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Variation blueprints: A prospective cohort study of chronic HIV infected subjects on HAART

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Abstract

This study was designed to evaluate a six-month variation in CD8, cytotoxic molecules, CD4, viral load, CD4/CD8 ratio, inflammatory cytokines and oxidative stress parameters in chronic HIV infection. A total of 58 HIV sero-positive subjects on HAART were recruited for this study. CDC staging method with CD4 count was used to classify the study subjects. After a baseline sampling, subjects were followed up for 6 months, samples were collected and evaluated every 3 months. The result of the study showed significant ($p<0.05$) differences in viral load of test subjects when comparing the baseline, 3-month and the 6-month follow-up values. Stage I individuals' PCV values showed a significant ($p<0.05$) change from baseline to six months, indicating a considerable potential fluctuation in six months. A significant variation was seen between 3months (32.91±1.51%) and 6months (35.18±2.44 %) PCV follow up values for stage II subjects. There was a steady significant ($p<0.05$) reduction in mean CD8 values for stage I participants from baseline to the 6-month follow-up. In stage II group, CD8 count decreased significantly ($p<0.05$) between three and six months follow up period. PEF and GRZM values for stage I individuals revealed significant ($p<0.05$) decrease within the first three months of the follow-up. Stage II participants likewise demonstrated a substantial ($P=0.022$) decline in PEF values in the first three months but no significant ($p>0.05$) difference was seen with GRZM. We observed a significant ($p=0.034$) variation in IL-10 values for stage I individuals between the baseline and 6-month follow-up. Stage II participants showed a substantial ($p<0.05$) change within the period. TNF-α values for stage I subjects showed no discernible change ($p>0.05$) over the course of the 6-month period. Nonetheless, there were notable ($p<0.05$) variations in TNF-α levels among stage II participants within the follow up period. TAC values for stage I individuals revealed a sizeable ($p<0.05$) difference between baseline and the 6-month. Likewise, for individuals in stage II, there were notable ($p<0.05$) modifications in their overall antioxidant capacity between three and six months follow up period. Conclusively, there may be notable changes in the health indices of seropositive individuals in three to six months.

Keywords: HIV infection; HIV stages; Cytolytic Molecules; Inflammation; Oxidative Stress; Variation

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1. Introduction

A range of disorders are associated with infection with the human immunodeficiency virus (HIV), including acquired immune deficiency syndrome (HIV/AIDS) [1, 2]. As the infection worsens, it causes additional immune system interference, raising the chance of opportunistic infections, cancers that sporadically attack healthy individuals, and common illnesses like tuberculosis [3].

The immune system is a sophisticated network of chemicals and cells that protects the organism's integrity by getting rid of anything that is deemed harmful. T cells in general and cytotoxic T lymphocytes (CTLs) specifically are the main actors in adaptive cellular immune responses. The importance of CD8+ T-cells in the suppression of HIV infection has long been recognized [4]. Due to their strong cytotoxic potential, circulating CD8+ T-cells rely on the expression of CD107a, which is a common indicator of degranulation capability, as well as the contents of their lytic granules, namely granzymes and perforin [5]. Mostly by the exocytosis of cytotoxic granules containing granzymes and perforin, cytotoxic T lymphocytes eliminate virally infected target cells. Perforin genetic mutations or deletions cause significant immunodeficiency and reduced cellular cytotoxicity [6].

Significant research has linked the cytotoxic activity of circulating HIV-specific CD8+ T-cells to the inhibition of viral replication and a postponed course, underscoring the significance of this effector function [7]. HIV-positive individuals have higher levels of activation and inflammation, which has an impact on CD8+ T-cells. Through the regulatory protein Tat and the envelope glycoprotein gp120, the human immunodeficiency virus causes the production of reactive oxygen species (ROS) [8]. The production and build-up of reactive oxygen species (ROS) are also facilitated by HIV-activated macrophages through TNF-α release and activated polymorphonuclear leukocytes [9]. Due in part to overconsumption of antioxidant molecules to shield cells from ROS-induced damage, the antioxidant capacity is subsequently reduced [10], which further amplifies the pro-oxidative status. This study evaluated a six-month variation in CD8, cytotoxic molecules, CD4, viral load, CD4/CD8 ratio, inflammatory cytokines and oxidative stress parameters in chronic HIV infection, using seropositive subjects visiting HIV clinic at Irrua Specialist Teaching Hospital and Central Hospital, Uromi Edo State.

2. Materials and Methods

A total of 58 HIV sero-positive subjects on highly active antiretroviral therapy (HAART) were recruited for this study. After a baseline sampling, subjects were followed up for 6 months. Samples were collected every 3 months from the subjects to evaluate the same parameters and determine changes over time. The CDC staging method with CD4 count was used to classify the study subjects; stage I (CD4 count ≥ 500 cells/µl) and stage II (CD4 count 200 to 500 cells/µl) [11, 12].

Subjects Selection Criterion: Recruited subjects were above 18 years, HIV seropositive and currently on HAART. 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 were collected from each patient and dispensed into plain container (5ml) and EDTA container (5ml). The samples in plain container were centrifuged and serum obtained for the determination of cytokines (IL-10, TNF-α), MDA (Malondialdehyde), 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 FBC, 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: Full blood count (FBC) was estimated using SYSMEX XE-2100™ haematological analyser, viral load was estimated using the COBAS C4800™, CD4 and CD8 counts were determined using the BD FACS Count™ System, ELISA was used for other parameters.

3. Result

The study participants comprised of 58 HIV sero-positive subjects visiting HIV clinic at two government hospitals in Edo central. The subjects were classified according to the Centre for Disease Control pattern, into stage I and II with 30 and 28 study subjects 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 while stage II had 6 (21.4%) male and 22 (78.6%) female. The subjects 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 subjects 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 level of VL, CD4, CD8, CD4/CD8, IL-10 and TNF-α at baseline, three- and six-months follow-up in stage I (mean±SD). The table showed significant ($p<0.05$) difference in mean values of viral load at baseline, 3months and 6months. While baseline showed the lowest value (28.18±3.16 copies/ml), 6 months showed the highest values (36.00±2.78 copies/ml). The CD4 values at baseline and 3months were significantly higher ($p<0.05$) than the 6months (756.77±237.89 cells/µl), there was no significant difference between the CD4 values at baseline (880.36±334.60 cells/µl) and 3months (959.27±374.68 cells/µl) samples. The CD8 values showed similar pattern with CD4. There was significant ($p<0.05$) difference when comparing baseline (817.77±349.90 cells/µl) and 3months (739.82±209.84 cells/µl) with the 6months (629.50±226.12 cells/µl) values. There was significant ($p=0.034$) difference in the IL-10 values between 3month (106.03±8.34 pg/ml) and 6month (104.24±7.37 pg/ml). The CD4/CD8 ratio and TNF-α showed no significant ($p>0.05$) difference across the different sampling time. However, CD4/CD8 ratio and TNF-α showed reversed pattern at baseline; lowest CD4/CD8 ratio and highest TNF-α values.

Table 2 shows the level of MDA, TAC, PEF and GRZM at baseline, three- and six-months follow-up in stage I (mean±SD). The mean MDA values at different sampling time showed no significant ($p>0.05$) difference. There was significant ($p<0.05$) difference in TAC when comparing baseline, 3 months and 6 months. At baseline serum perforin values (163.75±23.93 pg/ml) were significantly ($p=0.023$) higher than values at 3 months (148.98±16.35 pg/ml). There was no significant difference ($p>0.050$) when comparing baseline and 3 months with 6 months (151.78±23.67 pg/ml). Serum GRZM values at baseline (1.92±0.38 pg/ml) were significantly ($p<0.05$) higher than the values at 3months (1.64±0.23 pg/ml) and 6months (1.52±0.33 pg/ml). However, GRZM values at 3month was higher but not significantly ($p=0.107$) different from 6 months.

Table 1 Follow up values of VL, CD4, CD8, CD4/CD8, IL-10 and TNF-α for stage I (mean±SD)

Parameters	Baseline (A)	Three months (B)	Six months (C)	p-value A vs B	p-value A vs C	p-value B vs C
VL(copies/ml)	28.18±3.16	31.00±1.66	36.00±2.78	0.002	0.000	0.000
CD4(cells/µl)	880.36±334.60	959.27±374.68	756.77±237.89	0.215	0.037	0.000
CD8(cells/µl)	817.77±349.90	739.82±209.84	629.50±226.12	0.144	0.001	0.000
CD4/CD8	1.26±0.63	1.36±0.56	1.33±0.55	0.317	0.448	0.548
IL-10(pg/ml)	105.13±9.16	106.03±8.34	104.24±7.37	0.406	0.458	0.034
TNF-α(pg/ml)	17.04±1.90	16.44±1.85	16.43±2.38	0.158	0.166	0.995

Keys: VL=Viral load; CD= Cluster of differentiation; IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α SD = Standard Deviation; Significant level ($P<0.05$)

Table 2 Follow up values of MDA, TAC, PEF and GRZM for stage I (mean±SD)

Parameters	Baseline (A)	Three months (B)	Six months (C)	p-value A vs B	p-value A vs C	p-value B vs C
MDA(nmol/ml)	1.84±0.11	1.84±0.13	1.79±0.10	0.972	0.112	0.131
TAC(µmol/l)	895.87±128.49	1075.37±192.66	811.95±174.26	0.000	0.034	0.000
PEF(pg/ml)	163.75±23.93	148.98±16.35	151.78±23.67	0.023	0.127	0.605
GRZM(pg/ml)	1.92±0.38	1.64±0.23	1.52±0.33	0.005	0.000	0.107

Keys: MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF =Perforin; GRZM=Granzyme; SD = Standard Deviation; Significant level ($P<0.05$)

Table 3 shows the level of some haematological parameters at baseline, three- and six-months follow-up in stage I (mean±SD). The table indicated a significant ($p=0.031$) difference in PCV between baseline (36.55±5.35%) and 6months (33.59±1.79%), whereas the difference between baseline and 3month (34.36±2.61%) was not significant ($p=0.073$).

The same was also observed with 3months and 6months (p>0.05). For WBC, PLT, LYM, NEUT and MON, there were no significant differences in their mean values when comparing the baseline, 3 months and 6 months values obtained.

Table 3 Follow up values of some haematological parameters for stage I (mean±SD)

Parameters	Baseline (A)	Three months (B)	Six months (C)	p-value A vs B	p-value A vs C	p-value B vs C
WBC(x10^9/l)	5.55±1.39	5.27±1.17	5.26±1.20	0.425	0.387	1.000
PCV(%)	36.55±5.35	34.36±2.61	33.59±1.79	0.073	0.031	0.244
PLT(x10^9/l)	219.68±62.48	220.18±45.14	208.95±48.14	0.971	0.477	0.443
LYM(%)	47.45±8.80	48.41±7.34	48.18±7.74	0.663	0.763	0.909
N(%)	42.86±8.33	39.41±7.54	40.23±8.71	0.128	0.210	0.735
M(%)	9.68±2.75	12.18±5.70	11.59±6.34	0.066	0.213	

Keys: WBC=Total white blood cell count; PCV= Packed cell volume; PLT= Platelet count; LYM = Lymphocyte count; N=Neutrophile count; M=Monocytes count; SD = Standard Deviation; Significant level (P<0.05)

Table 4 shows the level of VL, CD4, CD8, CD4/CD8 ratio, IL-10 and TNF-α at baseline, three- and six-months follow-up in stage II (mean±SD). The table showed significant (p=0.009) difference in mean values of viral load (VL) between 3 months (29.64±2.42 copies/ml) and 6 months (33.55±3.83 copies/ml). However, there was no significant (p>0.05) difference when comparing the baseline (31.45±2.42 copies/ml) with 3 months values or baseline with 6 months values obtained. The CD4 values showed no significant (p>0.05) difference when comparing the baseline (400.09±68.03 cells/µl), 3 months (545.27±388.21 cells/µl) and 6 months (399.73±151.52 cells/µl) values. The CD8 values followed same pattern with VL, there was significant (p=0.022) difference between 3 months (808.36±396.16 cells/µl) and 6 months (626.82±243.48 cells/µl) values. The table showed no significant (p>0.05) differences when comparing the baseline (685.09±366.39 cells/µl) with 3 months or 6 months values. The CD4/CD8 ratio just like CD4, showed no significant (p>0.05) difference when the baseline (0.73±0.36), 3 months (0.77±0.43) and 6 months (0.71±0.29) values were compared. There was significant (p<0.05) difference in IL-10 values between the baseline (113.48±3.89 pg/ml) and 3 months (103.68±3.64 pg/ml) as well as between 3 months and 6 months (111.45±2.05 pg/ml) but there was no significant (p=0.290) difference between baseline and 6 months values. The baseline values of TNF-α (20.25±1.74 pg/ml) showed significant (p<0.05) difference when compared with 3 months (16.94±2.11 pg/ml) and 6 months (17.24±1.32 pg/ml), whereas there was no significant (p=0.780) difference between 3 months and 6 months.

Table 4 Follow up values of VL, CD4, CD8, CD4/CD8, IL-10 and TNF-α for stage II (mean±SD)

Parameters	Baseline (A)	Three months (B)	Six months (C)	p-value A vs B	p-value A vs C	p-value B vs C
VL(copies/ml)	31.45±2.42	29.64±2.42	33.55±3.83	0.099	0.236	0.009
CD4(cells/µl)	400.09±68.03	545.27±388.21	399.73±151.52	0.256	0.994	0.090
CD8(cells/µl)	685.09±366.39	808.36±396.16	626.82±243.48	0.240	0.282	0.022
CD4/CD8	0.73±0.36	0.77±0.43	0.71±0.29	0.770	0.875	0.414
IL-10(pg/ml)	113.48±3.89	103.68±3.64	111.45±2.05	0.000	0.290	0.000
TNF-α(pg/ml)	20.25±1.74	16.94±2.11	17.24±1.32	0.000	0.007	0.780

Keys: VL=Viral load; CD= Cluster of differentiation; IL-10= Interleukin 10; TNF- α = Tumor necrosis factor- α SD = Standard Deviation; Significant level (P<0.05)

Table 5 shows the level of MDA, TAC, PEF and GRZM at baseline, three- and six-months follow-up in stage II (mean±SD). The table shows no significant (p>0.05) difference in MDA values when compare baseline, 3 months and 6 months. The baseline (1.85±0.14 nmol/ml) showed the highest values while 6 months (1.82±0.20 nmol/ml) produced the least. For TAC, there was significant (p=0.027) difference between mean values at 3 months and 6 months. Serum perforin at baseline (167.21±18.12 pg/ml) was significantly (p=0.022) higher than mean values at 3 months (145.02±15.45 pg/ml).

No significant (p>0.05) difference was seen when compare 6 months (155.76±17.16 pg/ml) with baseline and 3 months. The table shows no significant (p>0.05) difference in GRZM when comparing baseline, 3 months and 6 months.

Table 6 shows the level of some haematological parameters at baseline, three- and six-months follow-up in stage II (mean±SD). As indicated in the table, WBC at baseline (4.17±1.07 x10^9/l) was significantly (p=0.023) lower than values at 6 months (5.19±0.79 x10^9/l). The WBC values showed increasing trend with baseline producing the lowest while 6 months was the highest. There was significant (p=0.034) difference between PCV values at 3 months (32.91±1.51%) and 6 months (35.18±2.44%), there were no significant (p>0.05) differences when comparing baseline (34.64±3.50%) with 3 months and 6 months values. No significant differences were seen with PLT, LYM, NEUT and MON when compare baseline, 3 months and 6 months values. Platelet (PLT) and MON showed a reversed pattern when compared with NEUT. While the duo showed a decreasing pattern from baseline to 6 months, NEUT showed an increasing pattern.

Table 5 Follow up values of MDA, TAC, PEF and GRZM for stage II (mean±SD)

Parameters	Baseline (A)	Three months (B)	Six months (C)	p-value A vs B	p-value A vs C	p-value B vs C
MDA(nmol/ml)	1.85±0.14	1.84±0.13	1.82±0.20	0.861	0.603	0.733
TAC (µmol/l)	806.35±150.86	811.42±83.11	749.10±40.50	0.869	0.174	0.027
PEF (pg/ml)	167.21±18.12	145.02±15.45	155.76±17.16	0.022	0.204	0.217
GRZM (pg/ml)	1.90±0.20	1.76±0.16	1.86±0.27	0.113	0.770	0.268

Keys: MDA= Malondialdehyde; TAC= Total anti-oxidant capacity; PEF= Perforin; GRZM=Granzyme; SD = Standard Deviation; Significant level (P<0.05)

Table 6 Follow up values of some haematological parameters for stage II (mean±SD)

Parameters	Baseline (A)	Three months (B)	Six months (C)	p-value A vs B	p-value A vs C	p-value B vs C
WBC(x10^9/l)	4.17±1.07	4.48±1.26	5.19±0.79	0.305	0.023	0.136
PCV(%)	34.64±3.50	32.91±1.51	35.18±2.44	0.175	0.548	0.034
PLT(x10^9/l)	223.45±55.69	213.27±65.41	210.18±70.24	0.288	0.399	0.777
LYM(%)	45.27±13.53	45.45±12.72	41.18±11.13	0.973	0.312	0.280
N(%)	45.00±15.42	45.36±14.17	49.36±12.18	0.954	0.344	0.425
M(%)	9.73±4.59	9.18±2.09	8.91±3.65	0.724	0.427	

Keys: WBC=Total white blood cell count; PCV= Packed cell volume; PLT= Platelet count; LYM = Lymphocyte count; N=Neutrophile count; M=Monocytes count; SD = Standard Deviation; Significant level (P<0.05)

4. Discussion

During chronic HIV infection, HIV-specific CD8+ T-cell pool exhibit reduced differentiation, decreased functionality, enhanced exhaustion, and little-to-no expression of perforin [7]. According to some research [7], one of the factors leading to the progression of HIV infection is the decrease of HIV-specific CD8+ T cell cytolytic capability during chronic infection. Moreover, persistent CD8+ T cell activation in the lack of potent antiviral action may exacerbate the illness and ultimately boost viral replication [13]. A condition of widespread immunological activation that lasts the duration of the chronic phase is consequently brought on by continuous HIV replication [14]. 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 [15]. 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 [16]. Assessing changes in these cytokine levels may provide insight into the pace of viral replication and the immune system's damage or recovery, particularly during HAART [17]. In addition to CD4+ T cell count, measurement of oxidative stress molecules may serve as a possible monitoring indicator for estimating the rate of infection progression, developing adverse treatment responses, and forecasting the likelihood of non-AIDS complications [17]. Also, a low CD4/CD8 ratio is indicative of immunological failure, and patients with this ratio also have a greater risk of

non-AIDS morbidity and death [17]. Hence, monitoring changes in CD4/CD8 ratio may potentially assist in preventing unfavorable disease outcomes.

Our study revealed that the mean value of the viral load increased consistently for stage I participants when compare baseline and the 6-month follow-up values, and there were significant ($p<0.05$) differences between the baseline and the 6-month follow-up as well as the 3-month follow-up. In the stage II group, there was a noteworthy ($p<0.05$) rise in VL from three months to six months. This result implies that the viral load of a sero-positive patient may vary significantly in 3 months. The report by de Siqueira-Filha *et al.* [18], which suggests routine viral load checks every three to six months once levels drop below 50 copies/ml, is consistent with our observation. The severity of a viral infection is generally correlated with a larger viral burden or viral load [19]. When discussing HIV/AIDS management, it is common to state that viral load monitoring is essential to the care of individuals living with HIV [19]. De Siqueira-Filha *et al.* [18] stated that a long-term plasma HIV-RNA level of less than 50 copies/ml is the intended result of treatment. A concentration of more than 400 copies/mL is considered inadequate control.

A substantial ($p<0.05$) difference was observed between the 3- and 6-month follow-up CD4 values for stage I individuals, which fluctuated in the opposite direction every three months, according to the present study when comparing their baseline and follow-up data. The CD4 count fluctuated similarly in stage II individuals, although the changes between baseline and six months was not statistically significant ($p>0.05$). Despite advancements in CD4 count monitoring, its use has recently sparked a heated discussion. According to studies conducted by Vogler *et al.* [20], CD4 counts remarkably fall below limits that are clinically significant in stable patients who achieve total virological suppression and immunological recovery while on antiretroviral therapy. Thus, in this case, care is rarely influenced by CD4 count monitoring. This claim appears to be consistent with our findings. If CD4 values do not stabilize within three to six months, it is possible that CD4 monitoring is not a viable means of tracking HIV infection, particularly in people who are virologically suppressed. The Department of Health and Human Services' most current set of guidelines also recognizes that the CD4 cell count offers only little information in stable individuals who have a satisfactory immunovirological response to antiretroviral therapy (ART). This expert group therefore recommends lowering the frequency of CD4 monitoring [21].

There was a steady reduction in mean value for CD8 values for stage I participants from baseline to the 6-month follow-up, with significant ($p<0.05$) differences observed between baseline and the 6-month follow-up and between the 3-month follow-up and the 6-month follow-up. In the stage II group, the CD8 count decreased significantly ($p<0.05$) between three and six months, but not significantly ($p>0.05$) from baseline to three months. Within three to six months, there may be a considerable fluctuation in the cytolytic lymphocytes and cytolytic capacity of sero-positive people, according to our finding. The reduction in CD8 count in this study is consistent with the findings of Parekh *et al.* [19], who reported that the chronic phase of HIV infection causes the immune system's capacity to produce new T cells to gradually drop. It is in agreement with the suggestion of de Siqueira-Filha *et al.* [18] for a routine check/monitoring every three to six months because the variation found was similar to our observation with viral load in three to six months.

No significant ($P>0.05$) difference found when comparing the CD4/CD8 ratio for stage I participants between baseline and the 6-month follow-up. A comparable pattern was noted for stage II participants; from baseline to six months, no significant ($p>0.05$) change was seen. This finding implies that the six-monthly check-ins for HIV patients on HAART may not yield enough data regarding immune reconstitution and risk assessment for non-AIDS related complication. A good response to antiretroviral therapy (ART) may necessitate both normalization of the CD4 count and the ratio of CD4+ to CD8+ T cells, since the later may aid in further differentiating the risk of disease progression of treated HIV-infected patients. The current clinical reality suggests that the CD4/CD8 ratio may be more beneficial than CD4 numbers, as most patients will be able to sustain long-term viral suppression [22].

It was observed that there was a significant ($p=0.034$) variation in IL-10 values for stage I individuals between the baseline and 6-month follow-up. In a similar vein, stage II participants showed a substantial ($p<0.05$) change between baseline and three months, as well as between three and six months. This finding might indicate that, in HIV participants, there might be a notable change in anti-inflammatory cytokines in three to six months. Such variation may have profound effect on disease progression following the report of Salehi *et al.* [23] who documented that HIV replication is the result of an imbalance between the effects of pro-inflammatory cytokines that increase viral replication and those of anti-inflammatory cytokines and chemokines that inhibit viral replication [23].

When stage I individuals' TNF-α values were compared between baseline and the 6-month follow-up, there was no discernible ($p>0.05$) change in mean values over the course of the 6-month period. Nonetheless, there were notable ($p<0.05$) variations in the average TNF-α levels among stage II participants when compare baseline and three months,

as well as baseline and six months, with a greater baseline value. The findings imply that there may be a notable change in pro-inflammatory cytokines within three months, particularly in patients whose diseases are more severe. This variation may also have a substantial effect on the course of the condition.

The results of comparing the MDA readings for stage I and II individuals at baseline with the 6-month follow-up revealed no significant ($p>0.05$) difference during the 6-month period. This discovery is in line with our submission regarding the CD4/CD8 ratio, which holds that a 6-month follow-up may not be enough to provide insight into the likelihood of non-AIDS complications in HIV individuals receiving HAART.

When TAC levels for stage I individuals were compared between baseline and the 6-month follow-up, the results revealed a substantial ($p<0.05$) difference. This finding may indicate that there is a considerable shift in the individuals' overall antioxidant capacity every three months in HIV stage I. Likewise, for individuals in stage II, there was notable ($p<0.05$) modifications in their overall antioxidant capacity between three and six months. This supports the finding with subjects in stage I. In summary, our research may indicate that, in order to effectively control oxidative stress and related complications, it may be necessary to routinely assess the total antioxidant capacity of HIV-positive persons every three to six months.

When PEF and GRZM values for stage I individuals were compared between baseline and six months of follow-up, the results revealed that the mean values had significantly ($p<0.05$) decreased within the first three months of the follow-up. Within a follow-up period of three to six months, there were no discernible ($p>0.05$) changes in their mean values. During the first three months of the follow-up, Stage II participants likewise demonstrated a substantial ($P=0.022$) decline in PEF values; however, no significant ($p>0.05$) difference was seen with GRZM. This finding is in line with our observations on the CD8 count and may indicate potential variations in cytolytic activity within three months. A routine checkup every three months may be optimal for a better prognosis because this variation may impact the host immunological response to HIV infection.

There was no significant ($p>0.05$) difference in the mean WBC count for stage I individuals between the baseline and 6-month follow-up. In contrast, within six months, stage II participants displayed significant ($P=0.023$) variance. Compared to stage I seropositive patients, the stage II finding may be related to increased white blood cell engagement because of higher HIV-RNA replication. In the six months, the sero-positive group's mean lymphocyte value did not differ significantly. It's possible that HAART compliance and effectiveness contributed to the stability of total lymphocytes during a six-month period.

Stage I individuals' PCV values showed a significant change from baseline to six months, indicating a considerable potential fluctuation every six months. A significant variation was seen between 3 months and 6 months for stage II subjects with the later being higher than the former. This may be due to medical intervention such as active use of multivitamins capsules and other food/blood supplements. This outcome may necessitate a routine assessment of packed cell volume for sero-positive subjects every 3 to 6 months. Human immunodeficiency virus infection impairs the nutritional status of those who are infected, and the disease can advance more quickly in those who have poor nutritional health [24]. According to Pecora *et al*, [25], there has been evidence that shortages in some nutrients might impact immune function and consequently change viral expression and replication. These factors can ultimately affect the course of HIV disease and the patient's death.

5. Conclusion

We conclude that there may be notable changes in the viral load, cytolytic capacity, total antioxidant capacity, serum levels of inflammatory cytokines and packed cell volume of sero-positive patients in three to six months. Serum levels of cytokines in HIV subjects vary more frequently in stage II subjects, which may lead to significant changes in inflammatory events in three to six months. In order to achieve appropriate management of anemia, oxidative stress, inflammation, and its associated complications, frequent evaluations of HIV-positive persons may be required every three to six months. Our study also shows that, in virologically suppressed patients, tracking CD4 counts and the CD4/CD8 ratio for just 3 to 6 months rarely yields enough information to direct treatment of the illness, and may not be used to determine immune status, nor assess the risk of non-AIDS complications.

Compliance with ethical standards

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Disclosure of Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Statement of ethical approval

Ethical approval was obtained from the ethics and research committee of Ambrose Alli University, Ekpoma (NHREC/12/06/2013), and informed consent of the patients was obtained before sample collection.

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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.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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