

Effectiveness of Anti-retroviral Therapy (ART) on HIV-1 Patients Using Viral Load and CD₄ Counts as Indices

Elujoba MK^{1,2*}, Ogbonna CIC¹, Nimzing L¹, Tijjani H^{2,3}, Chinyere F²,
Elujoba FO² and Ayanda E²

¹ Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria

² OLA Hospital Laboratory, Jos, Nigeria

³ DBR Laboratory, Bauchi State University, Gadau, Nigeria

Abstract:

Background: Human Immunodeficiency Virus (HIV) is a global phenomenon with an estimated population of 7.7% infected people in Plateau state, Nigeria.

Aim: This study was aimed at monitoring the effectiveness of Anti-retroviral Therapy (ART) on HIV-1 infected patients.

Procedure: A total number of 109 experimental volunteers' from Our Lady of Apostle (OLA) hospital, Jos, Nigeria were screened and detailed records were collected using a semi structured questionnaire for a period of 24 months at six months intervals. The effectiveness of ART was monitored using CD₄ count and viral loads of the volunteers. Samples were collected in EDTA containers and the baseline CD₄ and viral load were estimated. With high viral load (≥ 400 copies/ml) and low CD₄ count (≤ 200 cells/mm³), the volunteers were made to commence ART treatments based on their low immunity. The CD₄ count and the viral load values of the volunteers were then assessed at 6th, 12th, 18th and 24th month intervals.

Result: Significant improvement was recorded in CD₄ and viral load values of the volunteers; thus leading to an overall improvement in their physical health status and productivity. The active age groups (20 – 49 years) had a collective prevalence rate of 92.66% of the total study population. The highest percentage adherence of 84% was recorded in 40 – 49 years old volunteers. The patients between 50 – 59 years had a 4.59 % prevalence, while those at the two extremities of life had a combined prevalence of 2.75%. The positive response to ART improved steadily throughout the period of the study with an overall adherence rate of 84%.

Conclusion: The use of Anti-retroviral Therapy (ART) in the treatment of patients with HIV-1 infection proved to be effective among patients attending OLA hospital, Jos. It should be further encouraged and well advocated in the treatment and management of patient's with HIV-1 infection.

Keywords: Anti-retroviral Therapy, Viral load, CD₄, HIV-1, Adherence rate

I. Introduction

Human Immunodeficiency Virus (HIV) is a lentivirus and a member of the retroviridae family that causes Acquired Immune Deficiency Syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections and cancer to thrive [1]. Two types of HIV have been characterized; HIV-1 and HIV-2 with the former being more virulent, more infective and is the cause of the majority of HIV infections globally while the latter has a lower infectivity and is largely confined to West Africa. HIV infects vital cells in the human system such as helper T cells (specifically CD₄⁺ T cells), macrophages and dendritic cells. HIV infection leads to low levels of CD₄⁺ T cells through three main mechanisms; Firstly, by a direct viral killing of infected cells, secondly by increased rate of apoptosis in infected cells and lastly by killing infected CD₄⁺ T cells by CD₈ cytotoxic lymphocytes that recognize infected cells. When CD₄⁺ T cell number decline below a critical level, cell mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections [2].

Infections with HIV-1 are associated with progressive decrease of CD₄⁺ T cell count and an increase in viral load of HIV-infected patients. Most untreated people infected with HIV eventually develop AIDS. These individual mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system. Treatment with anti-retroviral therapy (ART) increases their life expectancies and this increase in life expectancy is being measured by the interaction between viral load and CD₄⁺ cell count in disease progression [3].

A special relationship exists between viral load and CD₄ cell count. The interaction between the viral load and the CD₄ count can predict how fast or slow the final stage of AIDS is reached in an HIV-positive patient, while viral load is the actual number of viruses in the blood, an indication of how sick an HIV infected person is, it is used to measure the person's response to anti-retroviral treatment which is indicated in copies per

mL. CD₄ cell count is an excellent indicator of how healthy the immune system is, which is indicated in cells per mm³. Viral load and CD₄ cell count vary together; a higher viral load will lead to a lower CD₄ count while a lower viral load will go hand in hand with a higher CD₄ cell count [4].

This study focused on the effectiveness of Anti-retroviral Therapy (ART) in HIV patients' response to treatment which was indicated by the viral load as well as the CD₄ cell counts in blood samples of the infected patients.

II. Materials And Methods

2.1 Study Area

The study was carried out in Our Lady of Apostles (OLA) Hospital located in Jos North Local Government Area of Plateau State, Nigeria. The State lies between latitude 80°24" north and longitude 80° 32" and 100° 38" east. Our Lady of Apostles Hospital Jos is one of the pioneer hospitals in the State Capital. It provides quality, preventive and curative health care services to the people of Plateau state and beyond.

2.2 Sample Size

A total number of 109 known HIV-1 infected patients, on Anti-Retroviral Therapy (ART) were used for the study. The minimum sample size of 109 was arrived at by using the standard (sample size) formula, $n = \frac{zxzpxq}{dxd}$ [5], [6] and putting into consideration the HIV-1 prevalence in Plateau State of 7.7%. Where n =desired sample size, z =standard normal deviation at 1.96 which correspond to 95% confidence limit, p =proportion of population with HIV, and $q=1-p$. A semi-structured questionnaire was also used to collect some data as sex, age, lab number and drug combinations.

2.3 Ethical Clearance

Ethical clearance was obtained from the Ethical Committee of OLA Hospital and the individual participants. Also, for minors, clearance was obtained from the parent(s) or guardian. They were assured of full anonymity and all information obtained from them will be used confidentially and exclusively for the purpose of this study.

2.4 Equipment

The following equipment were used during the study; Cyflow, Partec GmbH, D-48161 Munster, Germany; Eppendorf Centrifuge 5218, Hamburg, Germany; NUAIRE Labgard, Class II, NU-425-400E, Biological Safety Cabinet; GeneAmp, PCR System 9700, Singapore; Molecular Device, Emax, China and other standard laboratory equipment.

2.5 Drug Administered to the Patients during the Study

The following ART drugs were administered to the patients during the study:

| ART | Abbreviation |
|---------------------|---------------------|
| Nevirapine | NVP |
| Efavirenz | EFV |
| Lamivudine | 3TC |
| Tenofovir | TDF |
| Abacavir | ABC |
| Zidovudine | AZT |
| Lopinavir/Ritonavir | LPV/R |
| Atazanavir | ATZ |
| Ritonavir | RTV |

2.6 Sample Collection/Analysis

The infected patients were enrolled into the study after seeking their consent. Their blood samples were collected in EDTA containers for their baseline viral load and CD₄ cell estimations. Those with high viral load (≥ 400 copies/ml) and low CD₄ count (≤ 200 cell/mm³) were included in the study and commenced ART treatment. Their blood samples were again collected in EDTA containers after the commencement of the ART treatment at six (6) month intervals for two (2) years. The samples were investigated for CD₄ cell count and viral load; these were used as monitoring tools in studying the response of the patients to the anti-retroviral treatment.

2.7 Viral Load Assay

The AMPLICOR HIV-1 MONITOR test was with either of two specimen preparation procedures, the Standard procedure or the ultrasensitive procedure. In the standard specimen preparation procedure, HIV-1 RNA is isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by

precipitation of the RNA with alcohol. With the Ultra-Sensitive specimen preparation procedure, HIV-1 viral particles in plasma are concentrated by high speed centrifugation, followed by lysis of the virus particle with a chaotropic agent and precipitation of the HIV-1 RNA with alcohol. A known number of quantitation standard RNA molecules are introduced into each specimen with the lysis reagent. The HIV-1 Quantitation standard is carried through the specimen preparation, reverse transcription, amplification and detection steps and is used for the quantitation of HIV-1 RNA in the test specimen.

2.8 CD4 Cells Count

CD₄-PE FLUORESCENCE was used in analyses on a partec flow cytometer with an excitation light source of 488 nm (blue or green solid state laser). To count CD₄⁺ T-cells, the test tube with 840 µl of the already prepared blood sample were transferred to partec flow cytometer and a count analysis was carried out. The blood preparation allows one count procedure on the same sample. Following the above procedure the dilution factor is 42. In the cyflow counter II and cyflow SL 3, this dilution factor is already set or can be directly set in the instrument software. Counting result will be displayed automatically as CD₄⁺ T-cell per µl whole blood.

2.9 Statistical Analysis

Data were presented as Frequency, Percentage, Chart and Mean ± SEM of the group and were analyzed using one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc., Chicago, U.S.A) where appropriate. Differences at p<0.05 were considered significant.

III. Results

The age distribution of the volunteers is presented in Table 1 while Figure 1 shows their sex distribution.

Table 1: Age Distribution of HIV-1 Patients on ART

| Age (years) | Frequency | Percentage (%) |
|--------------|------------|----------------|
| 10-19 | 01 | 00.92 |
| 20 -29 | 34 | 31.19 |
| 30-39 | 42 | 38.53 |
| 40-49 | 25 | 22.94 |
| 50-59 | 05 | 04.59 |
| 60-69 | 02 | 01.83 |
| TOTAL | 109 | 100.00 |

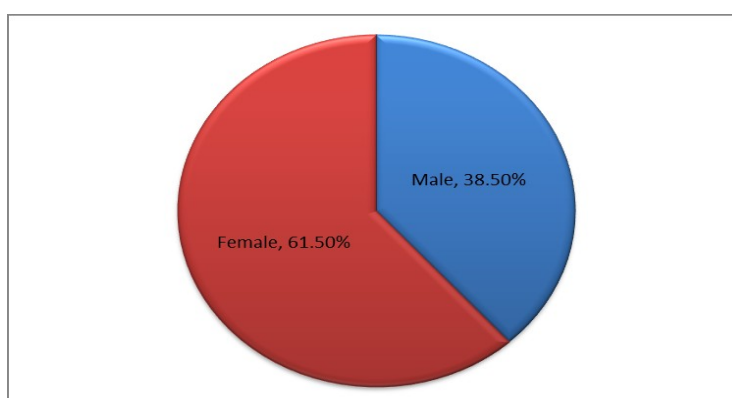


Figure 1: Sex Distribution of HIV-1 Patients on ART

Table 2 and 3 reveals the viral load and CD4 response of the HIV patients respectively to ART at six months intervals while Table 4 shows their adherence and non-adherence during the study.

Table 2: Viral Load Response of HIV-1 Patients to ART at Six Month Intervals

| Age (years) | Viral load (×10 ⁵ copies/mL) | | | | |
|-------------|---|------------------------|------------------------|------------------------|------------------------|
| | Baseline | 6 months | 12 months | 18 months | 24 months |
| 10-19 | 0.05±0.00 ^a | 0.01±0.00 ^b | 0.01±0.00 ^b | 0.01±0.00 ^b | 0.01±0.00 ^b |
| 20-29 | 1.89±0.56 ^a | 0.09±0.03 ^b | 0.12±0.06 ^b | 0.06±0.02 ^b | 0.09±0.08 ^b |
| 30-39 | 2.48±0.44 ^a | 0.14±0.05 ^b | 0.09±0.03 ^b | 0.14±0.06 ^b | 0.04±0.02 ^c |
| 40-49 | 1.33±0.37 ^a | 0.03±0.01 ^b | 0.05±0.03 ^b | 0.05±0.02 ^b | 0.01±0.01 ^c |
| 50-59 | 2.37±0.97 ^a | 0.04±0.03 ^b | 0.01±0.01 ^b | 0.04±0.03 ^b | 0.01±0.01 ^b |
| 60-69 | 1.32±1.13 ^a | 0.01±0.01 ^b | 0.01±0.00 ^b | 0.02±0.02 ^b | 0.01±0.01 ^b |

NOTE: Values are mean of group ± SEM, Values with different superscript are significantly different (p<0.05) across the row

Table 3: CD₄ Response of HIV-1 Patients to ART at Six Month Intervals

| Age (years) | CD ₄ (×10 ² cell/mm ³) | | | | |
|-------------|--|------------------------|-------------------------|-------------------------|------------------------|
| | Baseline | 6 months | 12 months | 18 months | 24 months |
| 10-19 | 2.15±0.00 ^a | 4.26±0.00 ^b | 5.16±0.00 ^c | 3.64±0.00 ^d | 4.30±0.00 ^b |
| 20-29 | 1.43±0.11 ^a | 2.53±0.15 ^b | 2.79±0.19 ^b | 3.32±0.19 ^c | 3.74±0.26 ^c |
| 30-39 | 1.55±0.09 ^a | 2.65±0.16 ^b | 2.78±0.14 ^b | 3.28±0.23 ^c | 3.46±0.23 ^c |
| 40-49 | 1.36±0.13 ^a | 2.50±0.16 ^b | 2.85±0.17 ^{bc} | 3.08±0.18 ^{bc} | 3.39±0.20 ^c |
| 50-59 | 1.38±0.29 ^a | 3.06±0.51 ^b | 2.87±0.39 ^b | 3.23±0.35 ^b | 3.76±0.48 ^b |
| 60-69 | 0.86±0.16 ^a | 1.55±0.01 ^b | 1.55±0.23 ^b | 2.72±0.66 ^b | 3.11±1.06 ^c |

NOTE: Values are mean of group ± SEM, Values with different superscript are significantly different (p<0.05) across the row

Table 4: Adherence of HIV-1 Patients to ART

| Age (years) | Total | Adherence | | Non-adherence | |
|-------------|-------|-----------|-----|---------------|----|
| | | Adherence | % | Non-adherence | % |
| 10-19 | 1 | 1 | 100 | 0 | 0 |
| 20-29 | 34 | 27 | 79 | 7 | 21 |
| 30-39 | 42 | 30 | 71 | 12 | 29 |
| 40-49 | 25 | 21 | 84 | 4 | 16 |
| 50-59 | 5 | 4 | 80 | 1 | 20 |
| 60-69 | 2 | 1 | 50 | 1 | 50 |

IV. Discussion

Sampling of HIV-1 infected patients as revealed in Table 1, shows that the age group with the highest HIV-1 prevalence rate was the 30 – 39 years which had 38.53%. This age group is closely followed in prevalence by the 20 – 29 years and 40 – 49 years with 31.19% and 22.94% respectively. These groups are sexually active groups. It is however, a known fact that sexual intercourse is not the only means of transmission of the disease. The active age groups (20 – 49 years) had a collective prevalence rate of 92.66% of the total study population.

Viral load response to ART after the initial commencement of treatment at the baseline time steadily improved as the course of treatment progressed throughout the study period of 24 months in all the studied age groups. A significant decrease (p<0.05) was recorded in these studied groups when compared with the baseline, with the 24th month being significant when compared to other months amongst the 30 – 39 years and 40 – 49 year groups respectively (Table 2). This result is also a reflection of the adherence status of these age groups. Highest Percentage adherence of 84% was recorded in 40 – 49 years (Table 4).

A better result was observed in the CD₄ response of the patients. Significant increases (p<0.05) were recorded when compared with the baseline treatment before commencement of ART. All groups also showed a significant response when compared with other months (6th, 12th and 18th) except for 10 – 19 and 50 – 59 years (Table 3). These age groups had an excellent adherence status, which however might not have been a true reflection due to their sample size (Table 4). Semi structured questionnaire revealed the adherence of the volunteers to the treatment regimen with 76.7% of them strictly adhering to the treatment instructions and periodicity. However, 23.3% of the test patients did not follow these instructions nor adhered to the periodicity of treatment schedule. Also 29.0% and 21.0% of those within the age range of 30 – 39 years and 20 – 29 years respectively failed to adhere to the given instructions (Table 4).

V. Conclusion

This study clearly shows the effectiveness of ART therapy in the treatment of HIV-1 infected individuals, especially those infected with HIV-1 strain. Migueles and Connors, [3] reported that treatment with Anti-retroviral therapy (ART) increased the life expectancy of people infected with HIV, and this increase in life expectancy is measured by the interaction between viral load and CD₄ count in disease progression. This study also indicated that non-adherence to ART may present a drop in CD₄ count and an increase in Viral load or a less significant response. These groups accounted for about 23.3% of the study population. Anti-retroviral Therapy (ART) presents a significant means of treatment and management regimen for HIV-1 infection while the use of viral load and CD₄ count values in the monitoring of the patients treatment and management is highly applauded and further advocated.

Acknowledgment

We wish to acknowledge the management of OLA Hospital, Jos, for the conducive working environment and to the Laboratory staff for their various contributions towards the success of this work.

References

- [1]. R.A. Weiss, How does HIV cause AIDS, *Science*, 260 (5112), 1993, 173-179.
- [2]. M.C. Miceli, and J.R. Parnes. Role of CD4 and CD8 in T Cell Activation and Differentiation. *Adv. Immunology*, 53, 1993, 59-122.
- [3]. S. Migueles and M. Connors. Long-term Non- Progressive Disease among Untreated HIV – Infected Individuals: Clinical implications of understanding Immune control of HIV. *Journal of American Medical Association*, 304 (2), 2010, 194-201.
- [4]. R.S. Hogg, B. Yip, and K.J. Chan. Rates of Disease Progression by Baseline CD4 Cell Count and Viral load after Initiating Triple-Drug Therapy, *JAMA*. 2001 Nov 28; 286 (20), 2001, 2568-77.
- [5]. W.W. Daniel, *Biostatistics: A foundation for Analysis in the Health Science*. 7 th edition. New York, John Wiley & Sons, 1999.
- [6]. M.O. Araoye, *Research Methodology with statistics for Health and Social Sciences*, Nathadex Publishers, 2003.