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Correlation Pattern of Selected Markers among Non-Naïve HIV Participants in Edo Central, Nigeria

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A B S T R A C T

This study was designed to define the correlation pattern ofCD4/CD8 ratio, inflammatory cytokines, oxidative stress parameters, and cytolytic molecules in chronic HIV infection, using seropositive participants visiting HIV clinic at two hospitals in Edo central, Nigeria. A total of 58 HIV sero-positive participants on HAART and 30 apparently healthy individuals (control) were recruited for this study. The CDC staging method with CD4 count was used to classify the sero-positive participants. The average duration of ART intake was 9.0±3.33 years and 8.18±4.19 years for stage I and II respectively. About 10mls of venous blood were collected from each patient and dispensed into plain container (5ml) and EDTA container (5ml) for analysis. Viral load was estimated using the COBAS C4800^{TM,} CD4 and CD8 counts were determined using the BD FACS Count™ System, ELISA was used for other parameters. The result of the study revealedthat the CD4 count for stage I has a strong positive correlation ($p<0.05$) with IL-10 ($r= 0.427$), PEF ($r=0.477$) and GRZM ($r=0.426$). Similarly, CD4/CD8 ratio showed a strong positive correlation ($p<0.05$) with GRZM. Contrary to CD4 count, viral load produced significant negative correlation ($p<0.05$) with IL-10 ($r= -0.428$) within stage I group. The TNF-α revealed a positive correlation with oxidative stress markers; MDA ($p=0.577$) and TAC ($p=0.014$) among stage I group. There was non significant correlation between studied parameters within stage II group. Furthermore, TNF-α values showed different correlation pattern between stage II and I with regards to MDA ($r= -0.462$; $r= 0.126$) and TAC ($r= -0.054$; $r= 0.514$) respectively. The control group result showed that IL-10 had a negative correlation with MDA ($p=0.000$) and TAC ($p=0.090$), and a positive correlation with PEF ($p=0.114$) and GRZM ($p=0.003$). We also observed among the control group, that TNF-α showed a strong negative correlation ($p<0.05$) with TAC. Conclusively, a higher anti-inflammatory status will result to a decline in viral replication. Therefore HIV subjects could be advised to engage in activities and lifestyle that would reduce inflammation. An improved CD4 count status is associated with improved cytolytic capacity of the immune effector T cells with increased release of cytolytic molecules. Also higher anti-inflammation status may result to improved CD4 count. This may have resulted from declined viral replication due to lower inflammation. We further conclude that CD4 count and viral load values which are usual tools for monitoring HIV infected patients could be used to predict other health measures such as inflammation and cytotoxic activities. This will enable care givers proactively arrest disease progression and provide timely interventions against other non AIDS pathology.

Introduction

Human immunodeficiency virus (HIV) is the cause of a number of illnesses known as acquired immune deficiency syndrome (AIDS) and human immunodeficiency virus (HIV) infection (Bhaskaran *et al.,* 2021; Farazmand, 2023). CD8+ T cells specific to HIV are able to inhibit HIV replication in vitro through both direct cytotoxicity and the secretion of soluble factors (Obregon-Perko *et al.,* 2018). The CD8+ T-cell pool is highly primed for strong cytotoxic effector activity during the acute phase of HIV infection, but this capacity declines during the chronic phase of infection (Baral *et al.,* 2019). In chronically HIV-infected individuals, this loss of CD8+ T cell-mediated control of virus replication due to a combination of virus escape and progressive T cell dysfunction and exhaustion is linked to the progression of the disease (Macatangay *et al.,* 2020).

Infection with HIV results in the production of cytokines by infected cells and cells of the immune system. Such cytokines regulate the immune function and affect viral replication (Balaji *et al.,* 2021). Changes in cytokine levels in HIV infected individuals can affect the function of the immune system and have the potential to directly impact the course of HIV disease by enhancing or suppressing HIV replication (Abe *et al.,* 2022). Human immunodeficiency virus infection is associated with chronic immune activation and dysfunctional cytokine production (Osuji *et al.,* 2018).

A related mechanism implicated in the pathogenesis of HIV disease and its complications is a pro-oxidative status associated with the infection and with antiretroviral therapy (ART) (Chauvin *et al.,* 2022). Human immunodeficiency virus induces the generation of reactive oxygen species (ROS) through the regulatory protein Tat and the envelope glycoprotein gp120 (Huang *et al.,* 2021). HIV-activated macrophages via TNF-α (tumor necrosis factor-alpha) release, and activated polymorphonuclear leukocytes, also contribute to the generation and accumulation of ROS (Singh *et al.,* 2019). As a consequence, there is a deficiency in antioxidant capacity, partly due to excessive consumption of antioxidant molecules in order to protect cells against ROS-induced damage (Kenmegne *et al.,* 2020), which further enhance the pro-oxidative status. Increased oxidative stress biomarkers have been documented in HIV-infected and in AIDS patients (Kenmegne *et al.,* 2020), as well as in patients receiving ART, with most studies being conducted in the era prior to currently recommended antiretroviral regimens (Butler *et al.,* 2018). Despite the theoretical etiopathogenic role of oxidative stress in HIV disease, evidence from clinical studies remains sparse.

The present study aimed at determining the correlation pattern of CD4/CD8 ratio, inflammatory cytokines, oxidative stress parameters, and cytolytic molecules, in chronic HIV infected participants on highly active antiretroviral therapy (HAART). We are hopeful that the result of this research will help in intensifying health interventions, predicting health status and prevention of severe episodes of inflammation, non AIDS complications and arrest avoidable disease progression to AIDS.

Materials and Methods

A total of 58 HIV sero-positive participants on HAART and 30 apparently health participants (control) were recruited for this study. The CDC staging method with CD4 count was used to classify the study participants; stage I (CD4 count ≥ 500 cells/µl) and stage II (CD4 count 200 to 500 cells/µl) (Kapogiannis *et al.,* 2020*;* Linley *et al.,* 2021).

Ethical Approval

Ethical approval was obtained from the ethics and research committee of Ambrose Alli University, Ekpoma (REF NO.: 014/23), and informed consent of the patients was obtained before sample collection.

Participants Selection Criterion

Recruited participants were above 18 years, HIV sero-positive and currently on HAART. The control participants were apparently healthy (HIV sero-negative) individuals above 18 years of age. Individuals with a history of diabetes mellitus, active opportunistic infections, inflammatory conditions, recent blood transfusion, diarrhoea or pregnancy were excluded.

Sample Collection

About 10mls of venous blood was collected from each patient and dispensed into plain container (5 ml) and EDTA container (5 ml). The samples in plain container were centrifuged and serum obtained for the

quantification of cytokines (IL-10, TNF-α), Malondialdehyde (MDA), Total anti-oxidant Capacity (TAC), Perforin (PEF) and Granzyme B (GRZM). The sera were separated and stored at ≤ -20 °C until analyses. The EDTA samples were used for the estimation of CD4+ T cells, CD8+ T cells and viral load (VL). These samples were analyzed at ONAMEC Research Laboratory Nnewi and Irrua Specialist Teaching Hospital HIV laboratory.

Sample Analysis

Viral load was estimated using the COBAS C4800™, CD4 and CD8 counts were determined using the BD FACSCount™ System, ELISA was used for other parameters.

Results and Discussion

The sero-positive group comprised of stage I and II with 30 and 28 study participants respectively. The age range of the participants was 20 years and above, with majority (>50%) being above 40 years. Stage I had 7 (23.3%) male and 23 (76.7%) female, stage II had 6 (21.4%) male and 22 (78.6%) female, while control group had 10 (33.3%) male and 20 (66.7%) female. The participants were mostly those with chronic HIV infection who have been on ART for a period ranging from 1 year to over 10 years. The average year of ART intake for stage I participants was 9.0±3.33years and stage II was 8.18±4.19years. All participants have had one or two sets of ART drug combination; Zidovudine + Lamivudine + Nevirapine and/or Lamivudine + Efavirenz + Tenofovir.

Table 1 shows the correlation between studied parameters for stage I participants. From the table VL showed a significant negative correlation with interleukin-10 (IL-10) (r= -0.428; p=0.047) and non significant negative correlation with TNF-α (r= -0.342; p=0.120). There was also a weak negative correlation between VL and PEF (r= -0.149; p=0.507). However, MDA, TAC and GRZM showed non-significant positive correlation (p>0.05) with VL. The CD4 count showed a strong positive correlation (p<0.05) with IL-10 (r= 0.427), PEF (r= 0.477) and GRZM (r= 0.426), and a weak positive correlation with TNF-α (r=0.205; p=0.361). CD4 however, revealed non-significant negative correlation (p>0.05) with MDA (r= -0.012) and TAC (r= -0.013). The CD8 count on the other hand produced weak negative correlation (p>0.05) with IL-10 (r= -0.123), TNF-α (r= -0.031) and GRZM (r= -0.333), and a weak positive correlation (p>0.05) with MDA (r= 0.084), TAC (r= 0.002) and PEF (r= 0.107). The CD4/CD8 ratio showed a strong positive correlation (r=0.427; p=0.048) with GRZN, weak positive correlation (p>0.05) with IL-10, TNF-α, MDA and PEF, and a weak negative correlation with TAC (r= -0.025; p>0.05).

Table 2 shows the correlation result of inflammatory markers, oxidative stress markers and cytolytic molecules for stage I participants. From the table, IL-10 showed negative correlation with MDA (r= -0.061, p=0.786) and TAC (r= -0.421, p=0.051); and a positive correlation with PEF (r= 0.205, p=0.360) and GRZM (r= 0.380, p=0.081). TNF-α showed a weak negative correlation (p>0.05) with GRZM (r= -0.218); a strong positive correlation with TAC (r= 0.514, p=0.014); and a weak positive correlation (p>0.05) with MDA (r= 0.126) and PEF (r= 0.055).

Table 3 shows the correlation between studied parameters for stage II participants. As indicated in the table, VL showed a weak positive correlation (r= 0.528, p=0.095) with IL-10; negative correction with TNF-α (r= -0.066, p=0.847); non-significant negative correction (p>0.05) with MDA (r= -0.003) and TAC (r= -0.516). Viral Load also revealed weak negative correction with PEF (r= -0.037, p=0.914) and a positive correction with GRZM (r= 0.097, p>0.05). CD4 count showed weak negative correlation (p>0.05) with IL-10 (r= -0.002), TNF-α (r= -0.377) and MDA (r= -0.013) whereas a positive non-significant correlation (p>0.05) was seen with TAC (r= 0.282), PEF (r= 0.166) and GRZM(r= 0.025). There was weak negative correlation (p>0.05) when CD8 was compared to IL-10, MDA, PEF and GRZM. However, TNF-α and TAC demonstrated positive non-significant correlation (p>0.05) with CD8. A contrast pattern to CD8 was seen with CD4/CD8 ratio. The later produced weak positive correlation (p>0.05) with IL-10 (r= 0.264), MDA (r= 0.188), PEF (r= 0.240) and GRZM (r= 0.064), and negative non-significant correlation (p>0.05) with TNF-α (r= -0.127) and TAC (r= -0.437).

Table 4 shows the correlation result of inflammatory markers, oxidative stress markers and cytolytic molecules for stage II participants. As indicated in the table, IL-10 showed non-significant negative correlation (p>0.05) with MDA (r= -0.285), TAC (r= -0.313), PEF (r= -0.281) and GRZM (r= -0.342). On the other hand, TNF-α showed positive correlation with PEF (r= 0.093,

p=0.785) and GRZM (r= 0.249, p=0.459); and negative correlation with MDA (r= -0.462, p=0.152) and TAC (r= -0.054, p=0.874).

Table 5 shows the correlation between studied parameters for control participants. The control group CD4 count revealed weak negative correlation (p>0.05) with IL-10 (r= -0.318), TNF-α (r= -0.261), PEF (r= -0.421) and GRZM (r= -0.299); and a positive correlation with MDA (r= 0.397, p>0.05) and TAC (r= 0.626, p=0.053). There was a negative CD8 correlate with TNF-α (r= -0.298, p=0.402) and MDA (r= -0.404, p=0.247) whereas a weak positive CD8 correlation (p>0.05) was seen with IL-10 (r= 0.186), TAC (r= 0.018), PEF (r= 0.208) and GRZM (r= 0.463). The ratio (CD4/CD8) showed almost similar pattern with CD4: there were weak negative correlation (p>0.05) with IL-10 (r= -0.346), PEF (r= -0.505) and GRZM (r= -0.580); and positive correlation with TNF-α (p=0.953), MDA (p=0.091) and TAC (p=0.172).

Table 6 shows the correlation result of inflammatory markers, oxidative stress markers and cytolytic molecules for control group. Figures from the table revealed that IL-10 is a strong negative correlate (p<0.05) of MDA (r= -0.907) and a weak negative correlate (p>0.05) of TAC (r= -0.564). In contrast, IL-10 appeared to be a strong positive correlate of GRZM (r= 0.826, p=0.003) and weak positive correlate of PEF (r= 0.531, p=0.114). TNF-α showed a significant negative correlation (p<0.05) with TAC (r= -0.640); and weak positive correlation (p>0.05) with MDA (r= 0.079), PEF (r= 0.554) and GRZM (r= 0.281).

Immune response to ongoing HIV replication and the activity of multiple HIV gene products result in immune activation, which is reflected in the increased activation state of immune cells and the release of pro-inflammatory cytokines (Younas *et al.,* 2019). The course of HIV infection may be directly impacted by changes in cytokine levels in HIV-positive persons, which can either enhance or repress HIV replication (Abe *et al.,* 2022).

Previous studies have demonstrated that TNF-α-releasing HIV-stimulated macrophages and activated polymorphonuclear leukocytes generate and accumulate ROS (Singh *et al.,* 2019). Reactive oxygen species (ROS) can be produced in the mitochondria by Granzyme B at cytotoxic levels, which can cause cell death (Paşatu-Cornea *et al.,* 2022). The loss in an individual's antioxidant capacity resulting from overconsumption of antioxidant molecules to protect cells from ROS-induced damage enhances the pro-oxidative condition in HIV infection (Kenmegne *et al.,* 2020). There has been documented evidence of existing relationship across parameters of inflammation, oxidative stress, and cytolytic activities. The present study set out to evaluate this relationship in HIV infection. From the study, with the exception of IL-10 for the stage I group, viral load did not correlate significantly (p>0.05) with the parameters under investigation. The results, however, revealed a specific pattern: stage II VL and IL-10 exhibited a weak positive association (p>0.05) whereas stage I VL and IL-10 showed a strong negative correlation (r=-0.428, p=0.047). Stage I VL showed a positive relationship with MDA and TAC, whereas Stage II VL revealed a negative correlation with the duo. Varying degrees of viral replication and immunological responses could be linked to this variance in the correlation pattern between stages I and II. It has been established that pro-oxidative state and inflammation are related to disease development and progression of HIV illness (Chauvin *et al.,* 2022; Lomeli-Martinez *et al.,* 2019). According to Gray *et al.,* (2021), taking HAART can reverse or slow the progression of the disease, which lowers inflammation. This could explain the substantial correlation between VL and IL-10 in stage I group. Effective HAART improves the immunological state of HIV-positive people, delays medication resistance, and inhibits viral replication at various stages of the HIV life cycle. The prognosis for HIV illness has improved significantly overall with the use of HAART (Corrilynn *et al.,* 2018).

Previous studies showed that perforin-mediated cytotoxicity as measured in blood is a consistent correlate of HIV immune control (Nguyen *et al.,* 2019; Perdomo-Celis *et al.,* 2019; Collins *et al.,* 2020), and the ability of CD8+ T cells to upregulate perforin following in vitro stimulation correlates inversely with viral load (Nguyen *et al.,* 2019). We observed from this study, a consistent correlation pattern (p>0.05) of VL with the cytolytic molecules; negative correlation with PEF and positive correlation with GRZM. The viral load pattern with PEF in this study is consistent with the report of Nguyen *et al.,* (2019). However, the positive correlation of VL with GRZM in such chronic infection may be linked to the fact that granzyme-B could also produced by a range of non-cytotoxic cells, such as basophils, mast cells, and smooth muscle cells (Nüssing *et al.,* 2022).

Table.1 Correlation between studied parameters for stage I participants

Parameters		IL-10 (pg/ml)	TNF-α (pg/ml)	MDA (nmol/ml)	TAC (µmol/l)	PEF (pg/ml)	GRZM (pg/ml)
VL(copies/ml)	r	-0.428	-0.342	0.201	0.056	-0.149	0.004
	p-value	0.047*	0.120	0.369	0.806	0.507	0.985
CD4(cells/µl)	r	0.427	0.205	-0.012	-0.013	0.477	0.426
	p-value	0.047*	0.361	0.959	0.956	0.025*	0.048*
CD8(cells/µl)	r	-0.123	-0.031	0.084	0.002	0.107	-0.333
	p-value	0.585	O.891	0.709	0.992	0.636	0.130
CD4/CD8	r	0.217	0.201	0.015	-0.025	0.294	0.427
	p-value	0.333	0.370	0.948	0.913	0.184	0.048*

Keys: VL=Viral load; CD= Cluster of differentiation; IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α; MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM= Granzyme B; Significant level (P<0.05), r= correlation coefficient; *= Significant values

Table.2 Correlation of inflammatory, oxidative stress and cytolytic markers for stage I participants

Parameters		MDA (nmol/ml)	TAC (µmol/l)	PEF (pg/ml)	GRZM (pg/ml)
IL-10 (pg/ml)	r	-0.061	-0.421	0.205	0.380
	p-value	0.786	0.051	0.360	0.081
TNF-α (pg/ml)	r	0.126	0.514	0.055	-0.218
	p-value	0.577	0.014*	0.808	0.330

Keys: IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α; MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM= Granzyme B; Significant level (P<0.05), r= correlation coefficient; *=Significant value

Table.3 Correlation between studied parameters for stage II participants

Parameters		IL-10 (pg/ml)	TNF-α (pg/ml)	MDA (nmol/ml)	TAC (µmol/l)	PEF (pg/ml)	GRZM (pg/ml)
VL(copies/ml)	r	0.528	-0.066	-0.003	-0.516	-0.037	0.097
	p-value	0.095	0.847	0.993	0.104	0.914	0.776
CD4(cells/µl)	r	-0.002	-0.377	-0.013	0.282	0.166	0.025
	p-value	0.996	0.253	0.969	0.401	0.625	0.941
CD8(cells/µl)	r	-0.229	0.236	-0.375	0.545	-0.360	-0.367
	p-value	0.499	0.485	0.256	0.083	0.277	0.268
CD4/CD8	r	0.264	-0.127	0.188	-0.437	0.240	0.064
	p-value	0.432	0.710	0.581	0.179	0.477	0.851

Keys: VL=Viral load; CD= Cluster of differentiation; IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α; MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM= Granzyme B; Significant level (P<0.05), r= correlation coefficient

Table.4 Correlation of inflammatory, oxidative stress and cytolytic markers for stage II participants

Parameters		MDA (nmol/ml)	TAC (µmol/l)	PEF (pg/ml)	GRZM (pg/ml)
IL-10 (pg/ml)	r	-0.285	-0.313	-0.281	-0.342
	p-value	0.395	0.349	0.402	0.304
TNF-α (pg/ml)	R	-0.462	-0.054	0.093	0.249
	p-value	0.152	0.874	0.785	0.459

Keys: IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α; MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM= Granzyme B; Significant level (P<0.05); r= correlation coefficient

Table.5 Correlation between studied parameters for control participants

Parameters		IL-10 (pg/ml)	TNF-α (pg/ml)	MDA (nmol/ml)	TAC (µmol/l)	PEF (pg/ml)	GRZM (pg/ml)
CD4(cells/µl)	r	-0.318	-0.261	0.397	0.626	-0.421	-0.299
	p-value	0.370	0.466	0.256	0.053	0.225	0.401
CD8(cells/µl)	r	0.186	-0.298	-0.404	0.018	0.208	0.463
	p-value	0.607	0.402	0.247	0.960	0.565	0.178
CD4/CD8	r	-0.346	0.021	0.562	0.469	-0.505	-0.580
	p-value	0.328	0.953	0.091	0.172	0.136	0.079

Keys: VL=Viral load; CD= Cluster of differentiation; IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α; MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM= Granzyme B; Significant level (P<0.05); r= correlation coefficient

Table.6 Correlation of inflammatory, oxidative stress and cytolytic markers for control participants

Parameters		MDA (nmol/ml)	TAC (µmol/l)	PEF (pg/ml)	GRZM (pg/ml)
IL-10 (pg/ml)	r	-0.907	-0.564	0.531	0.826
	p-value	0.000*	0.090	0.114	0.003*
TNF-α (pg/ml)	r	0.079	-0.640	0.554	0.281
	p-value	0.827	0.046*	0.096	0.432

Keys: IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α; MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM= Granzyme B; Significant level (P<0.05); r= correlation coefficient; *=Significant values

Stage I mean CD4 levels revealed significant positive correlations (p<0.05) with PEF (r=0.477) and GRZM (r=0.426); non-significant positive correlation with TNF-α (r=0.205, p=0.361); and strong positive correlations with IL-10 (r=0.427, p=0.047). Stage I CD4 counts were relatively normal because of a lower viral load and a corresponding decrease in inflammation, which is facilitated by enhanced anti-inflammatory cytokine release. Stage I events could be caused by the efficacy of HAART, which causes a markedly progressive increase in CD4+ T cell count that are comparable to those in people without HIV (Osuji *et al.,* 2018) and a drop in viral load. With MDA (r=-0.012, p=0.959) and TAC (r=-0.013, 0.956), on the other hand, a negative correlation was seen with regards to CD4. This correlation pattern agrees with the fact that increased CD4 count and reduced viral replication result in reduced inflammation (Muema *et al.,* 2020). There was a minor variation in correction pattern for stage I and stage II between CD4 counts and IL-10 (r=0.427, r= -0.002); TNF-α (r=0.205, r=-0.377); and TAC (r= -0.013, r= 0.282) respectively. The variation in virologic and immunologic events in both groups may have resulted to the dissimilarities observed in the present study. CD4 count correlation

pattern with PEF and GRZM for sero-positive groups is consistent with improved immune capacity/immune competence status of stage I group. This observation may also have been occasioned by effective HAART intake. Similar to stage II, the control group's CD4 count exhibited a positive association with TAC (r= 0.626, p=0.053); and a negative correlation (p>0.05) with TNF-α and IL-10. Nevertheless, in contrast to stages I and II, the CD4 count in control group displayed a negative connection (p>0.05) with the cytolytic molecules. This may be due to lack of HIV-related immune activation or growth in the control groups, which resulted in a comparatively low level of cytolytic activity.

The stage I mean CD8 levels exhibited a weak negative association (p>0.05) with GRZM (r = -0.333), TNF-α (r = -0.031) and IL-10 (r = -0.123); while there was a positive connection (p>0.05) with MDA (r = 0.084), TAC (r = 0.002) and PEF (r = 0.107). A non-significant correlation was also observed between Stage II CD8 count and other measures; the count exhibited a positive correlation with TNF-α(r =0.236) and TAC (r=0.545); whereas a negative correlation with MDA, PEF, GRZM and IL-10 was observed. There was a positive association (p>0.05) between the control group's CD8 count and the cytolytic molecules, anti-inflammatory cytokine, and overall antioxidant capacity. Nevertheless, CD8 count for control group demonstrated a negative connection (p>0.05) with the pro-oxidative stress parameter and pro-inflammatory cytokine. Persistent widespread immune activation during the chronic phase is caused by continuous HIV replication (Chauvin *et al.,* 2022). The immune system reacts to continued HIV replication by releasing inflammatory cytokines and increasing the activation state of immune cells, increasing immunological activation. Hence, chronic immunological activation and abnormal cytokine production are linked to HIV infection (Osuji *et al.,* 2018). The immunological dysregulation that characterizes the progression of HIV to AIDS has been partially attributed to an imbalance in cytokine production (Balaji *et al.,* 2021) and Chauvin *et al.,* (2022) had also documented that inflammation and oxidative stress are correlated in the pathogenesis of HIV disease indicators and its consequences.

There was a significant positive connection between the mean CD4/CD8 ratio and GRZM (r=0.427, p=0.048) in stage I. The ratio at stage I is frequently more than 1.0, indicating immunological competence or recovery. Strong positive correlation of CD4/CD8 ratio with cytolytic molecule (GRZM) is indicative of a robust immune system. Along with IL-10, TNF-α, MDA, and PEF, the ratio also displayed a slight positive connection (p>0.05). On the other hand, a weak negative relationship (r = -0.025, p =0.913) was seen with TAC. Stage II showed non-significant correlation across parameters with CD4/CD8 ratio; the ratio showed a negative link with total antioxidant capacity and the pro-inflammatory cytokine, but a positive correlation with the anti-inflammatory cytokine, MDA, and cytolytic molecules. As opposed to stage II, the control group's ratio demonstrated a positive link (p>0.05) with TAC and TNF-α whereas a negative correlation (p>0.05) with ant-inflammatory cytokine and cytolytic molecules was observed. Pro-oxidative stress and chronic inflammation have been linked to a number of chronic diseases, including cancer, non-alcoholic liver disease, neurodegenerative disorders, and cardiovascular disease, in addition to non-AIDS events like cellular senescence and aging (Sonia Zicari *et al.,* 2019; Wang *et al.,* 2021). In order to identify patients who require active care of concomitant risk factors for age-associated diseases or more thorough screening for non-AIDS episodes, CD4/CD8 ratio monitoring may therefore be utilized in this regard.

While the stage II mean IL-10 values showed non-significant negative correlations (p>0.05) with MDA, TAC, PEF, and GRZM, the stage I mean IL-10 values showed weak negative correlations (p>0.05) with MDA and TAC; and weak positive correlations (p>0.05) with PEF and GRZM. But in the control group, IL-10 had mild correlations (p>0.05) with TAC (r= -0.564) and PEF (r=0.531); strong positive correlation with GRZM (r=0.826, p=0.003); and strong negative correlation with MDA (r=-0.907, p=0.000). The association that has been identified between GRZM, PEF, TAC, MDA, and IL-10 in the control group may suggest that an increase in ant-inflammation marker may lead to corresponding decrease in inflammation occasioned by decreased viral replication with consequential immune recovery and robust immune capacity. The later is demonstrated by increased production of cytolytic molecules. The relationship equally showed that decreased inflammation may also be linked to reduced pro-oxidative status. Oxidative stress and inflammation are correlated and are related to disease development and progression of HIV illness (Chauvin *et al.,* 2022; Lomeli-Martinez *et al.,* 2019). This result agrees with previously documented study which revealed that amplified oxidative stress results from the release of several reactive species at the site of inflammation by inflammatory cells (Ramos-

González *et al.,* 2021). On the other hand, a number of reactive oxygen/nitrogen species can initiate intracellular signaling cascade that enhances proinflammatory gene expression (Sies & Jones, 2020; Ramos-González *et al.,* 2021). Thus, oxidative stress and inflammation are closely related pathophysiological events that are interconnected.

The mean TNF-α values in stage I demonstrated a strong positive correlation with TAC (r=0.514, p=0.014); a weak positive correlation (p>0.05) with MDA and PEF; and a negative correlation with GRZM (r=-0.218, p=0.330). In stage II, the mean TNF-α values demonstrated a non-significant negative correlation (p>0.05) with MDA and TAC; as well as a weak positive correlation (p>0.05) with the cytolytic molecules. The correlation result for stage I contradict previously documented reports that HIV-activated macrophages via TNF-α release, and activated polymorphonuclear leukocytes, contribute to the generation and accumulation of ROS (Singh *et al.,* 2019) which could lead to deficiency in the antioxidant capacity, due in part to excessive consumption of antioxidant molecules in order to protect cells against ROS-induced damage (Kenmegne *et al.,* 2020), which further enhance the pro-oxidative status in HIV infection. Nonetheless, the findings of this research might be connected to the impact of long-term HAART therapy, which can return health metrics to nearly normal levels (Gray *et al.,* 2021). The pattern seen may also be associated with a negative feedback mechanism emanating from raised pro-oxidative markers. On the other hand, the control group had a mild positive association (p>0.05) with MDA, PEF, and GRZM; and a substantial negative correlation with TAC (r=-0.640, p=0.046). The correlation result in the control group indicates that an increase in inflammatory event could result to an increase in accumulation of pro-oxidative markers leading to a pro-oxidative status.

The study revealed that for stage I participants, viral load had a strong negative correlation with IL-10. This implies that a higher anti-inflammatory status will result to a decline in viral replication. Therefore HIV participants could be advised to engage in activities and lifestyle that would reduce inflammation. The CD4 count for stage I participants showed significant positive correlation with IL-10, PEF, and GRZM. This implies that an improved CD4 count status is associated with improved cytolytic capacity of the immune effector T cells with increased release of cytolytic molecules. Also higher anti-inflammation status may result to improved CD4 count. This may have resulted from declined viral replication due to lower inflammation. We further conclude that CD4 count and viral load values which are usual tools for monitoring HIV infected patients could be used to predict other health measures such as inflammation and cytotoxic activities. This will enable care givers proactively arrest disease progression and provide timely interventions against other non AIDS pathology.

Authors' Contribution

Okparaku, S. O. wrote the manuscript and coordinated sample collection and analysis. Agbakoba, R. N. produced the research design and played supervisory role. Chukwuanukwu, R. C. proof-read the manuscript. Iyevhobu, K. O. sourced for materials.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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