Original Article

Prevalence and determinants of virological failure among adult antiretroviral treatment experienced HIV-1 patients in South Omo, Ethiopia

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Abstract

Background: Virological failure (VF) is a challenge which hinders the success of the antiretroviral treatment (ART) program. Although viral load is the preferred method used to detect and confirm treatment failure, research into its prevalence and determinants is limited.

Objective: This study was conducted to determine the prevalence of virological failure and its determinants among HIV-1 infected adults, who experienced first line ART in South Omo, Ethiopia.

Methods: A cross-sectional study was conducted on patients attending ART clinics at Jinka Zonal hospital and four rural health centers found in South Omo. A total of 253 adult patients who were on ART for ≥ 6 months were recruited sequentially from January 2015- December 2016. In addition to the socio-demographic and clinical data, 10 ml of blood was collected for the $CD4^+$ T cell count and the viral load measurements. CD4+T cell count was conducted using the FACS Caliber flow cytometer and the viral load was estimated using a quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR). Samples with a VL ≥1000 copies/mL were considered as virological failure. Logistic regression analysis was used to identify correlates of virological failure.

Results: The median duration of ART was 51 months. About 61% of the participants were females with a mean age of 33.5 years. Majority of the participants (70%) were taking the tenofovir (TDF) based ART, while 30% were taking a zidovudine (AZT) based regimen. Majority (71.9%) of the participants started ART in WHO III/IV stage, with a median base line CD4 count of 239 cell/µL. More than 40% of the participants reported poor ART adherence, and 11% had immunological failure.

From a total of 253 patients, 206(81.4 %) [95%CI: 76.7-86.2] had viral suppression, while 47 (18.6%) [95%CI: 13.8-23.3] had virological failure with a mean viral load of 185,506 copies/mL (95%CI: 39083-408100). None of the virological failures were switched to second line ART. According to the multivariate logistic analysis, active tuberculosis (aOR=13, 95% CI= 3.46-29.69), immunological failure (aOR=3.61, 95% CI=1.26-10.39), opportunistic infections (aOR=8.39, 95% CI= 1.75-40.19), and poor adherence were significantly associated with VF among the study participants.

Conclusion: The study revealed a considerable prevalence of virological failures with the associated risk indicators. Based on the results and the difficulty faced, of being unable to place patients who were treatment resistant in second line ART, indicates that immunological and clinical criteria are poor biomarkers of drug switching. Hence, regular virological monitoring should be implemented for better ART treatment outcomes in individuals who are HIV positive. [*Ethiop. J. Health Dev*: 2022: 36(1): 00-00]

Key Words: HIV-1, Virological Failure, viral load, ART experienced, South Omo, Ethiopia.

Background

According to the Global Burden of Disease Study 2015, the number of people living with HIV was estimated to be at 3.8 million, with 1.8 million new infections, of which 75% were in sub-Saharan Africa(1). There was a decrease in the global mortality rate from 1.8 million in 2005 to 1.2 million in 2015. This may be due to the rapid increase in the proportion of people receiving ART for the last 10 years among individuals living with HIV(1). As of December 2016, 19.5 million people living with HIV were accessing ART globally(2).

Ethiopia has a total population of 99.4 million people, which is the second largest population in Africa. Since the first incidence of the HIV infection found in Ethiopia in 1984, AIDS has taken the lives of millions and left behind an estimated 744,100 orphans(3). At the end of 2016, the national HIV prevalence stabilized at 1.12%, and 715,500 people were living with HIV/AIDS, with 27,288 AIDS related deaths, and 19,743 new infections(4). Since the introduction of free ART in 2005 in Ethiopia, a total of 386,123 estimated adults living with HIV have been receiving ART with an estimated 54% coverage (4).

In 2014, the United Nations Program on HIV/AIDS (UNAIDS) and its allies initiated the ambitious goal of increasing the estimates of diagnosis, ART provision and viral suppression to 90% of the population living with HIV, this in an attempt to attain the ultimate goal of ending the HIV epidemic as a public health problem by 2030(2).

According to WHO, a viral load of <1000 copies/mL defines treatment success for patients on combined

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antiretroviral drugs in the management of HIV/AIDS(2). Studies suggest that it takes at least six months of stable ART to reach an undetectable blood plasma viral load (5). Although retaining patients in care and suppressing their viral load (VL) are both crucial for improved outcome evaluation, utilization of VL monitoring is found to be more important than retention when assessing the effectiveness of health facilities delivering ART services (6). Several studies have suggested that investing and ensuring the sustainability of a VL-informed care and monitoring model must be a priority in low and middle income countries (7, 8).

Although total elimination of HIV infections is unlikely to be achieved with the ARVs available currently, due to the establishment of a pool of latently infected CD4+ T cells (9), it is well documented that early ART for people living with HIV can decrease AIDS associated morbidity, mortality and reduce the transmission of HIV to others (10-13).

Even if regular virological monitoring and HIVDR testing have proven useful in the continuum of HIV/AIDS care and treatment in both developing and developed nations, WHO does not recommend them for routine use in most resource poor countries due to it being costly(5). Instead, clinical and immunological criteria have been used to indicate the failure of ARTs (14). However, different studies, have indicated that there is a poor correlation between these parameters and VF in both developed and resource limited settings, and this may lead to unnecessary drug switching that in turn, induces drug resistance development (15-18). As it has been reviewed elsewhere, employing clinical and immunological criteria to initiate and monitor ARTs in resource limited settings, and the use of drugs with a low genetic barrier like abacavir (ABC), lamivudine (3TC), tenofovir (TDF) and didanosine (ddI) have been associated with the emergence and accumulation of HIV-1 drug-resistant variants(19).

Despite the absence of routine viral load testing in resource limited countries, several studies conducted in African settings indicated that the proportion of HIV/AIDS patients on ART who had overt VF ranged from 7 - 24%(20-23). These studies also identified that younger people, poor adherence, low CD4 cells counts, advanced WHO stages, and having active TB increased the odds of virological non-suppression.

A recent global study involving 36 countries and 1926 patients with treatment failure from 1998 -2015 reported that 36.3% of patients developed tenofovir resistance, where the highest (57%) was in Sub-Saharan Africa. A systemic review on virological follow-up of adult patients on ART programs in Sub-Saharan Africa reported 78% of virological suppression after 6 months,76% after 12 months, and 67% after 24 months of ART(5).

In Ethiopia, limited studies were conducted on the prevalence of VF and according to these studies, VF was observed to range between 5.3% - 18% among HIV/AIDS patients who were on ART for a median time of between 6 - 24 months (19, 24, 25). Another study conducted in Addis Ababa, reported 31% VF, out of which 70% were ADR mutations (26).

Furthermore, data on virological failures and its determinants among patients on ART in Ethiopia is scarce due to lack of routine laboratory monitoring of HIV viral load, which has not been reviewed in the present study, South Omo, Ethiopia(27).

Therefore, this study was designed to determine prevalence of HIV-1 virological failure and its determinant factors among HIV-1 infected adults on ART for ≥ 6 months in South Omo, Ethiopia.

Materials and Methods

Study design and setting

A cross-sectional study was conducted from January 2015 - December 20016 in South Omo, Ethiopia, among patients attending ART clinics in four health centers, namely: Omorate, Turmi, Key Afer, Gazer, Millennium and Jinka Zonal hospital. Adult Patients (age ≥18 years) who provided informed consent and who had been on ART for 6 or more months and were still on ART at the time of enrolment were considered for the study. Eligible participants were recruited sequentially (prospectively) at the study settings over the study period. Both an interviewer-based questionnaire and chart review was used for data collection. Information on socio-demographic characteristics, clinical data, date of HIV diagnosis, and ART commencement, TB diagnosis and treatment, opportunistic infections, sexually transmitted infections, anthropometric measurements, and ART regimens were collected using standard questionnaires during ART clinic visits by a trained health care provider. Patient records were reviewed for the previous results of CD4+ T cell count, WHO clinical stages and other clinical characteristics.

Sample size determination: Sample size was determined using a single population proportion formula assuming an HIV-1 VF prevalence of 21% after a median follow-up period of 12 months on ART in Sub-Saharan settings (5), with a 5% margin of error.

ART regimen and eligibility criteria

ART in Ethiopia consists of a generic low cost fixed-dose combination (FDC) of two NRTI and one NNRTI with first line regimens of lamivudine (3TC) combined with tenofovir (TDF) or zidovudine (AZT), and either nevirapine (NVP) or efavirenz (EFV). People living with HIV (PLHIV) with a CD4+T cell count of ≤500 cells/mm3 or a WHO clinical stage III/IV or HIV infection and active TB irrespective of CD4+T cell count were eligible for ART in Ethiopia at the time of this study(28). However, majority of the study participants had already initiated ART with a CD4+T cell counts of <350 cells/mm3, which was in accordance with the previous national guidelines(29).ART patients were followed clinically every month for the first 6 months and then every 2–3 months thereafter.

Specimen Collection Procedure

A Blood sample of 10mL was drawn into EDTA tubes for CD4+ T-cell counts and viral load assays. Plasma was temporarily stored in three aliquots at -30^{o}C at Jinka Regional Laboratory and then transported to the Addis Ababa University, Aklilu Lemma Institute of Pathobiology laboratory and stored at -80^{o}C, until it was shipped to Kampala, Uganda on dry ice for VL measurements at the Makerere University. All testes were done anonymously but were linked using a unique client code.

CD4+ T cell Counts, RNA Extraction and Viral Load Measurement

Baseline $CD4^{+}$ T cell counts was performed in Jinka Zonal Hospital within 4-6 hours of blood collection, using a FACS Caliber flow cytometer (Becton-Dickinson, NJ) equipped with automated acquisition and analysis software. Individual test results were reviewed to confirm accuracy of the automated software analysis.

HIV-1 genomic RNA extraction and viral load measurements were performed in the Makerere University Microbiology laboratory. HIV-1 genomic RNA was extracted from 140µl of the cryopreserved plasma using the QIAmp viral extraction kit (QIAGEN, Hilden, Germany) as per the manufacturer's protocol. Briefly, the sample was lysed using the Lysis Buffer AVL, and the lysate was passed through a spin column to allow binding of the viral RNA on to a column and then it was washed with Buffer AW1 and AW2. Finally, elution of the pure viral RNA was adsorbed on to the QIAmp membrane, which was performed by a double elution Buffer AVE (2X 40µL) which is reported to increase yields by up to 10%(30).

Viral load was estimated using QuantiTect Probe RT-PCR kit (QIAGEN, Hilden, Germany) as per the manufacture's protocol: this is a hydrolysis probe-based assay that targets the conserved portion of GAG and LTR genome of the HIV-1 virus. The kit is designed to facilitate both efficient reverse transcription and specific amplification in a one-tube format. It is based on the unique QIAGEN One Step RT-PCR buffer system and has been specifically adapted for real time RT-PCR using the following HIV1/2 sequence specific Primer and Probes: Forward Primer Gag 183UF: CTAGC AGTGGCGCCGACAG, Reverse Primer Gag187LR: CCATCTCTCTCCTTCTAGCCTCCGCTAGTCA, Probe Gag187PFAM–5' TCTCTCGACGCA GGACT CGCTTGCTG'3 –BHQ.

A PCR master mix of 30µL was prepared according to the manufacture's protocol and was aliquoted in a RNase free PCR tube. RNase-free water consisting of 20µL was added to one of the tubes as a No Template Control (NTC). In each test tube, a test RNA of 20µL was added. A AcroMetrix HIV-1 RNA Panel (AcroMetrix Inc., CA, USA), a commercial standard calibrated against the WHO international HIV RNA standard was used as the analytical standards. The AcroMetrix standards consist of HIV-1 standards at concentrations of 150,000, 15000 and 1500 viral copies/ml.

The PCR tubes were then loaded on a 36–well Rotor Gene Q PCR Machine (QIAGEN® California, USA) and run using the following program: (i) reverse transcription at 50°C for 30 min; (ii) PCR initial activation at 95°C for 15 min; (iii) 55 cycles of denaturation at 95°C for 15s, annealing / extension and fluorescence acquisition at 67°C for 60s. Following amplification, the report was generated by Rotor-Gene Q Series Software 2.1.0 and used to plot the standard curve using at least 3 RNA standards with CT values and the resultant viral copies. From the graph, using the C_T value, viral copies of the test sample were determined and normalized to viral copies per ml of sample. The assay has a detection probability of 95% or above at a concentration of ≥126 copies /mL (31). The run was considered valid if the efficiency = 1.01, M (Slope) = - 3.292, B (Y- intercept) =38.949 and all controls pass.

Virological failure was defined as HIV-1 RNA ≥1000 copies/mL after a minimum of 6 months of ART initiation. Prevalence of HIV-1 VF was considered as the percentage of plasma samples with a detectable viral load of ≥1000 copies/mL. Furthermore, immunological failure was defined as persistent CD4 levels which were below 100cells/mm3, while immunological recovery was defined as patients enrolled to care with CD4 counts below 200 cells/mm3 at baseline and who had achieved ≥500 CD4 cells/mm3 in recent CD4 measurements at the start of ART care.

Data Analysis

Descriptive statistics were employed to describe socio-demographic, clinical characteristics, and HIV-1 VF among the study participants. Continuous data was presented using descriptive such as the mean and medians. Frequencies and percentages were used to describe categorical data. Bivariate and multivariate logistic regression was conducted to determine the independent predictor of virological failure among the study subjects. Crude and adjusted odd ratios (OR), with 95% confidence intervals (CI) and p. values <0.05 were considered significantly associated. Predictors that indicated multi-collinearity were removed from the multivariate regression model after the variance inflation factor (VIF) was determined. Data analysis was performed using IBM SPSS for Windows, Version 23.0. Chicago, SPSS Inc.

Ethical Considerations

Institutional permission to conduct the study was obtained from Aklilu Lemma Institute of Pathobiology Institution Review Board, Addis Ababa University. Informed consent to participate in the study as well as permission to use isolates from samples provided was obtained from all enrolled participants. To ship the specimens to Kampala Uganda for laboratory analysis, permission was obtained from the Ethiopian Food, Medicine and Health Care Administration and Control Authority, as well as the material transfer agreement entered between Addis Ababa and Makerere Universities. In addition, ethical clearance was

obtained from the Ethiopian Federal Ministry of Science and Technology for the main project.

Results

Baseline characteristics of the study participants

A total of 253 adults on first line ART for a median duration of 51(IQR: 49-61) months were recruited sequentially from January 2015 - December 2016. Majority of them were females (60.5%), the mean age was 33.5 years ([95%CI: 32.5-34.5/]) (Table 1). Majority (70%) of the study subjects were taking tenofovire (TD4) based ART, while 30% were on a Zidovudine (AZT) based regimen during the study period. Majority (71.9%) of them started ART in the WHO III/IV stage, with a median base line CD4 count of 239 (95%CI: 204-284.5) and IQR of 228 (95% CI: 208-268). About 40% (102/253) of the participants reported poor ART adherence, and 11% (27/253) of them had an immunological failure (Table2).

Table 1: Socio-demographic characteristics of the study participants among first line ART patients in South Omo, Ethiopia (n=253).

Characteristics	Categories	Frequency (%)
Sex	Male	100(39.5%)
	Female	153(60.5%)
Age (years)	Mean	33.5(95%CI:32.5-34.5), Sd=8.4
Age Group(years)	≤ 30	103(40.7%)
	>30	150(59.3%)
	Married	131(51.8%)
Marital status	Divorced	59(23.3%)
	Single	25(10.3%)
	Widowed	37(14.6%)
	Orthodox Christian	191(75.5%)
Religion	Protestant	51(20.5%)
	Muslim	8(3.2%)
	Other	3(1.2%)
Educational status	Illiterate	126(49.8%)
	Read & write	19(7.5%)
	Primary	61(24.1%)
	Secondary	36(14.2%)
	Tertiary	11(4.3%)
	Agro pastoralist	30(15%)
Occupation	Housewife	76(30%)
	Government Employee	46(28.2%)
	Daily Laborer	93(36.8%)
	Underweight	37(14.6%)
Baseline BMI Group (Kg/m^2)	Normal	185(73.1%)
	Overweight	23(9.1%)
	Missing	8(3.2%)

Table 2: **Antiretroviral treatment related and clinical characteristics of the study participants among first line ART patients in South Omo, Ethiopia (n=253).**

Characteristics	Categories	Frequency (%)
First Line ART Regimen	1c(AZT-3TC-NEV)	48(19.0%)
	1d(AZT-3TC-EFV)	28(11.0%)
	1e(TDF-3TC-EFV)	119(47.0%)
	1f(TDF-3TC-NEV)	58(23.0%)
First line ART regimen Categorized	Tenofovir based	177(70%)
	Zidovudine based	76(30%)
Base Line WHO staging	I/II	71(28.1%)
	III/IV	182(71.9%)
Baseline CD4 count(cells/µL)	Median	239(95% CI: 204-284.5)
	IQR	228 (95% CI: 208-268)
	≤350	238(94.1%)
	>350	15(5.9%)
Current CD4 Count (cells/µL	Median	501(95% CI:462-540)
	IQR	337(95% CI:288-399)
Duration on ART (Months)	Median	51(95%CI:42-59)
	IQR	57(95%CI:49-60)
Follow up WHO staging	I/II	232(91.7%)
	III/IV	11(4.3%)
	Missing	10(4.0%)
Duration of ART group	≤ 12 months	27(10.7%)
	> 12 months	226(89.3%)
Active TB	Yes	38(15%)
	No	215(85%)
Any OIs diagnosed	Yes	38(15%)
	No	215(85%)
Any STIs Diagnosed	Yes	35(13.8%)
	No	215(85%)
Immunological Failure(n=235)	Yes	27(11.5%)
	No	208(88.5%)
Virological Failure	Yes	206(81.4 %) [95%CI: 76.7-86.2]
	N0	47 (18.6%) [95%CI: 13.8-23.3]

Prevalence of Virological Failure (VF)

The majority (94%) of study participants had base line CD4 cell counts ≤ 350 cells/µL at the start of the study. Based on the persistent CD4 levels, which were below 100 cells/mm3, severe immunological failure was observed in 11.5% (27/235) of the participants (table 2). Immunological failure was significantly associated with older age and malnutrition in the bivariate analysis (OR= 5.9, 95% CI: 1.7-20.1, P.= 0.005) and (OR= 3.1, 95% CI:1.22-8.10, P=0.02) respectively (data not shown).

Among the 253 study participants, 206 (81.4 %) [95%CI: 76.7-86.2] experienced viral suppression (<1000 copies/mL), and 47 (18.6%) [95%CI: 13.8-23.3] had VF (≥1000 copies/mL) with a mean VL of 185,506 copies/mL (95%CI: 39083-408100). Table 3 indicates the logistic regression analysis of predictor variables associated with virological failure. A bivariate logistic regression analysis indicated that subjects who were older than 30 years of age (cOR= 3.54, 95%CI=1.63-7.70) were more likely to have virological failure compared to subjects who were younger than 30 years of age. Patients with base line CD4 counts ≤ 100 cells/µl of blood (cOR=2.77, 95% CI=1.35-5.68) and baseline WHO staging III/IV (cOR=3.15,95 %CI=1.28-7.79) were more likely to experience virological failure than those who had base line CD4 counts >100 cells/µl and baseline WHO staging of I/II respectively. Patients with immunological failure (cOR=6.15, 95%CI=2.64-14.33), diagnosed with an active TB (cOR=19.31, 95% CI= 5.65-62.7) or who were diagnosed with any other opportunistic infections (cOR=6.68, 95% CI=3.16-14.14) and who were diagnosed with any sexually transmitted infections within the last 3 years (cOR=5.1, 95%CI=2.36-10.93) were more likely to have virological failure than their counter parts. Patients who reported not taking their ARV pills consistently within the last seven days were more likely to experience virological failure (cOR= 2.66, 95% CI=1.32-5.35) than patients who consistently took their medication. Variables such as sex, marital status and duration of ART were not significantly associated with virological failure (Table 3).

In the multivariate logistic analysis, being diagnosed with active TB (aOR=13, 95% CI= 3.46-29.69), immunological failure (aOR=3.61, 95% CI=1.26-10.39), the presence of any other opportunistic infections (aOR=8.39, 95% CI= 1.75-40.19), and individuals who reported not taking their ARV pills consistently within the last week were significantly associated with virological failure.

Table 3 **Logistic regression analysis describing associations of HIV-1 virological failure among first line ART patients in South Omo, Ethiopia (n=253).**

Risk Factor	**Categories**	**VL≥ 1000copies /mL N (%)**	**VL< 1000copies /mLN (%)**	**Bivariable Analysis**			**Multivariable analysis**		
				COR	95% C.I	P-value	AOR , 95% C.I		P-value
Sex	Male	22(22%)	78(78%)	1.44	0.76-2.73	0.26			
	Female	25(16.3%)	128(83.7%)	1:00					
Age Group	≤30 years	9(8.7%)	94(86.2%)	1:00			1:00		
	>30 years	38(25.3%)	112(77.8%)	3.54	1.63-7.70	0.001			
Marital Status	In union	23(17.6%)	108(82.4%)	1.15	0.67-2.17	0.67			
	Not union	24(19.7%)	98(80.3%)	1:00					
Active Tuberculosis	Yes	13(76.5%)	4(23.5%)	19. 3	5.65-62.70	<0.001	**13.0**	**3.5-29.7**	**0.001***
	No	34(14.4%)	202(85.6%)	1:00:					
Baseline WHO staging	I/II	6(8.5%)	65(91.5%)	1:00					
	III/IV	41(22.5%)	141(75.5%)	3.15	1.28-7.79	0.01			
Baseline CD4 count/mL	≤ 100	16(34.0%)	31(66.0%)	2.77	1.35-5.68	0.005			
	>100	30(15.76%)	161(84.3%)	1:00					
Immunological Failure	Yes	14(51.9%)	13(48.1%)	6.15	2.64-14.33	<0.001	**3.6**	**1.3- 10.4**	**0.01***
	No	31(14.9%)	177(85.1%)	1:00					
Any opportunistic Infections	Yes	19(50.0%)	19(50.0%)	6.68	3.16-14.14	<0.001	**8.4**	**1.8-40.1**	**0.008***
	No	28(13.0%)	187(87.0%)						
Sexually transmitted infections within last 3 years	Yes	16(45.7%)	19(54.3%)	5.12	36-10.93	<0.001			
	No	31(14.2%)	187(85.8%)	1:00					
Duration on ART in month	≤12	2(7.4%)	25(92.6%)	0.32	0.07-1,41	0.13			
	>12	45(19.9%)	181(80.1%)	1:00					
Missed ARV Pills within last week	Yes	29(28.4%)	73(71.6%)	2.94	1.53-5.64	0.001	**2.7**	**1.3 -5.4**	**<0.001***
	No	18(11.9%)	133(88.1%)	1:00			1:00		

Discussion

In this study,18.6% (47/253) of patients receiving ARTs had virological failure after a mean time of 51 months on ART, which is in line with the global report of 18% of the pooled prevalence of virological failure (2) which was the same in Gondar, Ethiopia(18%) (25), and a bit higher than in other Ethiopian studies: 10% reported from Addis Ababa (32), 12% from Gondar (19), 10.24% from North Shoa (33), 15% reported from Wollo (34), and 12.47% from Adigrat(35). However, the current report was much lower than the other African studies conducted in Cameron 23.2% (36), Kenya, 24% (37) and Tanzania 32%(38). This difference might be due to the differences among study participants, duration of follow-up, study design, and variations in the treatment adherence of antiretroviral treatment. The virological suppression (81.4%) reported in the present study was almost comparable with similar research outcomes from Sub-Saharan Africa and other resource limited countries where it ranged from 67%-78% after 6-24 months of ART (5). Similarly, a study from Rwanda found that 88.1% of patients suppressed their viral load after 12 months of ART (39). Virological suppression of 81.4% of the current study, suggested that Ethiopia could achieve the 90% virological suppression target of WHO by 2020, and that could contribute to the elimination of AIDS as a public health threat by 2030(2).

In this study, the diagnosis of active tuberculosis, immunological failure, the presence of any opportunistic infections, and the self-reported history of poor treatment adherence were found to be independent predictors of HIV virological failure. In line with our findings, several studies revealed that a high proportion of TB/HIV co-infected patients while on ART, were found to be more likely to have virological failure as compared to those who were on infected with HIV(23, 40, 41), this finding suggests that virological monitoring should be prioritized in TB/HIV co-infected patients in resource-limited settings. Research indicates that virological failure is more likely to occur in patients diagnosed with TB after initiating ART, and this double infection could result in the overlapping of drug toxicities, exacerbation of side effects, poor adherence, and in immune reconstitution inflammatory syndrome (IRIS)(42-44).

Similar to the findings of this study, immunological failure(45), poor ART adherence, (20, 22, 32, 46) and the development of any opportunistic infections were found to be independent predictors of virological failure (21, 47, 48). Virological and immunological failures are known to have a synergistic negative effect on the prognosis of HIV/AIDS patients on ART. Several studies have reported that poor ART adherence increased the chance of immunological recovery (49-52), which leads to increased viral replication which in turn increases the infection rate of new CD4+ T cells and ultimately the depletion of immune cells(53). Furthermore, as stated in other research, an elevated viral load indicates poor adherence or drug-resistance (9, 23). A similar study from Kenya indicated that poor treatment adherence was a strong correlate of virological failure(54).

In this study, an association was found between a history of opportunistic infections and virological failure. Likewise, a number of studies demonstrated that opportunistic infections resulted in an increased viral load among PLHIV(55, 56). It has also been reported that macrophages infected by opportunistic infections increased the production of the HIV virus(57). This observed association of opportunistic infections and an increased viral load signifies the importance of the timely management of opportunistic infections among HIV/AIDS patients.

Conclusion and Recommendation

The high instances of virological failure were observed among participants diagnosed with opportunistic infections, active tuberculosis, immunological failures, and poor treatment adherence. Therefore, special attention should be given to those who develop opportunistic infections including active tuberculosis, poor treatment adherence, and immunologically failed individuals to have better ARV treatment outcomes. It is also recommended that, at the very least, routine viral load measurements should be in place to inform the timely switch to second line treatment in case of virological failures. This will improve the quality of HIV/AIDS care and treatment programs in Ethiopia.

References

1. Wang H, Wolock TM, Carter A. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. Lancet HIV. 2016;3(8):e361-87.
2. WHO. HIV drug resistance report 2017. Geneva: World Health Organization; 2017 (http://www.who.int/hiv/topics/ , accessed on 20 Oct 2017). 2017.
3. EFMH. National Guidelines for Comprehednsive HIV prevedntion, Care and Treatment. Federal Ministry of Health(FMOH),Addis Ababa , Ethiopia.; 2014.
4. EPHI. HIV Related Estimates and Projections for Ethiopia–2017 Addis Ababa, Ethiopia: Ethiopian Public Health Institute; 2017.
5. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. Lancet Infect Dis. 2010;10(3):155-66.
6. Duber H, Roberts D, Ikilezi G, Fullman N, Gasasira A, Gakidou G, et al. Evaluating facility-based antiretroviral therapy program effectiveness: a pilot study comparing viral load suppression and retention rates. Tropical Medicine & Hygiene. 2016(doi: 10.1111/tmi.12694.).
7. Hoffmann CJ, Maritz J, van Zyl GU. CD4 count-based failure criteria combined with viral load monitoring may trigger worse

switch decisions than viral load monitoring alone. Trop Med Int Health. 2016;21(2):219-23.
8. Phillips A, Shroufi A, Vojnov L. Sustainable HIV treatment in Africa through viral-load-informed differentiated care. Nature. 2015;528(7580):S68-76.
9. Thaker H, Snow M. HIV viral suppression in the era of antiretroviral therapy. Postgrad Med J. 2003;79(927):36-42.
10. Engsig FN, Zangerle R, Katsarou O, Dabis F, Reiss P, Gill J, et al. Long-term mortality in HIV-positive individuals virally suppressed for >3 years with incomplete CD4 recovery. Clin Infect Dis. 2014;58(9):1312-21.
11. Grinsztejn B, Hosseinipour MC, Ribaudo HJ, Swindells S, Eron J, Chen YQ, et al. Effects of early versus delayed initiation of antiretroviral treatment on clinical outcomes of HIV-1 infection: results from the phase 3 HPTN 052 randomised controlled trial. Lancet Infect Dis. 2014;14(4):281-90.
12. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365(6):493-505.
13. West GR, Corneli AL, Best K, Kurkjian KM, Cates W, Jr. Focusing HIV prevention on those most likely to transmit the virus. AIDS Educ Prev. 2007;19(4):275-88.
14. WHO. Consolidated Guidelines on HIV Prevention, Diagnosis, Treatment and Care for Key Populations - 2016 Update. 2016.
15. Badri M, Lawn S, Wood R. Utility of CD4 cell counts for early rediction of virological failure during antiretroviral therapy in a resource-limited setting. BMC Infect Dis 2008;8(89).
16. Mee P, Fielding K, Charalambous S, Churchyard G, Grant A. Evaluation of the WHO criteria for antiretroviral treatment failure among adults in South Africa. . AIDS 2008;22:1971-77.
17. Messou E, Gabillard D, Moh R. Anthropometric and immunological success of antiretroviral therapy and prediction of virological success in west African adults. Bull World Health Organ. 2008;86 435-42.
18. Kantor R, Diero L, Delong A. Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. Clin Infect Dis 2009;49 (3):454-62.
19. Mulu A, Maier M, Liebert UG. Low Incidence of HIV-1C Acquired Drug Resistance 10 Years after Roll-Out of Antiretroviral Therapy in Ethiopia: A Prospective Cohort Study. PLoS One. 2015;10(10):e0141318.
20. Ruperez M, Pou C, Maculuve S, Cedeno S, Luis L, Rodriguez J, et al. Determinants of virological failure and antiretroviral drug resistance in Mozambique. J Antimicrob Chemother. 2015;70(9):2639-47.
21. Rangarajan S, Donn JC, Giang le T, Bui DD, Hung Nguyen H, Tou PB, et al. Factors associated with HIV viral load suppression on antiretroviral therapy in Vietnam. J Virus Erad. 2016;2(2):94-101.
22. Zoufaly A, Jochum J, Hammerl R, Nassimi N, Raymond Y, Burchard GD, et al. Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon. J Antimicrob Chemother. 2015;70(3):922-5.
23. Bulage L, Ssewanyana I, Nankabirwa V, Nsubuga F, Kihembo C, Pande G, et al. Factors Associated with Virological Nonsuppression among HIV-Positive Patients on Antiretroviral Therapy in Uganda, August 2014–July 2015. BMC Infectious Diseases 2017;17:326.
24. Abdissa A, Yilma D, Fonager J, Audelin AM, Christensen LH, Olsen MF, et al. Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia. BMC Infect Dis. 2014;14:181.
25. Mulu A, Liebert UG, Maier M. Virological efficacy and immunological recovery among Ethiopian HIV-1 infected adults and children. BMC Infect Dis. 2014;14:28.
26. Abegaz W. Genomic Characterization of HIV-1 Isolates from Ethiopian Patients: Baseline Studies on Antiretroviral Drug Resistance and Sub-type Variations. A Thesis Submitted to the school of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Medical Microbiology, Addis Ababa, Ethiopia. 2011.
27. Ssemwanga D, Lihana RW, Ugoji C, Abimiku A, Nkengasong J, Dakum P, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS Rev. 2015;17(1):3-20.
28. FDRE. National Guideline for Comprehensive HIV Prevention, Care and Treatment Ethiopian Federal Ministry of Health, Addis Ababa, Ethiopia. 2014.
29. HAPCO. Guidelines for implementation of the antiretroviral therapy programme in Ethiopia; . Available from: http://www.etharc.org/index.php/resources/download/finish/40/235. 2007.
30. QIAGEN. QIAamp® Viral RNA Mini Handbook For purification of viral RNA from plasma, serum, cell-free body fluids, and cell-culture supernatants. 2014.
31. Kaur P, Khong WX, Wee SY, Tan EL, Pipper J, Koay E, et al. Clinical evaluation of a low cost, in-house developed real-time RT-PCR human immunodeficiency virus type 1 (HIV-1) quantitation assay for HIV-1 infected patients. PLoS One. 2014;9(3):e89826.
32. Mekuria LA, Nieuwkerk PT, Yalew AW, Sprangers MA, Prins JM. High level of virological suppression among HIV-infected adults receiving combination antiretroviral

therapy in Addis Ababa, Ethiopia. Antivir Ther. 2016;21(5):385-96.
33. Derseh BT, Shewayerga B, Dagnew Mekuria A, Admasu Basha E. Virological Treatment Failure Among Adult HIV/AIDS Patients from Selected Hospitals of North Shoa Zone, Amhara Region, Ethiopia. Infect Drug Resist. 2020;13:4417-25.
34. Fentie Wendie T, Workneh BD. Prevalence and Predictors of Virological Failure Among Adults Living with HIV in South Wollo Zone, Northeast Ethiopia: A Retrospective Cohort Study. HIV AIDS (Auckl). 2020;12:393-402.
35. Negash H, Welay M, Legese H, Adhanom G, Mardu F, Tesfay K, et al. Increased Virological Failure and Determinants Among HIV Patients on Highly Active Retroviral Therapy in Adigrat General Hospital, Northern Ethiopia, 2019: Hospital-Based Cross-Sectional Study. Infect Drug Resist. 2020;13:1863-72.
36. Meriki HD, Tufon KA, MH A. Immuno-haematologic and virologic responses and predictors of virologic failure in HIV-1 infected adults on first-line antiretroviral therapy in Cameroon. Infect Dis Poverty. 2014;3(1):5.
37. Hassan AS, Nabwera HM, Mwaringa SM, et al. HIV-1 virologic failure and acquired drug resistance among first-line antiretroviral experienced adults at a rural HIV clinic in coastal Kenya: a cross-sectional study. AIDS Res Ther. 2014;11(9).
38. Ramadhani HO TN, Landman KZ, et al. . Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania Clin Infect Dis. 2007;45(11):1492-8.
39. Ndahimana J, Riedel DJ, Mwumvaneza M, Sebuhoro D, Uwimbabazi JC, Kubwimana M, et al. Drug resistance mutations after the first 12 months on antiretroviral therapy and determinants of virological failure in Rwanda. Trop Med Int Health. 2016;21(7):928-35.
40. von Braun A, Sekaggya-Wiltshire C, Scherrer AU, Magambo B, Kambugu A, Fehr J, et al. Early virological failure and HIV drug resistance in Ugandan adults co-infected with tuberculosis. AIDS Res Ther. 2017;14(1):1.
41. Rebouças M, da Silva M, Haguihara T, Brites C, Netto E. Tuberculosis incidence among people living with HIV/AIDS with virological failure of antiretroviral therapy in Salvador, Bahia, Brazil. Braz J Infect Dis 2017;21(5).
42. McIlleron H MG, Burman WJ, Maartens G. Complications of antiretroviral therapy in patients with tuberculosis: drug interactions, toxicity, and immune reconstitution inflammatory syndrome. . J Infect Dis. 2007;196(Suppl.):S63-75.
43. Ahoua L GG, Pinoges L, et al. Risk factors for virological failure and subtherapeutic antiretroviral drug concentrations in HIV-positive adults treated in rural northwestern Uganda. BMC Infect Dis 2009;9(81).
44. Singh A AA, Chakravarty J, kumari S, Rai M, Sundar S. Predictive markers of failure of first line anti retroviral treatment in HIV patients in India. J AIDS Clin Res. 2013(4).
45. Hailu GG, Hagos DG, Hagos AK, Wasihun AG, Dejen TA. Virological and immunological failure of HAART and associated risk factors among adults and adolescents in the Tigray region of Northern Ethiopia. PLoS ONE. 2018;13(5): e0196259.
46. Ayalew MB, Kumilachew D, Belay A, Getu S, Teju D, Endale D, et al. First-line antiretroviral treatment failure and associated factors in HIV patients at the University of Gondar Teaching Hospital, Gondar, Northwest Ethiopia. HIV AIDS 2016;8:141-6.
47. Jobanputra K, Parker LA, Azih C, Okello V, Maphalala G, Kershberger B, et al. Factors associated with virological failure and suppression after enhanced adherence counselling, in children, adolescents and adults on antiretroviral therapy for HIV in Swaziland. PLoS One. 2015;10(2):e0116144.
48. Bisetegn G, Arefaynie M, Mohammed A, Fentaw Z, Muche A, Dewau R, et al. Predictors of Virological Failure after Adherence-Enhancement Counseling among First-Line Adults Living with HIV/AIDS in Kombolcha Town, Northeast Ethiopia. HIV AIDS (Auckl) 2021;26(13):91-7.
49. Negash H LH, Tefera M, Mardu F, Tesfay K, Gebresilasie S, et al. The effect of tuberculosis on immune reconstitution among HIV patients on highly active antiretroviral therapy in Adigrat general hospital, eastern Tigrai, Ethiopia; 2019: a retrospective follow upstudy. . BMC immunology. 2019;20(1):45.
50. Zeleke A. Prevalence of antiretroviral treatment failure and associated factors in HIV infected children on HAART at Gondar University Hospital, retrospective cohort study. International J Med Med Sci. 2016;8(11):125-32.
51. Sang RKA MF. Factors Associated with Virological Failure amongst Adults on Antiretroviral Therapy in Nyanza Region, Kenya. IOSR-JDMS. 2016;15(7):108-21.
52. Teshome YY, Yalew AW. Magnitude and predictors of antiretroviral treatment failure in private health facilities in Addis Ababa, Ethiopia. PloS One. 2015;10(5):e0126026.
53. Heestermans T, Browne JL, Aitken SC, Vervoort SC, Klipstein- Grobusch K. Determinants of adherence to HAART among HIV-positive adults in sub- Saharan Africa: a systematic review BMJ Global Health. 2016;1(:e000125).
54. Hassan AS, Nabwera HM, Mwaringa SM, Obonyo CA, Sanders EJ, Rinke de Wit TF, et al. HIV-1 virologic failure and acquired drug resistance among first-line antiretroviral experienced adults at a rural HIV clinic in

coastal Kenya: a cross-sectional study. AIDS Res Ther. 2014;11(1):9.

55. Fang G, Burger H, Grimson R, Tropper P, Nachman S, Mayers D, et al. Maternal plasma human immunodeficiency virus type 1 RNA level: a determinant and projected threshold for mother-to-child transmission Proc Natl Acad Sci 1995;92(26):12100–4.
56. Mofenson LM, Lambert JS, Stiehm ER, Bethel J, Meyer WA, Whitehouse J, et al. Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. Pediatric AIDS clinical trials group study 185 team N Engl J Med. 1999;341(6):385–93.
57. Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections Science. 1997;276(5320):1871-61.