ORIGINAL ARTICLE CODEN: AAJMBG

# Serum levels of lipid peroxidation, nitric oxide metabolites and enzymatic antioxidants in subjects infected with HIV

**Adedeji D. Atere[1,2*], Victor O. Akinbola[2], Yekeen A. Kosamat[1], Oluwaseyi E. Bamisaye[3] and Adekunle A. Adesiyan[1]**

[1]Department of Medical Laboratory Science, College of Health Sciences, Osun State University, Osogbo, Nigeria, [2]Department of Medical Laboratory Science, Achievers University, Owo, Ondo State, Nigeria and [3]Department of Biomedical Laboratory Science, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

***Received:*** 18th January 2025; ***Accepted:*** 26th March 2025; ***Published:*** 01st April 2025

**Abstract:** *Background:* HIV/AIDS remains a major health challenge worldwide, particularly in resource-limited settings and where rates of oxidative stress, secondary to chronic immune activation and inflammation, may exacerbate its impact. This study examines the disparity between oxidative stress indices and enzymatic antioxidant activities in individuals infected with HIV and its possible role in disease progression. *Methods:* A cross-sectional study was carried out in 80 individuals (50 HIV-infected and 30 controls). Serum hydrogen peroxide ($H_2O_2$), nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels were quantified by spectrophotometric methods. SPSS was used for statistical comparisons and correlation analyses. *Results:* The mean serum levels of MDA (3.20±0.62 µmol/L), $H_2O_2$ (4.83±0.63 µmol/L), and NO (36.47±5.83 µmol/L) in HIV-infected patients were significantly elevated compared to the control group, which had levels of 1.70±0.31 µmol/L, 3.34±0.28 µmol/L, and 26.83±4.14 µmol/L, respectively ($p<0.001$). On the other hand, the activities of antioxidant enzymes (SOD: 1.99±0.39 U/ml, CAT: 39.66±1.38 U/L, GPx: 2.06±0.35 U/ml) were significantly reduced in HIV-infected individuals compared to controls ($p<0.001$). A significant positive correlation between MDA and GPx was identified ($r=0.317$, $p=0.025$). *Conclusions:* This study emphasizes the necessity of targeted antioxidant therapies to mitigate oxidative damage and enhance clinical outcomes in HIV-infected individuals, thereby emphasizing a critical oxidative stress imbalance.

**Keywords:** HIV Infection, Oxidative Stress, Lipid Peroxidation, Nitric Oxide Metabolites, Antioxidant enzymes.

## Introduction

HIV/AIDS continues to pose a substantial global health burden, particularly in underdeveloped regions, where 90% of cases are concentrated. Africa remains disproportionately affected, accounting for 70% of deaths due to HIV-1 infection, with Nigeria ranking second in the continent for the number of individuals living with HIV [1-2].

Beyond its immunological impact, HIV infection disrupts cellular metabolic pathways, fostering oxidative stress through chronic immune activation and inflammation. This form of oxidative stress is characterized by excessive generation of reactive oxygen species (ROS) and lower levels of antioxidant defenses. This makes the disease worse and its effects last longer [3-4].

The proportion of ROS generation and antioxidant defenses is disrupted, resulting in oxidative stress (OS). ROS originate from mitochondrial respiration, immune responses, and external factors like environmental agents and drugs [5-6]. Cellular signaling requires low levels of ROS, but elevated levels can lead to damage through lipid peroxidation, protein damage, and DNA oxidation, potentially promoting HIV pathogenesis [7].

The process of lipid peroxidation, induced by free radicals that attack polyunsaturated fatty

acids, is known to be especially detrimental in HIV infection, resulting in rigidity and dysfunction of cell membranes [8-9].

Antioxidant defenses, comprising enzymatic systems like SOD, CAT, and GPx, mitigate ROS to maintain cellular homeostasis. Nevertheless, reduced activity of these enzymes correlates with HIV infection, resulting in increased oxidative damage and enhanced facilitation of viral replication [10-11]. Nitric oxide (NO) introduces additional complexity to this equation; it is essential for cellular signaling yet may also exacerbate oxidative stress when interacting with ROS, leading to the production of peroxynitrite, a potent oxidizing agent [12].

Although increasing evidence demonstrate the involvement of oxidative stress in HIV pathogenesis, we have not sufficiently clarified the mechanisms by which oxidative imbalance contributes to the severity of disease. Moreover, there are conflicting reports regarding whether oxidative stress markers correlate with antioxidant enzyme activities in HIV-infected subjects [13-14]. Bridging these voids is essential for creating specific therapeutic approaches aimed at curbing oxidative injury and enhancing patient prognosis. Investigating the dysregulation of oxidative stress response and the consequential alterations in oxidative stress-related process, this study elucidates the biochemical basis of HIV-induced pathogenesis. Understanding these interactions may also help improve HIV management approaches, specifically in low-resource settings, where the HIV burden is highest.

## Material and Methods

*Study Design:* This study is a hospital-based cross-sectional analysis conducted between January and July 2024. This study analyzed oxidative stress markers and antioxidant enzyme levels in HIV-infected individuals compared to non-HIV-infected controls. The study comprised 80 subjects, including 50 HIV-infected individuals and 30 non-HIV-infected controls, matched by sex and age. Participants were recruited from the Heart-to-Heart Clinic at the Federal Medical Centre, Owo, Nigeria. Participants ranged in age from 18 to 70 years and provided written informed consent prior to enrollment.

*Inclusion Criteria:* The study included HIV-infected participants aged between 18 and 70 years. Some were receiving antiretroviral therapy for at least six months. HIV-negative controls who were not on any chronic medications that can impact oxidative stress levels and had no history of chronic disease were recruited for appropriate comparison. After understanding the study's purpose and procedures, all participants provided written informed consent.

*Exclusion Criteria:* Participants with diabetes mellitus, hypertension, or chronic kidney disease which could interfere with the results of oxidant and antioxidant metabolites were exempted from the study. We also excluded subjects who were taking antioxidant supplements or medications that are known to modulate oxidative stress. Pregnant and lactating women were excluded to avoid possible physiological differences in their oxidative stress and antioxidant levels caused by their aforementioned condition.

*Ethical Considerations:* The Federal Medical Centre, Owo Ethical Review Committee granted ethics approval (Reference: FMC/OW/380/VOL.CCXVII/08). Participants were provided with information about study purposes, procedures, and potential risks. Written informed consent was obtained prior to participation in accordance with the Declaration of Helsinki.

*Sample Collection and Storage:* Venous blood samples (8 mL) were aseptically collected from each participant following standard phlebotomy procedures. Samples were dispensed into sterile plain bottles and permitted to clot at ambient temperature. Subsequently, blood was centrifuged at 4,000 rpm for a duration of 5 minutes to obtain serum. Serum samples were aliquoted into cryovials and stored at −20 °C prior to analysis.

*Laboratory Assays:* Biochemical assays for oxidative stress markers and antioxidant enzymes were done accordingly. The levels of MDA were determined via the Thiobarbituric Acid Reactive Substances (TBARS) assay that quantifies lipid peroxidation

spectrophotometrically at λ: 532 nm [15]. $H_2O_2$ was measured by a dichromate/acetic acid method and absorbance was measured at 570 nm [16]. NO was determined by Griess reagent method and absorbance was measured at 548 nm [17]. SOD activity was determined by measuring its inhibition of the auto-oxidation of epinephrine at 480 nm [18]. CAT activity was assessed based on its ability to convert dichromate to chromic acetate in the presence of $H_2O_2$ [16], and the absorbance was measured at 570 nm. The activity of GPx was assayed as the pyrogallol oxidation, followed by measuring absorbance at 430 nm [19].

*Quality Control:* All strict quality control measures were enforced for reliability of results. All reagents were freshly prepared, and all equipment was calibrated prior to performing the assays. Duplicate analyses were performed on all assays to assess the reproducibility of measurements, and control samples were included in every batch to assess overall performance and consistency. These safeguards ensured the accuracy and precision of the laboratory analyses.

*Statistical Analysis:* Data were analyzed with the IBM SPSS version 25. Continuous variables were described as means and standard deviations, and categorical variables as frequencies and percentages. Mean values of OS markers and enzymatic antioxidant between HIV-infected and control groups were analyzed using independent sample t-tests. In the HIV-infected group, Spearman's correlation analysis was performed to examine relationships between OS markers and enzymatic antioxidants. Statistical significance was set at $p < 0.05$.

## Results

A total of 80 participants were included in the study: 50 individuals infected with HIV and 30 non-infected controls. The mean age of HIV-infected patients (42.14±10.79 years) did not significantly differ from that of the control group (39.20±9.23 years). The comparison of the two groups regarding body mass index (BMI) and blood pressure revealed no statistically significant differences. Individuals infected with HIV exhibited a BMI of 25.00±4.12, whereas the control group demonstrated a BMI of 25.49±2.54 (Figure 1 & 2).

**Fig-1:** Sociodemographic characteristics of study participants

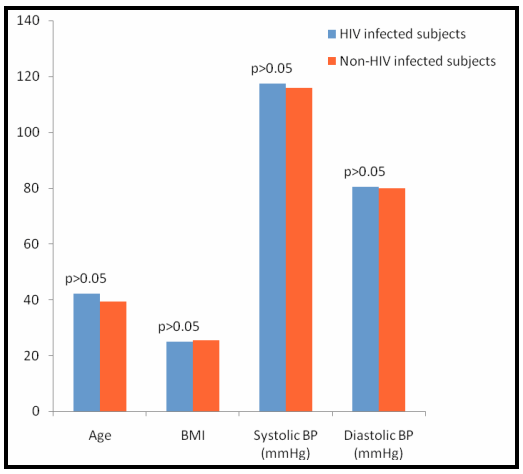



**Fig-2:** Gender profile of the study population

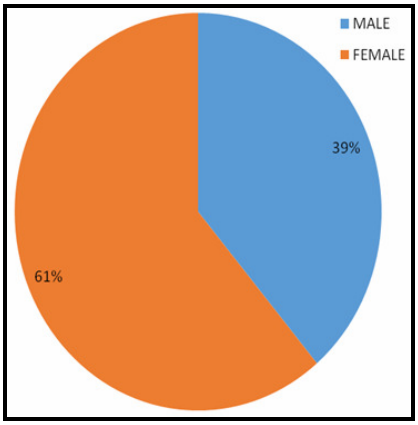



Table 1 presents the variations in OS markers and antioxidant enzyme levels between HIV-infected and non-infected individuals. MDA, $H_2O_2$, and NO levels were significantly elevated in HIV-infected participants compared to controls. The mean level of MDA in HIV-infected individuals was 3.20±0.62 µmol/L, compared to 1.70±0.31 µmol/L in the control group ($p<0.001$). In addition, concentrations of $H_2O_2$ and NO were markedly elevated in HIV-infected individuals compared to control individuals ($p<0.001$).

On the other hand, antioxidant enzyme activities were significantly reduced in individuals infected with HIV. The mean SOD level in the HIV-infected group was 1.99±0.39 U/ml, which was significantly lower than the 3.30±0.67 U/ml observed in the control group ($p<0.001$). CAT activity was diminished in HIV-infected individuals, showing a mean

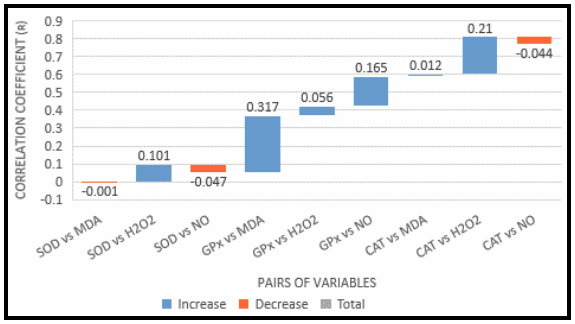
value of 39.66±1.38 U/L, in contrast to 53.74±3.08 U/L in the control group (p<0.001). Furthermore, GPx activity was markedly reduced in HIV-infected individuals, with a mean of 2.06±0.35 U/ml compared to 3.43±0.40 U/ml in controls (p<0.001). The correlation between OS markers and antioxidant enzyme levels in HIV patients are shown in figure 3.

**Table-1: Comparison of mean levels of oxidative stress markers in HIV infected subjects and non-infected subjects**

| Parameters | HIV infected subjects (n = 50) | Non-HIV infected subjects (n = 30) | P-value |
|---|---|---|---|
| SOD (U/ml) | 1.99±0.39 | 3.30±0.67 | 0.000* |
| MDA (µmol/L) | 3.20±0.62 | 1.70±0.31 | 0.000* |
| GPx (U/ml) | 2.06±0.35 | 3.43±0.40 | 0.000* |
| CAT (U/L) | 39.66±1.38 | 53.74±3.08 | 0.000* |
| $H_2O_2$ (µmol/L) | 4.83±0.63 | 3.34±0.28 | 0.000* |
| NO (µmols/L) | 36.47±5.83 | 26.83±4.14 | 0.000* |

*significant at p≤0.05; Key: n= Sample size

**Fig-3:** Correlation of serum level of oxidants with antioxidant in HIV infected subjects




We found a significant positive correlation between the MDAs and GPx (r = 0.317, p = 0.025), which may indicate a compensatory increase in GPx activity parallel to a rise in lipid peroxidation. In other words, MDA was not significantly correlated with SOD or CAT, on the contrary.$H_2O_2$ had positive but not statistically significant correlations with SOD, GPx, and CAT. However, NO was positively correlated with GPx (r=0.165, p=0.252) but negatively correlated with SOD (r=–0.047, p=0.748) and CAT (r=–0.044, p=0.760). These results emphasize the intricate interaction between oxidative stress markers and antioxidants during HIV infection.

## Discussion

This study investigated the relationship between OS markers and antioxidant enzyme activities in HIV-infected subjects compared to non-infected controls. The findings indicated a striking imbalance between oxidants and antioxidants in HIV subjects, which present with high levels of OS markers and low activities of antioxidant enzymes. This study emphasizes the importance of OS in HIV pathogenesis and its potential impact on disease progression and management.

The high levels of oxidative stress biomarkers including MDA, $H_2O_2$, NO detected among HIV-infected individuals as compared to controls serve as an addenda to the increased oxidative stress burden associated with this population. Higher levels of MDA, an indicator of lipid peroxidation, reflect increase cellular membrane lesion and loss of cellular integrity [7, 9]. Hydrogen peroxide, the most important ROS, causes more oxidative damage via the formation of more powerful oxidants like hydroxyl radicals [5].

The elevated NO levels also indicate an alteration in nitric oxide metabolism, a process that can create oxidative stress in the form of the damaging oxidant peroxynitrite [12, 14]. These results are in agreement with the literature that highlights that chronic immune activation/inflammation during HIV infection leads to overproduction of ROS [3]. Increased oxidative stress observed in the present study corroborates the contribution of oxidative stress to the development of these HIV-associated conditions including neurocognitive and cardiovascular disorders [4, 20].

This decrease in antioxidant enzyme activity, namely SOD, CAT and GPx, in HIV positive individuals suggests that they are already less capable of dealing with ROS. In a multitude of phenomena associated with cell damage and death by oxidative stress, these enzymes act best as scavengers for ROS to neutralize oxidative stress [21-22].

Decreased antioxidant homeostasis adds to the oxidative imbalance, leading to a vicious cycle of ROS generation and impaired protective pathways. We demonstrated that MDA and GPx were positively correlated with each other in HIV-infected people, indicating a compensatory response in which increased lipid peroxidation induces GPx activity to balance oxidative stress. Nonetheless, the absence of notable correlations for other oxidative stress markers and antioxidant enzymes accentuate the complexity surrounding all these interactions. This result is consistent with previous studies that demonstrated differential relationships between measures of oxidative stress and antioxidant defenses in HIV-infected groups [23-24].

The OS imbalance observed in this study highlights the importance of implementing targeted therapeutic measures to counteract oxidative damage in HIV-infected patients. However, since ROS play a significant role in the development of HIV-1 infection complications, it has been suggested that supplementation of antioxidants such as vitamins C and E may be investigated as an adjuvant therapy to augment antioxidant defenses and potentially diminish the complications related to this infection [22, 25]. So acquiring routine information on markers of oxidative stress and levels of antioxidant enzymes can also be passionate, pointing out the evolution of the disease, its worsening, and the action of antioxidant-based therapies.

## Conclusion

Elevated MDA, $H_2O_2$, and NO, and reduced SOD, CAT, and GPx activities in HIV-infected individuals indicate a remarkable disturbance in oxidative stress in these subjects. Our findings highlight the important role of OS in HIV pathogenesis and possible contribution to disease progression. This imbalance can be addressed with antioxidant therapies and more frequent biomarker monitoring, potentially translating to improved clinical outcomes for HIV-infected individuals. Studies should be conducted based on long-term antioxidant interventional therapy on oxidative stress and disease progression of HIV infected patients.

### Acknowledgments

We would like to appreciate all staff of Federal Medical Center, Owo for providing assistance.

**Financial Support and sponsorship:** Nil

**Conflicts of interest:** There are no conflicts of interest.

## References

1. Atere AD, Akinbo BD, Okafor AM, Egbuchulem KI, Akinola EA. Evaluating correlation between total lymphocyte counts and CD4 counts in monitoring HIV patients. *Arch Appl Sci Res*. 2016; 8(3):22-28.
2. Emokpae MA, Mbonu I. Blood levels of some toxic metals in human immunodeficiency virus (HIV) type 1 infection. *Ann Health Res*. 2018; 4(1):75-81.
3. Buckley S, Byrnes S, Cochrane C, Roche M, Estes JD, Selemidis S et al. The role of oxidative stress in HIV-associated neurocognitive disorders. *Brain Behav Immun Health*. 2021; 1:100235.
4. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018; 13:757-772.
5. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J*. 2012; 5(1):9-19.
6. Borgmann K, Ghorpade A. Methamphetamine augments concurrent astrocyte mitochondrial stress, oxidative burden, and antioxidant capacity: Tipping the balance in HIV-associated neurodegeneration. *Neurotox Res*. 2018; 33(2):433-447.
7. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014; 2014:360438.
8. Ibeh BO, Obidoa O, Nwuke C. Lipid peroxidation correlates with HIV mRNA in serodiscordant heterosexual HIV partners of Nigerian origin. *Indian J Clin Biochem*. 2011; 26(3):249-256.
9. Obeagu E, Nkwocha B, Nwanna C, Nwanjo H. 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. *Innov Assoc J Biol Innov*. 2016; 5(1):24-30.
10. Quaye O, Kuleape JA, Bonney EY, Puplampu P, Tagoe EA. Imbalance of antioxidant enzymes activities and trace elements levels in Ghanaian HIV-infected patients. *PLoS One*. 2019; 14(7):e0220181.
11. Shibata T, Yamada T, Sato H. Reactive oxygen species and antioxidant activities in HIV-infected patients: Implications for redox status and immunosuppression. *Biochem Biophys Res Commun*. 2019; 261(3):421-424.
12. Soccal RM, de Carvalho JAM, Bochi GV, Moresco RN, da Silva JEP. Nitric oxide levels in HIV-

infected, untreated patients and HIV-infected patients receiving antiretroviral therapy. *Biomed Pharmacother.* 2016; 79:302-307.
13. Khabaz RF, Heneine W, George JR, Parekh B, Rowe T, Woods T et al. Infection of a laboratory worker with simian immunodeficiency virus. *N Engl J Med*. 1994; 330(3):172-177.
14. Costiniuk CT, Mehraj V, Routy J-P, de Castro C, Wasef N, Jenabian MA et al. Nasal nitric oxide levels in HIV infection: A cross-sectional study. *AIDS Res Treat*. 2018; 2018:3074507.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979; 95(2):351-358.
16. Sinha P. Induction of oxidative stress and antioxidant enzymes by excess cobalt in mustard. *J Plant Nutr*. 1971; 35(6):952-960.
17. Wolff SP. Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol*. 1994; 233:182-189.
18. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972; 247(10):3170-3175.
19. Reddy KP, Brown TL. Kinetics of surface processes for Mo (CO) 6 on partially dehydroxylated alumina and hydroxylated alumina. Observation of Mo (CO) 5 (ads). *J Am Chem Soc*. 1995; 117(10):2845-2854.
20. Valcour VG, Shikuma CM, Watters MR, Sacktor NC. Cognitive impairment in older HIV-1-seropositive individuals: Prevalence and potential mechanisms. *AIDS* 2014; 18(1):79-86.
21. Popoola T, Awodele O. Interplay between antiretroviral therapy and oxidative stress in HIV seropositive patients. *Afr J Med Med Sci*. 2016; 45:5-21.
22. Kumar A, Chaturvedi MM. HIV-1 infection, oxidative stress, and antioxidants: A review. *Clin Exp Immunol*. 2020; 160(1):45-55.
23. Ngondi JL, Oben JE, Forkah DM. The effect of different antiretroviral therapy regimens on oxidative stress in HIV-infected patients in Cameroon. *J Clin Biochem Nutr*. 2016; 39(2):105-110.
24. Coco-Bassey SB, Asemota EA, Okoroiwu HU, Etura JE, Efiong EE, Inyang IJ et al. Glutathione, glutathione peroxidase and some hematological parameters of HIV-seropositive subjects attending clinic in University of Calabar Teaching Hospital, Calabar, Nigeria. *BMC Infect Dis*. 2019; 19(1):1-9.
25. Wang L, Zhang L, Niu Y, Sitia R, Wang C. Glutathione Peroxidase 7 utilizes hydrogen peroxide generated by Ero1α to promote oxidative protein folding. *Antioxid Redox Signal*. 2014; 20(4):545-556.

**Cite this article as:** Atere AD, Akinbola VO, Kosamat YA, Bamisaye OE and Adesiyan AA. Serum levels of lipid peroxidation, nitric oxide metabolites and enzymatic antioxidants in subjects infected with HIV. *Al Ameen J Med Sci* 2025; 18(2): 105-110.



*All correspondences to: Dr. Adedeji D. Atere, Lecturer, Department of Medical Laboratory Science, College of Health Sciences, Osun State University, Osogbo, Nigeria. E-mail: adedeji.atere@uniosun.edu.ng