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Virological and Immunologic Outcome of HIV Infected Drug Experienced Adults in North Central Nigeria

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Abstract

Background: Antiretroviral therapy (ART) has decreased the mortality and morbidity among people living with HIV and AIDS (PLWH). Viral load (VL) has been used by clinicians as primary tool recommended by WHO to monitor patients progress on High Antiretroviral active therapy (HAART). There is a paucity of information on Virological and immunological outcomes of HIV infected drug experience adults' patients in north central Nigeria. We conducted a tertiary hospital based cross-sectional study at federal medical centre in Nasarawa state between December 2019 to march 2020.

Method: A total of 474 HIV positive adult were enrolled using a Systematic random technique. Blood specimen for CD+4 T cells count and viral load determination were obtained and PCR-Real time and Flow-cytometry were used to estimate plasma viral load and CD4+ T cell count respectively. Frequency was used to determine percentage and logistic regression was used to determine the associated factors with Virological suppression and immunology outcome in patients on HAART 95% CI and odd ratio (OR) was used to measure strength of association.

Results: From the 474 cohort that were enrolled 34.6% were on WHO baseline clinical stage IIV and 57.8% of cohort were on HAART, started treatment regimen in less than a year with 42.2% diagnosed and confirmed with HIV infection between 1-5years. 57.8% were transferred into the centre as a major reason for enrolment. 57.8% has history of TB treatment in the past while 42.2% of the study participants who were eligible for treatment initiation were determine by CD4+ counts with a median interquartile range of 180 (92-300) cells/mm^3. virological suppression (VL level < 1000 copies/ml) was found in 85% (95% CI 77.7, 86.1) of study participants, and it has been associated with CD4 cell count between 250 and 400 cells/mm^3 (adjusted odds ratio (AOR) = 2.56; 95% CI 1.14, 5.75) and > 499 cells/mm^3 (AOR = 7.71; 95% CI 3.48, 17.09) at VL testing and current age > 40 years old (AOR = 5.40; 95% CI 2.3, 10.01). Similarly, favourable immunological status (≥250 cells/mm^3 for male and ≥ 600 cells/mm^3 for female) was observed in 52.9% (95% CI 47.4, 58.8) of the study participants. Baseline CD4 cell count of > 200 cells/mm^3, age at enrolment of 26 through 40 years old, and urban residency were significantly associated with favourable immunological outcome.

Conclusion: Low Immunological recovery among study cohort was observed although viral suppression was shown in majority of the HIV infected adult who are on HAART. Early initiation on HAART should be encouraged in other to achieve immunological recovery and viral suppression in order to achieve the USAIDS- 90-90-90 to end HIV pandemic by 2030.

Keywords: Suppression • Virological • Immunological • Mortality • Morbidity

Abbreviations: AIDS: Acquired Immunodeficiency Syndrome • HIV: Human Immunodeficiency Virus • HAART: High Antiretroviral Active Therapy • ART: Antiretroviral Therapy • VL: Viral Load • WHO: World Health Organization

Introduction

The human immunodeficiency virus (HIV) has continues to become a global public health burden with 39.7 million of people living with HIV globally in 2020 [1]. In Sub-Sahara Africa region, it's estimated that over 70% of HIV new infection occurs annually with increase in morbidity and mortality [2]. Over the years, high active antiretroviral therapy (HAART) has been used to reduce HIV related morbidity and mortality [3]. There has been great transformation in HIV infection management from been fatal to a manageable chronic disease due to HAART [4]. In resource limited countries, rapid scale-up of anti-retroviral therapy (ART) for HIV (AIDS) and it has been successful [5]. In Nigeria, significant progress has been in terms of HIV prevention and control with programs been implemented that has reduced related HIV complications [6]. This program includes scale-up of ART treatment with optimal access. Nigeria also helped the "test & treat" approach a 2016 WHO consolidated guideline on the use of anti-retroviral drugs for the management for HIV infection. There has been usable decline. In the annual new infection in Nigeria as a result of scale-up of HIV care related services. Despite rapid scale up of HIV related service delivery in Sub-Sahara Africa and other resource constrain countries, there has been increase in HIV drugs resistances which has become a challenge in many national ART roll-out programs [6], it has become pertinent to have a robust treatment monitoring response approach [7].

Plasma HIV RNA titer and CD4+ cell count is used in clinical practice to monitor treatment response; they are also used as diagnostic Manuel to evaluate disease progression. In the absence of viral load testing platform in resources constrain settings, CD4+T cell count is used to monitor the ART. Increase in CD4+ T cell count is associated with decrease in HIV viral load for a sustained response to HAART [7].

In clinical practice, Virological and immunological response to ART can be measured by the ability of the immune system to suppress viral replication which is time dependent. However, it's critical evident for policy makers in

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Nigeria to review treatment response which it has become a challenge due to lack of adherence [8].

In order to meet up with the joint United Nations program on HIV/AIDS (UNAIDS), "90-90-90" initiative whose mandate was to achieve 90% viral suppression by 2020 among people receiving treatment [9], which was reviewed and updated for 2030 [10]. Nigeria Government alongside partners have in recent times implemented viral load in tertiary and secondary health care facilities, however there're paucity of data and information regarding immunological status and viral suppression outcome of patients on HAART. There's need to generate evidence base data to inform policy makers and to serve as a guide for improved treatment [11].

What is already known on this topic?

- The primary goal of ART is to suppressed HIV RNA lower than the detectable level.
- Due to lack of HIV RNA monitoring platforms in resource constrain settings, patients are supposed to continue on first line ART until virological failure progresses to a 50% decrease.

What this study adds

- Introduction of ART Program in Nigeria has showed good response in reducing HIV-RNA viral load to undetectable level.
- Our research showed better outcome of Nevirapine based regimen than Efavirenz based.

Research Methodology

Study design

We enrolled 474 HIV infected adults who are on HAART seeking for health care services at Federal Medical Center, Keffi in North Central Nigeria between December 2019 to march 2020. Considering assumption of 95% level of confidence 15% marginal error we used simple proportion formula to determine. Sample size from a previous study [12], by taking 30% base on this assumption, we calculated a sample size to be 474 during the 4-month study period. HIV RNA viral load was done on all the participants enrolled. We used a system random sampling technique to enroll participants.

Patient's data collection tools procedure

We used key informant interview for a face to face interview and a structured questionnaire to collate socio-demographic data. We review medical records to determine duration since ART initiation, History of opportunities infection, history of TB treatment, as eligibility criteria which forms basis for clinical data.

Clinical operational definition

At any point within in time within 6 months after HAART commencement, a Virological suppression level of <1000 copies Viral load (VL) was consider for HIV-RNA level in Nigeria, CD4+ cell count is defined base on CD4+ cell reference range as favorable (≥466 cell/mm^3 for female and ≤ 400 cell/mm^3 for male) and unfavorable (≤ 400 cells/mm^3 for male and < 466 cell/mm^3 for female).

Sample collections and preparation

A treatment phlebotomist was assigned to collect 5ml of blood sample using a K3 BD EDTA vacutainer disposal tube. The specimen was well labelled with patient ID and separated into two vails, one for HIV viral load testing and the former for CD4+ cells count. For viral load analysis, a whole blood sample tube was centrifuged for 10min at 1500 rpm where plasma was separated and aliquot and store at -20.

Plasma viral load quantification

A (QIAGEN Gm6H, Hilden, Germany) Q1 Amp viral RNA Mini-Kit was used to extract Hiv-1 DNA following Standard operational procedure according manufactures instruction. The HIV RNA was quantify using Real-time PCR (COBAS Amplipre/COBAS Taq-MAN Analyzer (Roche Diagnostic USA). A three phase process was employed which include HIV-1 RNA isolation, formation of complementary deoxyribonucleic acid (CDNA) generation from reverse transcription of target RNA and amplification of targeted cDNA by Simo Haneous polymerase chain reaction. Specific probes of oval-labelled target florescence oligonucleotide were used for the quantification and detection, and amplification of target CDNA.

CD4+ T-lymphocytes using flow cytometry

A BD Bioscience San Jose, CA USA flow cytometry system was used to enumerate CD4+ T Lymphocytes following standard operation procedure according to the manufacturers instruction. A reagent (Tro-count) containing CDA5, CDA, CD3 monoclonal antibodies labelled with (FITC, Res.p, PE, PerCD) dyes are mixed with anti-coagulated lysed red blood cells and whole blood using lysing solution A (BD Bioscience) multi-set BD software is used to enumerate absolute CD4+ cells count.

Data quality

Throughout our laboratory procedure, we used data quality control (QC) measure to guaranteed reliability of our findings. All materials, equipment and laboratory process were controlled adequately. Pre-analytical and post analytical quality assurance was carried out as part of control check on the COBAS Amplipre/COBAS Taqman and The FACS calibur TM analyzers. We used 16 HIV positive as a pretest to check for validity of the questionnaire before actual data collection.

Ethical considerations

Ethical approval was obtained from the Ethical Review Board of Federal Medical Center, Keffi. Prior to enrollment in the study, all participants were informed as consent on the objectives and background of the study. Information was provided toward the risks and benefits of the current study. Similarly, a designated questionnaire and data were collected after obtaining returned informed consent. Anonymity and confidentiality of the study participants were maintained.

Statistical analysis

All data were cleaned and checked for completeness in excel version 2019 before they were exported to Epi-info version 7.1 Descriptive analysis were done and presented as tables. We used binary logistics regression to identify associated risk factors and strength of association.

Results

A total of 474 HIV infected adults placed on HAART were enrolled into the study. Urban residency (20.3%) and male (69.1%) were majority among the study cohort with a median ae of 31 years (29-40years interquartile range (IGR) age of the study cohort with 18 and above 60years as minimum and maximum ages respectively. From the 474 cohort that were enrolled 34.6% were on WHO baseline clinical stage IIV and 57.8% of cohort were on HAART, started treatment regimen in less than a year with 42.2% diagnosed and confirmed with HIV infection between 1-5years. 57.8% were transferred into the center as a major reason for enrolment. 57.8% has history of TB treatment in the past while 42.2% of the study participants who were eligible for treatment initiation were determine by CD4+ counts with a median interquartile range of 180 (92-300) cells/mm^3 (Table 1). From our result using the bivariate binary logistic regression analysis, baseline CD4+ counts, Residency, WHO baseline stage and duration on HAART were significantly associated with favorable immunological outcome Virological suppression. However, in multivariable binary logistics regression analysis, CDA4+ T cell count of <200 cell/mm^3 (aOR: 0.40 j 95% CI (0.21-2.1), Urban residency (ADR: 1.44; (1.0-2.1) were significantly associated with favorable outcomes (Table 2). Viral Load testing at age between 10-30 years (aOR= 1.22 95% CI (0.57-1.67), CD4+ T cells absolute counts (aOR= 2.3 95% CI (1.2-4.1), duration HAART were significantly associated with viral suppression.

From Tables 3 and 4 stating the immunological status of people with HIV

Table 1. Treatment and clinical characteristic of 474 HIV infected adult on HAART accessing care at Federal medical center, keffi, north central Nigeria.

Variables	Category	Frequency (%)
Period since HIV Diagnosis (years)	1 to 5	200 (42.2)
	6 to 10	150 (31.6)
	>10	120 (25.3)
Period from enrollment to eligibility (years)	<1	274 (57.8)
	≥1	200 (12.2)
WHO Baseline Stage	Stage I	100 (21.1)
	Stage II	150 (31.6)
	Stage III	160 (33.7)
	Stage IV	164 (34.6)
Reasons for enrolment on HAART	Viral load suppression low CD4+ counts	120 (25.3)
	Low total lymphocytes	100 (31.6)
	Transfer in	100 (33.7)
	Transfer out	274 (34.6)
Anemic baseline Status	Non-Anemic	274 (57.8)
	Anemic	200 (42.2)
CD4+ at viral load testing (cell/mm^3)	<250	120 (25.3)
	250-600	150 (31.6)
	>600	200 (42.2)
Baseline CD4+ T cells	<200	200 (42.2)
	250-600	150 (31.6)
	≥600	124 (25.3)
TB Treatment History	No	200 (42.2)
	Yes	274 (57.8)

Table 2. Factors Associated with immunological outcome and Virological suppression of seropositive 474 HIV patients attending Nasarawa State, Federal Medical Centers.

Variables	Virological outcome			Immunological Outcome		
	No Viral suppression	Viral Suppression	aOR (95% CI)	Unfavorable immunological outcome	Favorable immunological outcome	aOR (95% CI)
Age (years)						
18-30	60	140	1.44 (1.0-0-21)	70	70	1
31-40	100	50	1	75	75	1.22 (0.57-1.67)
>40	25	95	3.4 (3.0-4.1)	95	25	4.1 (1.5-5.6)
Age at HAART enrollment						
18-30	70	150	1	150	70	1
31-40	100	57	1.4 (0.9-2.1)	57	100	2.2 (1.6-2.7)
>401	60	140	1	140	60	3.1 (3.0-4.1)
Residency						
Urban	120 154	154	2.1 (1.43-2.5)	154	120	4.3 (3.9-6.1)
Rural	180 20	20	3.1 (2.1-4.5)	20	180	5.2 (4.0-5.1)
WHO Baseline						
Stage I	40	60	2.3 (2.1-4.1)	160	40	7.1 (6.0-7.5)
Stage II	50	100	1	100	50	5.1 (2.1-6.2)
Stage III	60	100	1	100	60	4.1 (1.4-4.4)
Stage IV	64	100	1	100	64	2.1 (2.0-3.1)
Duration Since HAART initiation (years)						
18-30	140	60	2.1 (1.4-2.6)	60	140	3 (3.1-4.2)
6 to 10	90	60	1	60	90	1.2 (0.9-2.1)
>10	60	60	3.1 (4.1-4.1)	40	80	4.3 (3.9-4.0)
Baseline CD4+ count (cell/mm^3)						
<250	100	100	1	150	70	1
250-600	100	50	0.40 (0.30-0.40)	57	100	2.2 (1.6-2.7)
<600	40	84	3.1 (2.5-4.5)	140	60	3.1 (3.0-4.1)

in North Central Nigeria, 211 out of 474 participants which accounts for 44.5% has viral load <500 copies/mL with CD4+ T cells cell/mm^3 mean of 308 at 95% CI (300-360). 178 cohorts had their CD4+ cells between 201-250 cells/mm^3 with 140 copies of viral loads less than 400 copes accounting for 78.6% with 18% viral loads above 1000 copies/mL. Out of the 474 participants enrolled into the study, 142 had viral load less than 400 copies/mL with 121 between 400-999 and 211>1000 copies/mL. In our study we discovered a decrease in CD4+ T Cells count with increase in age at average CD4+ cells of 400 cells/mm^3 at age 31-40years in Table 2 COR: ft.22 (0.57-1.67). In this study, we correlate the immunological and Virological response outcome of different

ART regime currently been prescribed for eligible HIV infected adult who are enrolled for commence treatment as described in Tables 5 and 6. Among the common ART regimes combinations are Nevirapine/lamivudine/Tenofavirines (NUP/3TC/TDF) was found to be more effective in terms of viral load response with minimal Virological failure [13].

Discussion

One of the greatest achievements in Nigeria HIV program the last two decades is the scale up of ART services, which has consistently suppressed HIV RNA to an undetectable level reducing rise of clinical progression [14]. Despite the success recorded, there're challenges been faced by the program which include; poor adherence and drug resistance which have led to treatment failure? For this reasons stated above, WHO recommended routine viral load testing for ART monitory [15].

Despite achieving 72% Virological suppression in our study, it differs from other previous studies. It's consistent with studies in Ethiopia [15] with 90%, South Africa (94%) and Ghana (89.6%) respectively. Similarly studies in Ethiopia reveal 88.1% of patients enrolled on ART shows Virological suppression. Our study also reveals treatment outcome can be impaired if ART is initialized at advance stage of the disease [16]. From our study, remarkably 50.2% of cohorts were enrolled at stage 3 or 4 WHO baseline conditions which were recommend to achieve Virological suppression by 2020 [17].

Remunerable, from our study, we characterize and analyze with comparison Virological and immunological response in sex and age our results reveals older age is more susceptible to Virological failure (p=0.02) compared to participants who are young. The immunological recovery was seen in 60.8 to 90.2% of the total study population out of the different regimen adopted in Nigeria, NVP/3TCTDF and NUP/3TC/ART had better immunological and Virological responses among people living with HIV (AIDS) in North central Nigeria. Our study concord with a study done among Chinese population, where 12.1% Virological failure rate was observed with 3TC combined drugs [18].

Our study revealed better outcome with Nevirapine based regimen than Efivirenz based regimen (p=0.03) which is in line with a similar study in India [19]. Keeping insight similar geographical, socio-cultural and similar HIV epidemics Nigeria and India share in common. Comparison between our study and that of India is realistic, so we can infer that Nevirapine base combination is better than Efavirenz base regimen. Although a study in Botswana suggest that Efavirenze combination drugs are better than Nevirapine regimen combination drugs are better than Nevirapine regimen [20]. Discrepancy in prevalence of HIV type 1 and 2 subject in Nigeria and Botswana might account.

Table 3. Immunological status of 474 HIV infected drug experienced patients and its relationship with virological characteristics.

Viral Load (copies/mL)	Number of Patients and percentage n (%)	CD4+ T cell (cell/mm^3) Mean: 95% CI
<500	211 (44.5)	308 (300-360)
500-1000	121 (25.5)	400 (350-490)
>1000	142 (29.9)	500 (400-610)

Table 4. CD4+ T cell level in different Viral Load status of Seropositive 474 HIV infected drug experienced Patients accessing care at Federal Medical Center, Keffi, Nasarawa State Nigeria.

CD4+ cell count (cell/mm^3)	Total Cases	Number of patients with viral load		Viral load >1000 copies/mL (%)
		Viral load <400 copies/mL (%)	Viral load (VL) =500-1000 (%)	
<100	40	15 (37.5)	15 (37.5)	10 (25)
100-200	135	120 (88.9)	15 (11.1)	0
201-250	178	140 (78.6)	20 (11.2)	18 (10.1)
250-400	68	20 (29.4)	30 (44.1)	18 (26.5)
>400	53	10 (18.9)	30 (56.6)	13 (24.5)
Total	474	305 (64.3)	110 (23.2)	59 (12.5)

Table 5. ART regimen and virological characteristics of Seropositive 474 HIV patients receiving care at Federal Medical Center, Keffi.

ART Regimen	Number of patients with viral load			Total	Viral load failure rate (%)
	>1000 copies/mL	400-999 copies/mL	<400 copies/mL		
EFV/ABC 3TC	20	25	35	80	9.5
EFV/ART/3TC	13	43	40	96	8.15
EFV/ART/3TC	32	10	50	92	12.34
NVP/TDF/3TC	34	20	36	90	12.89
NVP/TDF/3TC	43	23	50	116	22.15
TOTAL	142	121	211	474	11.93

TDF: Tenofovir, ART: Zidovudine NVP: Nevipapine, 3TC: Lamivudine VL: Viral Load
This table reveals different patient regimen at different viral load level (the assay detects limits at 400 copies copies/mL)

Table 6. ART treatment regimen and immunological Characteristics of Seropositive 474 HIV drug experienced patients accessing care Federal Medical Center, Keffi, Nasarawa state.

ART Regimens	Total Cases	Number of patients with percentage with varying absolute CD4T count (cell/cm^2)				
		<99	100-200	<250	250-400	400-600
EFV/ABC/3TC	80	15 (18.7)	20 (25.0)	30 (37.5)	7 (8.7)	8 (10)
EFV/AZT/3TC	96	25 (26.0)	15 (15.6)	20 (20.8)	26 (27.1)	10 (10.4)
EFV/AZT/3TC	92	0	40 (43.5)	42 (45.7)	5 (5.4)	5 (5.4)
NVP/TDF/3TC	90	0	20 (22.2)	40 (44.4)	15 (16.7)	15 (16.7)
NVP/TDF/3TC	116	0	40 (34.5)	46 (39.7)	15 (12.9)	15 (12.9)
Total	474	40 (8.4)	135 (28.5)	178 (37.6)	68 (14.3)	53 (11.1)

The study conducted in India looked at Virological and immunological outcome in terms of significant effects on ART, but we didn't look at the above the above clinical presentation [21].

Conclusion

Our study reveals sub-optimal virological level of suppression that requires strong commitment by all USAIDS program in achieving the 90% target of USAIDS 90-90-90 BY 2020. Low immunological status was observed at age (>40years) with CD4+ cell counts of 250 cells/mm^3 which are associated with Virological suppressions. Age enrollment was negatively associated with immunological outcome at age 18-30years. Urban residency and CD4+ baseline of 200 cells/mm^3 was positively associated. We recommend enrollment on HAART at higher CD4 cell count levels in order to achieve the USAIDS plan of 90-90-90.

Author's Contributions

M.B.E conceptualized and designed the study, A.I A, K.A and JRA analyzed the data, D.T and A.C.O revised the manuscript for intellectual and scientific content, and developed the results and discussion section. A.I.A and J.R.A reviewed and revised the manuscript. All Authors read & agreed to publish the version of the manuscript.

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Limitations

The Data analyzed in this study is for a sub-population, and may influence the generalizability of this results, compared in a larger population.

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Conflict of Interest

The author declared that there is no Conflict of Interest.

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