Original Article

Prospective Evaluation of p24 Antigen and HIV-1 Protease Assays at 6 Months and 12 Months Initiation of Antiretroviral Therapy in HIV Infected Participants at Federal Medical Center, Lokoja, Nigeria

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Abstract

Background: This was a cross-sectional study designed to evaluate the potential use of p24 antigen and HIV-1 protease assays in treatment monitoring of HIV-1 infection at Federal Medical Center, Lokoja Nigeria. **Materials and Methods**: 154 participants aged 18-64 years were randomly recruited. 40 (group A) participants on efavirenz, Lamivudine, and tenofovir, 35 (group B) participants on nevirapine, lamivudine, and zidovudine served as a test, while 79 (group C) participants not antiretroviral therapy (ART) served as control. **Results**: P24 antigen, HIV-1 protease and viral load were significantly decreased in test participants than in control participants (f=14.59, p=0.002), (f=22.9, p=0.005) and (f=117.541, p=0.001 respectively). Antigenic markers in test participants with 6 months of therapy were significantly higher than 12 months of therapy (P<0.05). Plasma levels of tenofovir, lamivudine, and efavirenz in test participants were significantly lower while nevirapine was significantly higher at 6 months than in 12 months of therapy (P<0.05 respectively). Strong positive correlations were observed between p24 antigen, HIV-1 protease, and viral load while negative correlations were observed between p24 antigen, protease, viral load, and CD4+ t-cell counts, then between lamivudine, nevirapine, tenofovir and viral load. **Conclusions**: Decreased p24 antigen and HIV-1 protease with undetectable viral load in HIV infected participants indicated successful suppression of HIV viremia and strongly suggests good antigenic markers for treatment monitoring in resource-poor settings where regular viral load monitoring is unavailable. A combination of HIV-1 protease enzyme and p24 antigen assays with viral load and possibly CD_4^+ count can serve as tools for identifying participants with clinically insignificant symptoms of treatment failure.

Keywords: HIV, ART, p24 antigen, HIV-1 protease, Nigeria.

Introduction

Human immunodeficiency virus infection has progressively persisted despite vigorous researches and prophylactic measures on HIV/AIDS infection. [1] Because many infectious agents are endemic in sub-Saharan Africa, many immune complexes are formed and the presence of circulating immune complexes (CICs) in HIV/AIDS individuals stimulates many immune-pathological conditions ranging from chronic inflammatory responses to organ tissue damage. [2] Antiretroviral therapy (ART) restores immune function and reduces HIV-related outcomes but when treatment fails, increased morbidity and compromised quality of life in HIV patients are observed. [3] In Almost all EMS emergency staff and staff members reported worrying about the risk of developing HIV and hepatitis [4]. According to UNAIDS, in 2018 in Ukraine 240,000 people were living with HIV; 13,000 people were newly infected with HIV; and 6,200 people died from an AIDS-related illness [5]. The immune system is known to be very sensitive to changes in the body and general health [6].

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There are different antiretroviral drugs combinations used in the treatment of HIV at first-line therapy: these drugs combinations are being removed at intervals to get a better combination with good therapeutic effect and minimal toxicological effects. The purpose of the act was to suppress viral load and improving the immune system. [7] The principal aim of antiretroviral therapy (ART) is the suppression of circulating plasma virus to undetectable levels, thereby prolonging the life span of infected individuals. [8] Expanding access to ART in resource-limited settings along with close monitoring is expected to yield successful treatment outcomes. This is achieved by performing quantitative viral load monitoring every 3 – 6 months in high-income settings. [9] However, in resource-limited settings, a therapeutic outcome is evaluated based on CD_4^+ T-cell count or clinical findings which has been found to have its setbacks or limitations [10] Viral load assays are based on the amplification of HIV-RNA that requires infrastructural facilities for molecular diagnosis, expensive equipment, and skilled scientist which are often unavailable in low-income settings such as Nigeria. An alternative to HIV-RNA load using Enzyme-Linked Immunosorbent Assay (ELISA) for HIV treatment monitoring in resource-limited settings which is easier than polymerase chain reaction is, therefore, strictly advocated.

Materials and Methods

Study Design

This was a longitudinal cross-sectional study that recruited all participants enrolled for ART first-line regimens and those not yet on drugs at the time of the study. For HIV-1 infected participants on HAART, blood samples were collected at 2 different points: at 6 months into HAART and 12 months into HAART while blood samples were collected at 1 point for HIV-1 infected HAART naïve participants who were newly diagnosed as HIV-1 positive in the antiretroviral therapy clinic and served as HIV-1 positive control.

The HIV status of the subjects was confirmed using Geenius HIV Confirmatory System. The diagnosis of HIV and the criteria for the commencement of ART was based on Nigeria National guidelines for adult HIV and AIDS treatment and care. [11]

Study Area

The study was carried out at the Anti-Retroviral Therapy (ART) Clinic of Federal Medical Centre Lokoja. Federal Medical Centre Lokoja (FMCL) is located at number 1 Salihu Ibrahim way, Lokoja. FMCL was established on 9th November 1999. The ART Clinic of FMCL provides comprehensive HIV care services for the city of Lokoja which is located in the Kogi Central Senatorial district in Lokoja Local Government Area of Kogi State as well as some neighboring states such as Kwara, Benue, and Ekiti State. Lokoja is situated at 7.8^0 North Latitude, 6.74^0 East Longitude, and 55 meters elevation above the sea level. Lokoja is a town in Nigeria, capital of Kogi State, having about 60,579 inhabitants

Study Population

The study population consisted of 154 HIV-1 infected individuals aged 18-64 years (37 ± 0.74) which consist of 75 HIV-1 infected individuals that have been on drug combination (HAART) for a period of 6 – 12 months while the remaining 79 HIV-1 infected individuals have not commenced drug combination therapy (HAART naïve). For HIV-1infected individuals on HAART, blood samples were collected at 2 different points: at exactly 6 months into HAART and exactly 12 months into HAART while blood samples were collected at 1 point for HIV-1 infected HAART naïve individuals who were newly diagnosed as HIV-1 positive in the antiretroviral therapy clinic served as HIV-1 positive control. The HIV-1 infected subjects on drug combinations were divided into 2 groups based on the type of drug combinations. They were administered:

(1) Group A: 40 participants placed on a once-daily fixed-dose combination of tenofovir (300mg), lamivudine (300mg), and efavirenz (600mg).
(2) Group B: 35 participants placed on a once-daily fixed-dose combination of zidovudine (300mg), lamivudine (150mg), and nevirapine (200mg).

While the participants not yet on antiretroviral drugs that were newly diagnosed as HIV positive in the antiretroviral therapy clinic served as HIV-1 positive control.

Regrouping of participants based on HIV-1 antigenic suppression

The further classification was made based on evidence of HIV-1 antigenic suppression. The following indexes were used for HIV-1 antigenic suppression;

(i) HIV-1 viral load results in less than 1000 copies/ml for two consecutive times within 3-6 months apart with good adherence. [12]
(ii) HIV-1 protease enzyme results < 7.0 mU/mg for two consecutive times within 3-6 months apart with good adherence. [13]
(iii) HIV-1 p24 antigen results < 6.4 ng/ml for two consecutive times within 3-6 months apart with good adherence. [14]

A decrease in viral load with HIV-1 protease and or p24 antigen or three combined (decreased VL, Pr, and or p24 antigen) qualify as antigenic suppression. [14]

Viral load value above 1000 copies/ml, HIV-1 protease level above 7.0 mU/mg and HIV-1 p24 antigen level above 6.4 ng/ml for two consecutive times and within 3-6 months apart with good adherence were seen as non-suppression or non-antigenic suppression.

Group A participants placed on Tenofovir, lamivudine, and efavirenz were those individuals with baseline investigations

with hepatic impairment while group B participants placed on zidovudine, lamivudine, and nevirapine were those individuals with baseline investigations with evidence of renal impairment and psychiatric manifestations and these served as the basis of segregation into group A and B.

Regrouping of participants based on World Health Organization criteria.

Participants in each group were staged based on World Health Organization (WHO) criteria for HIV staging as follows: WHO clinical stage I (performance scale 1: asymptomatic, normal activity), WHO clinical stage II (performance scale 2: symptomatic, normal activity), WHO clinical stage III (performance scale 3: Bed-ridden <50% of the day during the past one month), WHO clinical stage IV (performance scale 4: bed-ridden >50% of the day during the last one month).

Sample Technique

The sampling technique adopted for recruiting participants in the study was a simple random sampling technique while the type of drug combination was used to segregate the participants into sub-groups.

Inclusion and Exclusion Criteria

Participants aged between 18 and 64 (37±0.74) years on the two different drugs combination must have taken the drugs for six months before qualifying for participation. Any HIV infected individuals that had co-morbidity were excluded. Also, any HIV infected individuals on contraceptives, smokers, and alcoholics were excluded.

Ethical Clearance and Informed Consent

Approval to carry out the study was obtained from the board of Ethics Committee of Federal Medical Centre, Lokoja with reference number FMCL/MED/115/II/271. Informed consent was obtained from each of the participants for the study following the guidelines of the Ethics Committee of Federal Medical Centre, Lokoja.

Specimen Collection

Eight milliliters of venous blood was collected from each subject at the point of joining the research and six months after joining the research. 4ml of blood was dispensed into Ethylene diamine tetra-acetic acid (EDTA) bottle and was used for CD_4 count, Viral Load, and antiretroviral drug concentrations. 3mls were dispensed into a Lithium heparin bottle and was used for p24 antigen and HIV-1 protease. Sample, when taken, were centrifuged immediately at 3000rpm for 3 minutes, plasma obtained were stored at -20^{0}C before analysis. For CD^+_4 counts; whole blood samples were used to measure the CD_4+ count immediately after samples were collected.

Laboratory for Analysis

CD^+_4 count analysis was carried out at the Federal Medical center, Lokoja, Kogi State. The Viral Load was analyzed at Asokoro General Hospital, Abuja. HIV-1 protease, ARVs concentrations were analyzed at the Biotechnology Research Centre of Nnamdi Azikiwe University, Awka, Anambra State.

Methods

HIV Viral Load Assay was done by Polymerase Chain Reaction (RT PCR using COBAS Ampliprep/COBAS Taqman). [15]

CD^+_4 T-cell count was estimated by flow Cytometry using BD Facscount. [16]

HIV-1 protease assay was done by Fluorometry using fluorescent Microplate Reader. [17]

P24 Antigen was assayed using Enzyme-Linked Immunoassay. [14]

ARVs (Antiretroviral drugs) concentration was measured by high-performance liquid chromatography. [18]

Statistical Analysis

Values obtained were expressed as mean ± standard deviation (SD) using SPSS version 17.0. All numerical results were analyzed with one-way ANOVA with post hoc multiple comparisons test while paired student t-test was used to compare means from the same group at different times. Immunologic pattern and HIV-1 antigenic suppression were analyzed with the Chi-Square test using SPSS for windows version 20.0 statistical program. Spearman's correlation analysis between the antigenic index and plasma antiretroviral drug concentrations was done within groups. P values below 0.05 were considered statistically significant.

Results

Characteristics of the study population

The characteristics of the study population (mean age, sex, and WHO clinical stage) are shown in table 4.1. The mean values of age in group A subjects on TDF + 3TC + EFV was 37± 0.73 years, while that of group B subjects on AZT + 3TC + NVP was 38 ± 0.63 years were significantly higher (P<0.01) than similar value in the controls (36 ± 0.71 years). However, women were the majority of subjects (71.43%). In total 108/154 (70.13%) of subjects were living in rural areas.

The majority of subjects were classified on WHO clinical stage I 57/154 (37%) followed by WHO clinical stage II 47/154 (30.52%) with no remarkable differences between subjects on WHO clinical stage III and Stage IV.

Mean values of some antigenic index in those on drug combinations (Group A and B) at 12 months and HIV-positive Controls (Group C) (mean + SD)

The p24 antigen was significantly different amongst the three groups (f=14.59, p=.002). The difference was due to higher values of p24 antigen in the HIV participants not on the

antiretroviral drug. The HIV-1 protease enzyme level was significantly different amongst the three groups (f=22.90, p=.015). The difference was due to higher values of protease enzyme level in the HIV-1 participants not on the antiretroviral drug. The HIV-1 viral load was significantly different amongst the three groups (f=117.541, p=0.001). The difference was due to higher values of viral load in the HIV-1 participants not on antiretroviral drugs. The CD_4^+ cell count showed no significant difference between the three groups (f=0.218, p=0.804).

Levels of some immunologic parameters in HIV-1 infected individuals at 6 and 12 months of antiretroviral therapy (Tenofovir, lamivudine, and efavirenz) in Group A participants

The p24 antigen was significantly different between 6 months and 12 months measurements within the same individuals (t=7.902, p=0.001). The difference was due to the lower values of p24 antigen measured at 12 months. The HIV-1 protease was significantly different between 6 months and 12 months measurements within the same individuals (t=4.733, p=0.001). The difference was due to lower values of protease enzyme measured at 12 months. The HIV-1 viral load was significantly different between 6 months and 12 months measurements within the same individuals (t=2.948, p=0.005). The difference was due to lower values of viral load measured at 12 months. The CD_4^+ cell count was not significantly different between 6 months and 12 months measurements within the same individual (t= -5.541, p=.211). The plasma tenofovir (TDF) was significantly different between 6 months and 12 months measurements within the same individuals (t= -9.454, p=.001). The difference was due to the higher values of TDF measured at 12 months. The plasma lamivudine (3TC) was significantly different between 6 months and 12 months measurements within the same individuals (t= -5.144, p=.001). The difference was due to higher 3TC values measured at 12 months. The plasma efavirenz (EFV) was significantly different between 6 months and 12 months measurements within the same individuals (t= -4.158, p=0.001). The difference was due to the higher values of efavirenz measured at 12 months.

Levels of some immunologic parameters in HIV-1 infected Individuals at 6 and 12 months of antiretroviral therapy (Zidovudine, lamivudine, and nevirapine) in Group B participants

The p24 antigen was significantly different between 6 months and 12 months measurements within the same individuals (t=7.776, p=.001). The difference was due to the lower values of p24 antigen measured at 12 months. The HIV-1 protease enzyme level was significantly different between 6 months and 12 months measurements within the same individuals (t=6.291, p=0.001). The difference was due to lower values of protease enzyme measured at 12 months. The HIV-1 viral load was significantly different between measurements at 6 months and 12 months within the same individuals (t=6.672, p=0.001). The difference was due to lower values of HIV-1 viral load measured at 12 months. The CD_4^+ cell count was not significantly different between measurements at 6 months and 12 months within the same individuals (t=-3.147, p=0.063). The plasma Zidovudine was not significantly different between measurements at 6 months and 12 months within the same individuals (t=-0.872, p=0.389). The plasma lamivudine was significantly different between measurements at 6 months and 12 months within the same individuals (t=-4.212, p=0.034). The difference was due to the higher values of Lamivudine measured at 12 months. The plasma nevirapine was significantly different between measurements at 6 months and 12 months within the same individuals (t=2.294, p=0.028). The difference was due to lower values of nevirapine measured at 12months.

Correlation analysis of antigenic index and plasma antiretroviral drugs in all the group Group A, B, and C participants

Table 5 shows the association between antigenic index and plasma antiretroviral drugs in all the groups. There was significant positive correlation between p24 and protease (r=.594, p=.000), p24 and VL (r=.448, p=.004), Pr and VL (r=.688, p=.000), TDF and 3TC (r=.502, p=.001), TDF and EFV (r=.478, p=.002), EFV and 3TC (r=.374, p=.017) while significant negative correlation was observed between Pr and 3TC (r= –.582, p=.032), VL and TDF (r= –.672, p=.041), 3TC and Pr (r= –.582, p=.032).

In group B, there was significant positive correlation between p24 and Pr (r=.575, p=.000), p24 and VL (r=.502, p=.002), Pr and VL (r=.696, p=.000), VL and 3TC (r=.783, p=.008), VL and NVP (r=.581, p=.006). All were found significant (P<0.05).

Similarly, in group C, there was significant positive correlation between p24 and pr (r=.458, p=.000), p24 and VL (r=.439, p=.000), pr and VL (r=.474, p=.000), pr and CD4 (r=.249, p=.027).

Discussion

The present study focused on some alternative index that could be used to monitor the management of HIV/AIDS infected participants in resource-limited settings such as Nigeria. One of the indices used in the present study for evaluating HIV-1 infected individuals was the ultrasensitive p24 antigen. The study observed that the p24 antigen level in participants on antiretroviral drugs was significantly lower when compared with HAART naive participants. This is an indication of the effectiveness of HAART. The reduced value of the p24 antigen in HIV infected participants is an indication of successful suppression of HIV viremia. The findings of this study also indicated that reduced p24 antigen is synonymous with reduced viral load. It is well noted that a rising p24 antigen level is an indication of the progression of HIV/AIDS in participants on HAART and is an indication of drug failure. [19] In other studies, it has been reported that elevated capsid protein (p24 antigen) is associated with a

faster progression to AIDS and death. Most of the association can be explained by increased HIV RNA levels in those with high p24 antigenemia. However, an association between p24 antigenemia and progression to AIDS that cannot be explained fully by changes in either HIV RNA level or CD_4^+ cells count remain, which suggest that p24 antigenemia still may have an important role in assessing patient prognosis, even in countries where HIV-1 viral load testing is performed routinely. [20]

In this study, a significant drop in p24 antigen was observed in participants on efavirenz and nevirapine based regimens respectively. Thus, it is possible to say that antiretroviral drugs used in this study inhibited reverse transcription of HIV-1 RNA in the replication cycle as seen in the significant drops in p24 antigenemia at 12 months. Although, this was not the case for all the participants because some of the participants had raised p24 antigen value. This finding is supported by the work done by Omar *et al.,* [21] that persistent undetectable viremia plasma p24 antigen levels match with undetectable viremia after 12 and 24 months of HAART. This observation is probably attributed to the reasons why HIV-1 p24 antigen assay is used as an alternative to HIV-RNA testing for monitoring treatment in poor resource settings where HIV-1 RNA testing is unavailable. [22] Other studies have shown that elevated HIV-1 p24 antigen in plasma is related to progression to Acquired Immune Deficiency Syndrome (AIDS) in participants not on HAART; as all non-suppressed viral load correlated with elevated plasma HIV-1 p24 antigen. [23] It could be hypothesized that high plasma p24 antigen is a surrogate marker associated with higher immune activation in an on-going HIV-1 RNA replication. [24] And that p24 prognostic strength and p24 correlation with HIV-1 RNA load predicted clinical-stage better than CD_4 count. [25]

In this study, HIV-1 protease enzyme activities in participants on antiretroviral drugs were significantly lower when compared with HAART naive participants. This was because HAART exerted a reducing effect on HIV-1 protease enzyme activities due to inhibition of viral replication at the reverse transcription phase by reverse transcriptase inhibitors. This HAART reducing effect on protease enzyme activity caused impaired viral Gag-Pol polyprotein processing resulting in the production of a non-infectious virus particle. However, this was not the case for all the participants because some participants on HAART had raised HIV-1 protease enzyme value. Since elevated HIV-1 protease activity was found in some participants on HAART, it can be hypothesized that treatment failure could be due to the absence of protease inhibitors in the drug combination therapy. These findings are supported by the work done by [13] that there is increased HIV-1 protease enzyme activities despite the use of nucleoside and non-nucleoside reverse transcriptase inhibitors; this is the basis for the introduction of protease inhibitors into the second-line – antiretroviral drugs combination. A similar study by Idoko *et al.,* [11] reported that viral assembly and maturation can be blocked by HIV-1 protease inhibitors. The introduction of protease inhibitors alongside nucleoside or non-nucleoside reverse transcriptase inhibitors is an excellent therapeutic strategy of second-line highly active antiretroviral therapy (HAART) that will enhance treatment outcomes.

The study showed that the HIV-1 viral load in participants on antiretroviral drugs was significantly lower when compared with HAART naive participants. The low viral load at 12 months of therapy is an indication of the efficacy and suitability of the regimen for an individual client. In this study, viral load was predominantly undetectable in participants on efavirenz and nevirapine based regimens. This result is similar to that predicted by UNAIDS, [26]

The study observed no statistical difference in CD_4^+ cells counts at 12 months for participants on antiretroviral drugs when compared with treatment-naive participants. Since the CD_4^+ T cell count was the same between those on drugs and those not on drugs. This is an indication of CD_4^+ count limitation in the monitoring of patients on antiretroviral therapy. This finding is supported by the work of Onyenekwe *et al.* [2] The author stated that the reduction in $CD4^+$count that is not merely a result of HIV infection but could be caused by other factors and accumulation of circulating immune complexes which is a great burden to HIV/AIDS individuals. It was hypothesized that malaria reduces the CD_4^+ count more than HIV infection. [27] In a similar development, Onyenekwe *et al.* [2] reported that after antimalarial treatment in normal and HIV infected individuals, the median CD_4 count at follow-up stages increases. Hence, CD_4^+ T-lymphocytopenia is caused majorly by circulating immune complexes due to malaria parasite, Salmonella species, and other microbial agents than HIV-1 infection. [2]

The present study also reveals that high CD_4^+ count was associated with high HIV-1 p24 antigen and high HIV-1 protease enzyme activities while low CD_4^+ count was associated with low HIV-1 p24 antigen and low activities of HIV-1 protease enzyme in participants with the suppressed viral load on HAART. This finding is similar to the report by Vijayan *et al.* [28] that higher T-cell activation in HIV-1 infection is associated with decreasing CD_4^+ cells during antiretroviral therapy. Thus, the role of p24 antigen and HIV-1 protease enzyme as markers of worse immunological evolution despite successful ART must be clarified in future studies. [28]

In this study, a higher significant difference was observed in antigenic markers between 6 months and 12 months of therapy. However, no significant difference was observed in CD_4^+ cells count between 6 months and 12 months of therapy. Also observed in the study, no significant difference was observed in CD_4^+ count between participants on antiretroviral therapy and HAART naive HIV-1 infected individuals. This may confer limitation of CD_4^+ cells count in the monitoring of response to antiretroviral therapy. This finding is supported by the work done by Onyenekwe *et al.* [29] that no significant difference was observed in CD_4^+ count between participants

on antiretroviral drugs (ARV's) and HAART naive seropositive participants. This may be due to higher T-cell activation in HIV-1 infection which is associated with decreasing CD_4 cells during inflammatory processes. [29]

The study observed that the mean values of antigenic markers of participants on antiretroviral drugs were significantly lower at 12 months of therapy when compared with the mean values of antigenic markers at 6 months of therapy. These results indicated a reduction in plasma levels of p24 antigen and HIV-1 protease enzyme activity due to suppressed HIV-1 viral load. The study also observed no significant difference for the immunologic marker at 6 months and 12 months of antiretroviral therapy. This was also a sign of limitation of CD_4^+ cell count in the monitoring of patients on antiretroviral therapy as observed in this study.

In this study, we observed that the antigenic index correlated with immunologic markers. These results support the fact that a decrease in antigenic markers used in this study correlated with an increase in immunologic marker. This is evidence that with successful suppression of viral load, the immunologic responses recover. The study also observed a good correlation between antigenic markers and antiretroviral drug concentrations. These supported the fact that virologic suppression is associated with a therapeutic level of antiretroviral drug concentrations.

In conclusion, Decreased HIV-1 p24 antigen and protease enzyme with undetectable viral load in HIV infected participants indicated successful suppression of HIV viremia and hence, strongly suggests good antigenic markers for monitoring of treatment success or failure in resource-poor settings where regular viral load monitoring is unavailable. HIV-1 protease enzyme and HIV-1 p24 antigen may also be combined with viral load and possibly CD_4^+ cell count as tools in identifying participants with clinically insignificant symptoms of treatment failure who could be candidates for active surveillance. it is recommended that these parameters be considered and included in the lists of routine monitoring panels for participants on antiretroviral therapy in resource-limited settings where viral load and CD4 T-cell count is unavailable. The focus in resource-limited settings has been almost exclusively on increasing access to drugs. Attention must now be paid to laboratory monitoring to limit the costs associated with the widespread use of expensive second-line therapy and to provide optional treatment to participants.

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Conflict of interests

The authors declare no conflict of interest

Authorship

CCO and IPE conceptualized and designed the study. IPE, NRU, AOK, and JEA acquired, analyzed, and interpreted the data. CCO, IPE, and NRU drafted and revised the article critically for important intellectual content. All authors read and approved the final version of the manuscript.

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Table 1: Characteristics of the Study Population

Variables	All Subjects N = 154	Group A (TDF + 3TC + EFV) n = 40	Group B (AZT + 3TC + NVP) n = 35	Group C (those not on drugs as control) n = 79	P values
Mean age in years (±SD)	36.68 (±0.74)	37.3 (±0.73)	38.11 (±0.63)	35.86 (±0.71)	0.005
Male	44 (28.57%)	11 (27.5%)	9 (25.71%)	24 (30.38%)	0.001
Female	110 (71.43%)	29 (72.5%)	26 (74.29%)	55 (69.62%)	0.001
Residential Status:					
(i) Semi-urban	46 (29.87%)	13 (32.5%)	12 (34.29%)	21 (26.58%)	0.05
(ii) Rural	108 (70.13%)	27 (67.5%)	23 (65.71%)	58 (73.42%)	0.001
WHO Clinical Stage:					
(i) Stage I	57 (37.01%)	14 (35%)	10 (28.57%)	33 (41.77%)	0.04
(ii) Stage II	47 (30.52%)	13 (32.5%)	13 (37.14%)	21 (26.58%)	0.04
(iii) Stage III	29 (18.83%)	9 (22.5%)	4 (11.43%)	16 (20.25%)	0.03
(iv) Stage IV	21 (13.64%)	4 (10%)	8 (22.86%)	9 (11.39%)	0.01

*Values differ significantly from controls (P<0.05)
*Values differ significantly within stages (P<0.001)
A: TDF + 3TC + EFV
B: AZT + 3TC + NVP
TDF: Tenofovir
3TC: Lamivudine
EFV:Efavirenz Standard Deviation
C: Control group not yet on drugs
N: Total Sample Size
n: Group Sample Size
AZT: Zidovudine
NVP: Nevirapine
%: Percent

Table 2: Levels P 24 antigen, HIV-1 Protease, Viral Load and CD4 in HIV-1 infected individuals at 6 and 12 months of ART in group A and B and C participants (Mean + SD)

GROUP	N	P24 (ng/ml)	Pr (mU/mg)	VL (copies/ml)	CD4 (cell/µl)
A	40	3.35±1.83	4.32±1.32	94±18	515±81
B	35	5.28±1.31	6.13±1.3	160±30	579±53
C	79	14.04±4.76	25.50±21.40	17429±1351	584±27
f-value		14.59	22.9	117.541	0.218
p-value		0.002*	0.005*	0.001*	0.804
A vs B		1.000	1.000	0.037*	1.000
A vs C		0.001*	0.001*	0.008*	1.000
B vs C		0.001*	0.001*	0.001*	1.000

*Significant
p24: HIV-1 p24 Antigen
A = TDF + 3TC + EFV drug combination group
B = AZT + 3TC + NVP drug combination group
TDF = Tenofovir
3TC = Lamivudine
EFV = Efavirenz
C= Control group not yet on drugs
AZT = Zidovudine
SD: Standard Deviation
Pr: HIV-1 Protease enzyme
LPL: Lipoprotein Lipase
VL: Viral load
CD_4: Cluster of differentiation
ALB: Albumin
NVP = Nevirapine
HL: Hepatic Lipase
n= Group Sample Size

Table 3: Levels of immunologic Parameters in HIV-1 infected individuals at 6 months and 12 months of antiretroviral therapy (tenofovir, lamivudine, and efavirenz) in Group A (Mean ± SD)

Parameters	N	6 months	12 months	t-test	p-value
P24 (ng/ml)	40	13.77±9.37	3.35±1.81	7.902	0.001*
Protease (mU/mg)	40	27.44±9.02	4.32±1.32	4.733	0.001*
Viral load (copies/ml)	40	9490±184	94±3	2.948	0.005*

CD4 (cell/µl)	40	509±30	520±40	-5.541	0.211
TDF (µg/ml)	40	54.61±25.69	97.30±21.27	-9.454	0.001*
3TC (µg/ml)	40	17.39±5.89	22.83±2.42	-5.144	0.001*
EFV (ng/ml)	40	1182.70±488.38	1573.26±487.45	-4.158	0.001*

* Significant, A = TDF + 3TC + EFV, CD_4: Cluster of differentiation, TDF = Tenofovir, n=Group Sample Size, 3TC = Lamivudine, p24: HIV-1 p24 Antigen, EFV = Efavirenz, SD: Standard Deviation.

Table 4: Levels of Immunologic Parameters in HIV-1 infected individuals at 6 months and 12 months of antiretroviral therapy (zidovudine, lamivudine, and nevirapine) in Group B (Mean + SD)

Parameters	N	6 months	12 months	t-test	p-value
P24 (ng/ml)	35	11.82±5.97	5.28±2.78	7.776	0.001*
Protease (mU/mg)	35	15.78±3.08	6.13±1.25	6.291	0.001*
Viral load (copies/ml)	35	18036±83	160±21	6.672	0.001*
CD_4^+ (cell/µl)	35	551±53	571±30	-3.147	0.063
AZT (ng/ml)	35	1944.18±68.71	2085.56±63.27	-.872	0.389
3TC (µg/ml)	35	12.17±4.97	14.64±5.07	-4.212	0.034*
NVP (ng/ml)	35	40.46±9.15	35.93±6.60	2.294	0.028*

* Significant
B = AZT + 3TC + NVP
AZT= Zidovudine
NVP= Nevirapine
p24: HIV-1 p24 Antigen
n: Group Sample Size
3TC = Lamivudine
CD_4: Cluster of differentiation
SD: Standard Deviation

Table 5: Correlation of antigenic index and plasma antiretroviral drugs in group A, B and C participants

Parameters	Group	N	r- value	P-value	S	Sig
P24 and Pr	A	40	.594	.000*	S	P<0.05
P24 and VL	A	40	.488	.004*	S	P<0.05
Pr and VL	A	40	.688	.000*	S	P<0.05
Pr and 3TC	A	40	–.582	.032*	S	P<0.05
VL and TDF	A	40	–.672	.041*	S	P<0.05
TDF and 3TC	A	40	.502	.001*	S	P<0.05
TDF and EFV	A	40	.478	.002*	S	P<0.05
3TC and EFV	A	40	.374	.017*	S	P<0.05
P24 and Pr	B	35	.575	.000*	S	P<0.05
P24 and VL	B	35	.502	.002*	S	P<0.05
Pr and VL	B	35	.696	.000*	S	P<0.05
VL and 3TC	B	35	–.783	.008*	S	P<0.05
VL and NVP	B	35	–.581	.006*	S	P<0.05
P24 and Pr	C	79	.458	.000*	S	P<0.05
P24 and VL	C	79	.439	.000*	S	P<0.05
Pr and VL	C	79	.474	.000*	S	P<0.05
Pr and CD_4	C	79	–.249	.027*	S	P<0.05

P<0.05 were considered significant
S: Significant