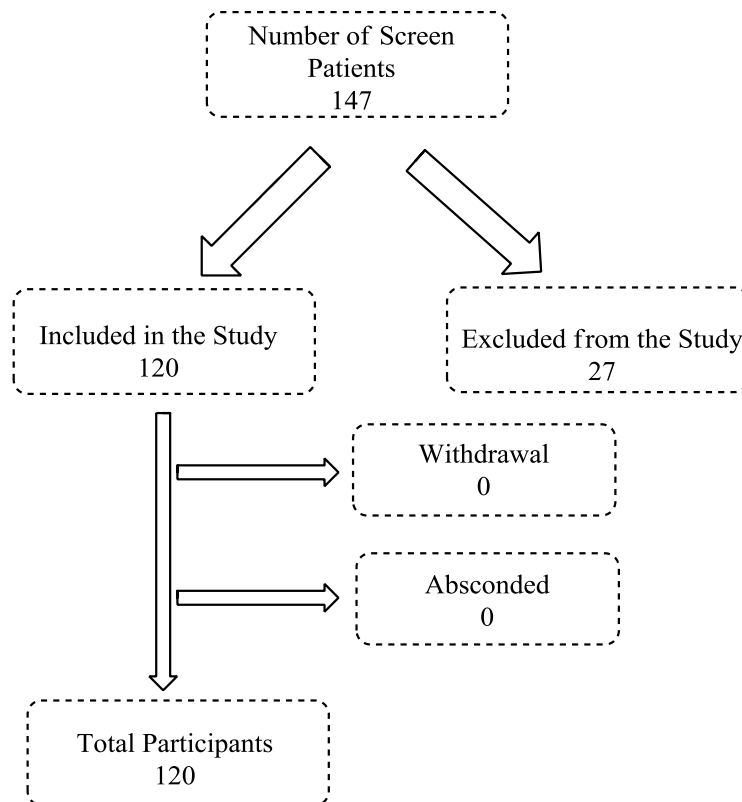


Fig. 1. Flow chart of the study design

### 3. RESULTS

#### 3.1 Response of Superoxide Dismutase (SOD) Enzymes

The response of superoxide dismutase (SOD) enzymes showed a reduction in the HIV positive patients on HAART as compares to the HIV negative individuals, which showed an increase SOD enzyme (Fig. 2). However, with no significant difference at  $p>0.05$ .

#### 3.2 Response of Reduce Glutathione (GSH)

In the results of the response of glutathione to the test patients, it showed a significant decrease ( $p<0.05$ ) in the level of glutathione in HIV positive patients on HAART while an increase in the level of glutathione was observed in the control (Fig. 3).

#### 3.3 Response of Lipid Peroxidation (MDA)

Lipid peroxidation (MDA) level was not significantly different ( $p>0.05$ ) in HIV positive patients on HAART compared with the control none HIV patients (Fig. 4).

### 4. DISCUSSION

The human body produces reactive oxygen species (ROS) which are free radicals that lead

to oxidative stress and the body also produces antioxidant to have a counter effect on the free radicals being released by the body thereby ensuring haemostasis. There must be a stable balance between the free radicals and the antioxidant *in vivo*. However in a disease condition, more free radicals are produced than the body antioxidant can balance up resulting into oxidative stress. The use of Anti-retroviral Therapy (ART) in the treatment of patients with HIV-1 infection is effective and encouraged to avoid further complications [18]. The results obtained from our studies shows that HIV positive patients produces more free radicals (ROS) than the HIV negative individuals and furthermore those on HAART also produces more free radicals than the HIV positive patients not on HAART (HAART Naïve).

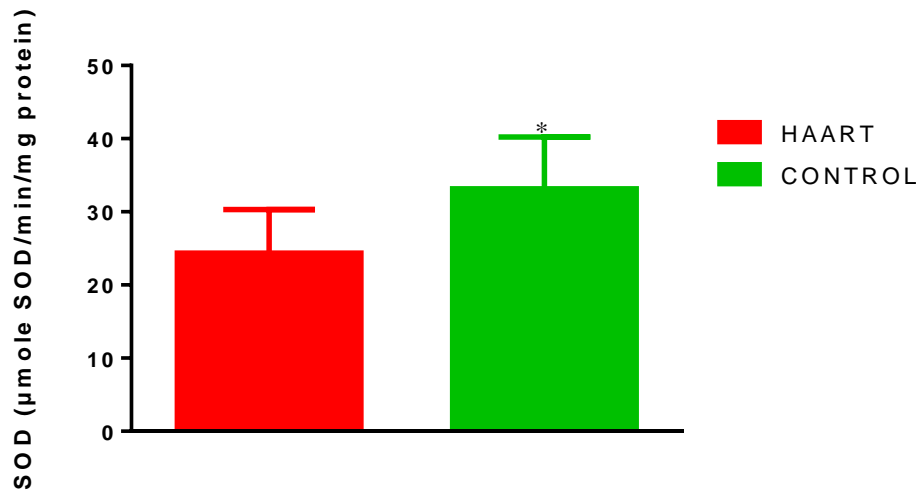
HIV infection and HAART are associated with low grade hepatotoxicity, with no impairment of renal functions [19]. Our results showed that both HIV positive on HAART and HIV HAART naïve had higher levels of MDA and lower level of SOD and GSH compared to HIV negative control subjects. Also in contrast to some studies, that higher levels of ROS and lower antioxidant levels have been reported in HIV subjects on HAART compared to HAART naïve HIV positive individuals. Individuals who strictly adhered to HAART have been shown to display higher oxidative stress than those with intermittent HAART adherence [20].

**Table 1. Gender distribution of HIV/AIDS patients on HAART treatments**

HAART		
Gender	Frequency	Percentage (%)
Male	17	42.5
Female	23	57.5
Control		
Gender	Frequency	Percentage (%)
Male	21	52.5
Female	19	47.5

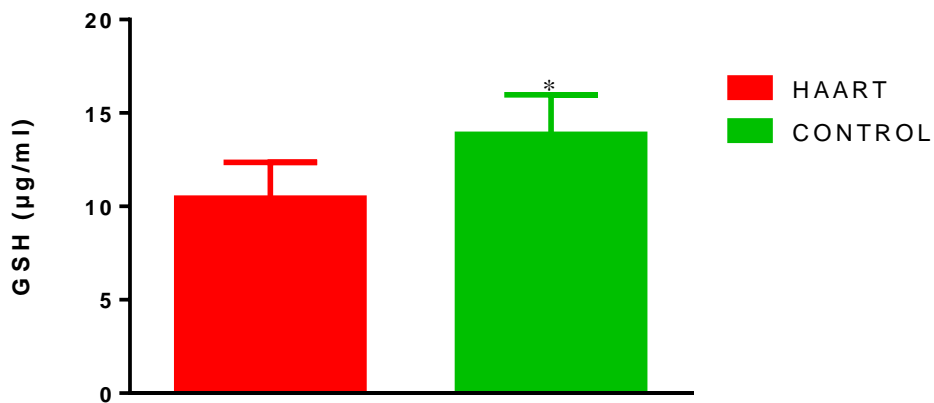
**Table 2. Age distribution of HIV/AIDS patients on HAART treatments**

Age (years)	HIV/AIDS patients		Control	
	Frequency	Percentage (%)	Frequency	Percentage (%)
20-29	8	10	18	45
30-39	17	21.25	12	30
40-49	29	36.25	7	17.5
50-59	26	32.5	3	7.5
<b>TOTAL</b>	<b>80</b>	<b>100</b>	<b>40</b>	<b>100</b>



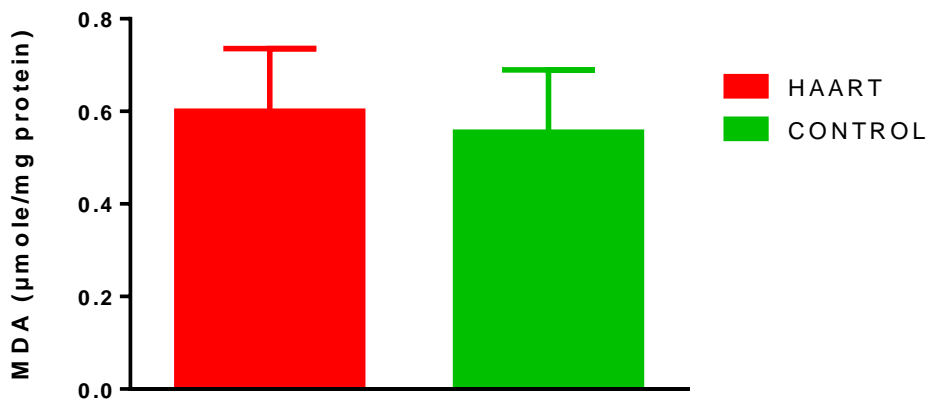
**Fig. 2. Superoxide dismutase (SOD) activities of HIV/AIDS patients on HAART treatments**

*Values are mean ± SD, n = 40*



**Fig. 3. Reduced glutathione (GSH) concentration of HIV/AIDS patients on HAART treatments**

*Values are mean ± SD, n = 40*



**Fig. 4. Malondialdehyde (MDA) concentration of HIV/AIDS patients on HAART treatments**

*Values are mean ± SD, n = 40*

SOD is an enzyme found in all living cells that help break down potentially harmful oxygen molecules which are called ROS in cells, they prevent damage to tissues and are important antioxidant defense against oxidative stress in the body. In HIV naïve patients, there is more production of ROS, which lead to oxidative stress. However, in the HIV positive patients on HAART there is less production of ROS compared to that of the naïve patients. SOD is responsible for the dismutase activity of the harmful oxygen molecules, thereby preventing damage to tissues and this action lead to reduction in the level of SOD.

The mechanism of operation of glutathione is similar to that of SOD in the breakdown of harmful oxygen molecules in cells. Glutathione just like SOD also help in the breakdown of harmful oxygen molecules in cells due to reactive oxygen species produced by the presence of HIV virus and administration of HAART leading to oxidative stress. These breakdowns of the harmful oxygen molecules in cells prevent damage to tissues, which lead to reduction in the level of glutathione in the body.

There was a significant increase in the level of lipid peroxidation in HIV positive patients on HAART, with a decrease in the control subjects. Free radicals are produced in patients with a disease condition and especially the HIV positive patients on HAART. This is an indication that the production of MDA is as a result of increase production of free radicals in the body due to the disease condition and drug intake. Malondialdehyde (MDA) is an enzymatic indicator that shows the presence of high-level production of free radicals in the body leading to oxidative stress. The significantly increased level of MDA in HIV positive patients as observed in this study is in keeping with the generally high levels of MDA found in some studies including that of Ikepeazu et al. [9]. This increase was observed with decrease in the levels of antioxidant (SOD and GSH) which is indicative of oxidative stress and lipid peroxidation. In this study, there was an observed negative correlation between HIV positive patients and the control group, 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.

## 5. CONCLUSION

The study indicated that HIV/AIDS positive patients without HAART treatments express

reduced levels of SOD and GSH, with an increase level of MDA while the HIV/AIDS positive patients with HAART treatments expressed higher levels of SOD and GSH with lower level of MDA. This could further lead to oxidative stress complications and thus HAART-naïve patients are encouraged to used HAART to prevent further complication that could arise from oxidative stress.

## ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Review Committee of Jos University Teaching Hospital (JUTH) before the commencement of the study with the reference number JUTH/DCS/IREC/127/XXX/2082. A semi-structured questionnaire was used to seek for the patient's informed consent and to collect relevant data related to the study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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