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Original Research Article

Comparison of oxidative stress and viral load in HIV infected individuals with and without antiretroviral therapy

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Abstract

Background: This study investigates the relationship between oxidative stress and viral load in HIV-infected individuals undergoing antiretroviral therapy (ART) compared to those not receiving ART. Oxidative stress, characterised by an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences, plays a critical role in the pathogenesis of HIV and its related complications. This study aims to compare oxidative stress levels and HIV viral load in individuals receiving ART with that not on therapy, to assess the impact of ART on oxidative stress markers and HIV replication. And also to understanding the relationship between ART, oxidative stress, and viral load is crucial for evaluating the long-term biochemical effects of HIV treatment and optimizing therapeutic strategies.

Materials and Methods: In this study, 30 samples were included. The viral load was measured using real-time PCR, and the CD4 count was found using the flow cytometric technique Additionally, ELISA was used to detect the oxidative stress biomarker protein carbonyl. It is evaluated by comparing the viral load and CD4 count with and without ART, and then measuring the protein carbonyl.

Result: The study indicate that CD4 cell counts were significantly higher in HIV patients receiving antiretroviral therapy (ART), with a ($p< 0.002$) while insignificant difference was observed in those not on ART ($p<0.83$). A comparison of oxidative stress markers revealed that levels of protein carbonyl a key indicator of oxidative damage were elevated in HIV-1 individuals, who were not undergoing ART, compared to those receiving treatment. Additionally, untreated participants exhibited a higher viral load than those receiving ART.

Conclusions: The study concluded that oxidative stress is higher in HIV-1 including those who are not taking ART then the participant taking ART. Our study findings are opposite to other studies hence concluding research in a higher number of samples will help to understand the role of oxidative stress in HIV-1 individuals.

Keywords: Oxidative stress, HIV, Protein carbonyl assay.

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1. Introduction

Human immunodeficiency virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), leads to progressive immune system failure by targeting key immune cells such as $CD4^+$ T lymphocytes, macrophages, and dendritic cells. Despite significant advancements in antiretroviral therapy (ART), HIV remains a major global health challenge, particularly due to persistent immune dysfunction and associated complications.[1] While ART effectively suppresses viral replication and improves immune function, emerging evidence suggests that certain antiretroviral drugs may contribute to increased oxidative stress a biochemical imbalance between reactive oxygen species (ROS) and antioxidant defences. Oxidative stress plays a critical role in HIV pathogenesis by promoting inflammation, apoptosis, and immune system decline. Objective if the study aims to compare oxidative stress levels and HIV viral load in individuals receiving ART with that not on therapy, to assess the impact of ART on oxidative stress

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markers and HIV replication. Understanding the relationship between ART, oxidative stress, and viral load is crucial for evaluating the long-term biochemical effects of HIV treatment and optimizing therapeutic strategies. Although antiretroviral therapy (ART) has significantly reduced HIV-related morbidity and mortality by suppressing viral replication and enhancing immune function, its long-term biochemical effects remain a subject of concern. Several studies suggest that ART, particularly certain drug classes, may induce oxidative stress by increasing the production of reactive oxygen species (ROS) or impairing antioxidant defence systems.[2] This oxidative imbalance can contribute to cellular damage, immune dysfunction, and the development of comorbid conditions such as cardiovascular and neurodegenerative diseases, even in virologically suppressed individuals. Additionally, oxidative stress itself plays a key role in HIV pathogenesis by promoting viral replication, immune cell depletion, and systemic inflammation. Therefore, investigating oxidative stress markers alongside viral load provides a more comprehensive understanding of the impact of ART—not just on viral suppression, but also on cellular health. Given these implications, it is essential to assess whether ART contributes to or mitigates oxidative stress in HIV-infected individuals.[3] This knowledge can help refine treatment strategies, monitor long-term toxicity, and potentially guide the development of adjunct therapies aimed at reducing oxidative damage and improving overall patient outcomes.[4,5]

1.1. Protein carbonyl ELISA

Protein carbonyl ELISA is a sensitive immunoassay that detects carbonyl groups introduced into proteins due to oxidative stress. These carbonyls serve as biomarkers of oxidative damage caused by reactive oxygen species (ROS) reacting with amino acid side chains. The assay involves derivatisation of carbonyls with 2, 4-dinitrophenylhydrazine (DNPH), forming stable DNP hydrazone adducts.[6] These modified proteins are immobilised on microplate wells, followed by incubation with a primary anti-DNP antibody and an enzyme-conjugated secondary antibody.[7] A colorimetric or chemiluminescent signal is produced upon substrate addition, which is proportional to the protein carbonyl content.[8,9] This ELISA offers high specificity and sensitivity and can process multiple samples simultaneously, providing quantitative data on oxidative damage.[10] It is widely used to evaluate oxidative stress in conditions like diabetes, cancer, cardiovascular, and neurodegenerative diseases. The assay is also useful for studying antioxidant effects and therapeutic responses. Accurate results depend on proper sample preparation, protein concentration, and strict adherence to protocols.[11] Overall, protein carbonyl ELISA is a reliable tool for oxidative stress biomarker analysis in both clinical and research applications.[12,13]

2. Materials and Methods

2.1. Research design

A cross-sectional study was conducted over a period of five months (June to October 2022) at the Clinical Microbiology Laboratory. A continuous sampling method was used to recruit 30 HIV-positive individuals. The participants were divided into two groups: 15 individuals receiving antiretroviral therapy (ART) and 15 individuals not receiving ART. Ethical approval for the study was obtained from the Institutional Ethics Committee (IEC) (CSP/22/JUN/112/341).

2.2. Sample selection and processing

Blood samples were collected from HIV-positive patients attending the Sri Ramachandra Hospital. These samples were submitted to the central laboratory for routine CD4 count and viral load testing. CD4 counts were determined using flow cytometry (Sysmex CyFlow Counter), and viral load was measured using a real-time polymerase chain reaction (RT-PCR) method.

Plasma was separated from whole blood by centrifugation and stored at –20°C until further analysis. Oxidative stress was assessed by measuring protein carbonyl levels using the ELISA method, following standard protocols.

2.3. Inclusion criteria

1. Samples received for HIV ELISA and viral load testing in the central laboratory or clinical virology unit.
2. HIV-positive individuals either receiving or not receiving antiretroviral therapy (ART).

2.4. Exclusion criteria

1. Individuals with chronic conditions such as hepatitis, diabetes, renal disorders, cardiovascular or neurological diseases, and psychiatric illnesses.
2. Individuals with lifestyle risk factors including heavy smoking, alcoholism, or tobacco chewing.

2.5. Various treatment plans according to NACO guidelines

Table 1: Indian HIV-positive patients were given the following list of antiretroviral medications:

First Line Therapy	Second Line Therapy
Tenofovir	Tenofovir
Lamivudine	Lamivudine
Efavirenz	Ritonavir
	Atazanavir

2.6. Protein carbonyl ELISA method

The ELISA procedure begins with washing the wells of the ELISA plate with 250µl of wash buffer to ensure a clean surface. Subsequently, 100 µl of standards, controls, and

samples are added to their respective wells. The plate was then securely covered and incubated at room temperature for 1 hour to facilitate antigen binding. Following incubation, the wells were washed five times with 250µL of wash buffer to remove unbound components. Next, 100µl of detection antibody is added to each well, followed by another 1-hour incubation at room temperature. After this step, the wells are washed again, and 100µl of conjugate is added to each well, with an additional incubation for 1 hour at room temperature. After the incubation, the contents of each well were discarded, and the wells were washed five additional times with 250µL of wash buffer to ensure the removal of unbound conjugate. Following the washing step, 100µl of substrate (SUB) is added to each well and incubated for 10–20 minutes at room temperature (15–30°C) in the dark to allow colour development. Finally, 100µl of stop solution (STOP) is added to each well, and the mixture is gently mixed using the shake mode of the microtiter plate reader. The absorbance is then measured immediately using an ELISA reader at 450 nm to quantify the results.[14,15]

3. Result

In this study, out of 30 HIV-positive patients, 15 were undergoing antiretroviral therapy (ART) while the remaining 15 were not receiving ART. A significant difference in CD4 count was observed in patients with ART ($p < 0.002$), whereas the change was statistically insignificant in patients without ART ($p < 0.83$) (Table 1). Figure 2 illustrates the comparison of oxidative stress between HIV patients with and without ART. Protein carbonyl levels, an indicator of oxidative stress, were found to be higher among HIV-1 individuals without ART (mean 24.6 ± 0.27nmol/mg) compared to those on ART (mean 9.4 ± 0.17nmol/mg). Similarly, patients not receiving ART exhibited higher viral loads, as shown in Figure 1. However, one of the major limitations of this study is the absence of baseline measurements at the time of ART initiation for the treated group. Without initial values, it is difficult to accurately assess the longitudinal impact of ART. To address this limitation, baseline values were estimated by using the measurements from the untreated group, which is assumed to represent the natural course of untreated HIV infection. Accordingly, the estimated baseline CD4 count for ART patients was approximately 147 cells/mm³, baseline viral load was around 400,000–500,000 copies/mL, and baseline protein carbonyl levels were estimated to be 2.5–2.8nmol/mg. These inferred values support the observed therapeutic impact of ART on immune function and oxidative stress in HIV-infected individuals. Nonetheless, future studies should include actual baseline data collection prior to ART initiation to enable more robust and accurate longitudinal comparisons.

Table 2: Summary of observed and estimated baseline values in HIV patients with and without ART

Parameter	With ART (observed)	Estimated Baseline (Before ART)	Without ART (observed)
CD4 Count (cells/mm³)	270.1 ± SD	~147 cells/mm³	147.3 ± SD
Viral Load (copies/mL)	~100,000–150,000	~400,000–500,000	~400,000–600,000
Protein Carbonyl (nmol/mg)	9.4 ± 0.17	~2.5–2.8 (estimated)	24.6 ± 0.27

Table 3: Compare of CD4 count for HIV infected individual with and without ART

S. No.	CD4 count with ART(n=15)	S. No.	CD4 count without ART (n=15)
1.	295 lymphocytes/mm^3	1.	165 lymphocytes/mm^3
2.	270 lymphocytes/mm^3	2.	154 lymphocytes/mm^3
3.	257 lymphocytes/mm^3	3.	146 lymphocytes/mm^3
4.	276 lymphocytes/mm^3	4.	122 lymphocytes/mm^3
5.	326 lymphocytes/mm^3	5.	134 lymphocytes/mm^3
6.	348 lymphocytes/mm^3	6.	170 lymphocytes/mm^3
7.	229 lymphocytes/mm^3	7.	180 lymphocytes/mm^3
8.	278 lymphocytes/mm^3	8.	195 lymphocytes/mm^3
9.	280 lymphocytes/mm^3	9.	185 lymphocytes/mm^3
10.	376 lymphocytes/mm^3	10.	120 lymphocytes/mm^3
11.	221 lymphocytes/mm^3	11.	115 lymphocytes/mm^3
12.	230 lymphocytes/mm^3	12.	130 lymphocytes/mm^3
13.	245 lymphocytes/mm^3	13.	125 lymphocytes/mm^3
14.	250 lymphocytes/mm^3	14.	135 lymphocytes/mm^3
15.	266 lymphocytes/mm^3	15.	140 lymphocytes/mm^3
P value	0.002	0.83	

P value<0.05 considered as significant

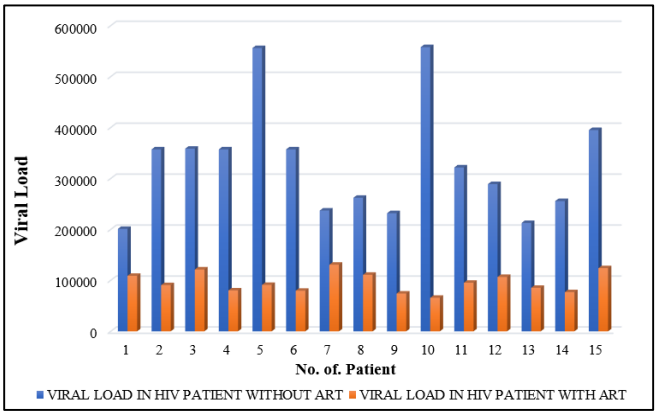

Figure 1: Shows that comparison of viral load in HIV infected patients under with and without antiretroviral therapy

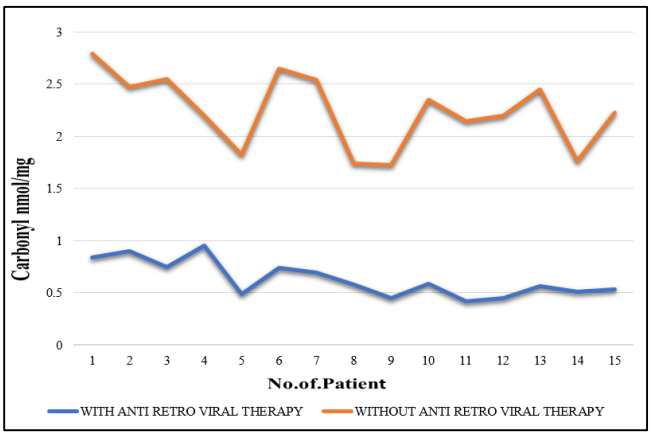

Figure 2: Shows that comparison of oxidative stress in HIV infected patient under with and without antiretroviral therapy

4. Discussion

Antiretroviral therapy (ART) remains the cornerstone in the management of HIV-1 infection, primarily through the suppression of viral replication and enhancement of immune function. In the absence of ART, patients experience persistent viral replication, which contributes to increased oxidative stress resulting from the overproduction of reactive oxygen species (ROS) by both infected and immune cells. This oxidative stress, characterised by an imbalance between ROS generation and antioxidant defence mechanisms, is known to contribute significantly to HIV-1 pathogenesis, accelerating immune deterioration and disease progression.[16,17]

Among the various biomarkers used to evaluate oxidative stress, protein carbonyl content is a well-established indicator of oxidative protein damage.[18] Elevated levels of protein carbonyls reflect increased oxidative stress and cellular injury, making it a reliable marker for assessing redox imbalance in HIV-1-infected individuals.[19,20]

In the present study, we assessed and compared the levels of protein carbonyl in HIV-1-positive individuals receiving ART and those not on therapy. Our findings revealed significantly higher protein carbonyl levels in individuals not receiving ART, suggesting that ART contributes to the reduction of oxidative stress.[21] This may be attributed to ART's ability to suppress viral replication and immune activation, thereby limiting ROS production and subsequent protein oxidation.[22]

However, our results contrast with findings from a study by Kolgiri *et al.*, which reported higher protein carbonyl levels in ART-treated individuals compared to untreated individuals.[12] While ART reduces viral burden, some antiretroviral drugs have been associated with mitochondrial toxicity and ROS generation, potentially contributing to oxidative stress as a side effect of long-term treatment.[23]

Additionally, other studies have reported that oxidative stress in HIV-1 patients may remain within normal ranges, regardless of ART status, underscoring the complexity of oxidative stress regulation and the influence of multiple biological and environmental factors.[24]

These findings highlight the need for larger, multicentre studies with standardised methodologies to better understand the interplay between HIV infection, ART, and oxidative stress. Future research should also explore additional oxidative biomarkers alongside protein carbonyl to provide a comprehensive view of redox dynamics in HIV-1 pathogenesis and treatment.

In conclusion, our study suggests that ART may help mitigate oxidative stress in HIV-1-infected individuals, but further investigation is necessary to clarify the long-term biochemical impact of ART and optimise treatment strategies for improved clinical outcomes.[25]

5. Conclusion

Our study concludes that oxidative stress, as indicated by elevated protein carbonyl levels, is significantly higher in HIV-1-positive individuals not receiving antiretroviral therapy compared to those undergoing ART. These findings support the role of ART not only in suppressing viral replication but also in reducing oxidative damage. However, given that our results contrast with some previous studies, further research involving larger sample sizes and standardised methodologies is essential to better understand the complex relationship between oxidative stress and ART in HIV-1-infected individuals.

6. Source of Funding

None.

7. Conflict of Interest

None.

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