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Persistent Low Level Viraemia In Antiretroviral Treated Patients: Associated Factors and Virologic Outcome Among HIV-1 Infected Patients at Nyakach County Hospital

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ABSTRACT

Purpose: Antiretroviral treatment (ART) assists patients in controlling their HIV infection and ensuring long-term survival. Despite this advantage, a significant number of HIV-positive individuals do not attain full virologic suppression. The purpose of this research was to determine the prevalence of low-level viraemia, its impact on ART

outcome, and related factors in HIV-1 infected patients receiving ART at Nyakach County Hospital in Kisumu, Kenya.

Patients and Methods: This observational cohort research included HIV-1-infected persons who were registered at Nyakach County Hospital between January 2005 and February 2018 and were receiving WHO-recommended antiretroviral therapy (ART) regimens and viral load monitoring. Persistent low-level viremia is defined in the 2018 Kenya ART guidelines as having a detectable VL (above the low detectable threshold) but less than 1,000 copies/mL on two or more consecutive tests. The World Health Organization's (WHO) recommendations for low and middle-income countries (LMIC) define virologic failure as plasma HIV-RNA concentrations more than 1000 copies/mL. The outcomes of this study were viral failure and its related factors. Cox proportional hazard models were used to assess risks.

Results: The analysis comprised 738 individuals on first-line antiretroviral therapy, of whom 81 encountered virologic failure and 657 did not. The median duration of ART in the virologic failure group was 6.6 years (IQR 3.5–9.5), while in the non-failure group it was 7.2 years (IQR 4.4–9.7). Increased likelihood of virologic failure was related with higher levels of low-level viraemia (hazard ratio [HR] 9.87, 95% confidence interval [CI] 4.32-22.54; p0.001) compared to virological suppression of fewer than 50 copies/mL. A longer duration of antiretroviral therapy was related with a 38% reduction in the probability of virologic failure (hazard ratio [HR] 0.62, 95% confidence interval [CI] 0.55-0.68; p0.001). Low-level viraemia occurred at a rate of 35.4 per 100 person years of follow-up.

Conclusion: Low-level viremia of 500–999 copies/mL at baseline is linked with an increased probability of virologic failure during follow-up in this group.

Keywords: *Viraemia, Antiretroviral therapy, HIV, Kenya*

Background

The widespread use of highly active antiretroviral treatment (ART) among people living with HIV/AIDS (PLWH) has resulted in a considerable decrease in HIV-related morbidity and mortality globally [1] and a rise in life expectancy. As a consequence, HIV has been converted from a lethal progressing infection into a chronic manageable condition [2]. By the end of 2018, around 37.9 million individuals worldwide were HIV positive, the overwhelming majority of whom lived in low- and middle-income countries, mainly in Sub Saharan Africa. Around 770,000 individuals died of AIDS-related causes in the same year, while 1.7 million people were newly infected. In 2018, 36.2 million persons living with HIV were adults and 1.7 million were children under the age of 15 years. By the end of 2018, it was projected that over 1.5 million persons in Kenya were living with HIV and 1.0 million were receiving antiretroviral therapy [1].

Antiretroviral therapy's primary goal is to reduce and sustain viral load to undetectable levels (suppress viral replication) and to enhance immunity (CD4 levels) in such a way that severe HIV-related illness is improbable. [2]. Despite these advantages, a significant proportion of patients do not have a sustained virological response to antiretroviral therapy[3] and hence face treatment failure. While some individuals receiving antiretroviral therapy achieve optimal viral suppression, defined broadly as a viral load that remains consistently below the detection level, typically less than 50 copies/mL, depending on the assay used to measure viral load [4], others do not, and some individuals also experience transient detectable HIV RNA levels (viral blips). After at least 12 months on ART, a significant number of persons who do not achieve maximal viral

suppression may retain persistent low level viraemia defined by HIV RNA levels of fewer than 1000 copies/mL on two or more consecutive assessments. The predictive usefulness of low-level viremia and the optimal clinical treatment remain unknown. This is because there is no conclusive evidence that patients with viral loads fewer than 200 copies per mL are more likely to have virologic failure. [5]. Persistent low-level viraemia is caused by HIV particles being released from latently infected cells or by viral replication continuing [2,6].

There is a dearth of data on the virologic fate of patients with chronic low-level viremia, and so the threshold of viremia at which the risk of virological failure becomes considerable remains a point of contention. Additionally, there is variation in the definitions of low level viraemia and virologic failure between studies [5], making it difficult to interpret the findings of investigations. Definitions that are consistent with research findings are not necessarily appropriate to actual practice. [5]. The World Health Organization applies a less strict definition of virologic failure, requiring a viral load of >1000 copies per mL on two occasions before recommending a transition to alternative antiretroviral therapy [7]. Additionally, most research on low-level viremia have been conducted in high-income countries with improved HIV treatment and management. Given the expansion of HIV treatment programs in Sub Saharan Africa and Kenya in general, it is critical to understand the relationship between persistent detectable low-level viremia and virologic failure, particularly in resource-poor settings with limited ART alternatives. Improved reporting and assessment of viral outcomes across a broader variety of settings is necessary to assist efforts to enhance HIV care and treatment [3].

Documented factors that can possibly contribute to persistent low level viraemia hence lead to increased risk of virological failure include ongoing viral replication at sanctuary sites, viral mutations hence primary infection with drug resistant strains of HIV, patient factors such as poor adherence, certain ART regimen combinations, age, high baseline plasma viral load, low baseline CD4 count, drug –drug interactions, toxicity and HIV WHO stage at ART initiation[8,9]. When ART is not monitored well, patients who experience virologic failure may develop drug resistance mutations which

may compromise future treatment options [10] and result in increased morbidity and mortality. As a practise, patients failing first line therapy are switched to second line therapy to suppress viral load [11]. Those who fail second line therapy have limited options left as agents used to construct salvage third line regimen are more expensive, with increased pill burden and more side effects [12]. Thus, there is need for monitoring to achieve complete virologic suppression and as a result prevent development of treatment failure.

In line with the above facts, this study assessed the effect of four categories of persistent low level viraemia (defined as HIV RNA levels less than 50 copies per mL, 50–199 copies per mL, 200–499 copies per mL and 500–999 copies per mL) on treatment outcome as well as associated factors among antiretroviral treated HIV-1 infected patients at Nyakach County Hospital, Kisumu, Kenya.

Methodology

Study Design and Participants

This was a retrospective cohort study, in which HIV-1 infected adults, (≥18 years) who experienced low level viraemia and on ART between January 2005 and February 2018, at Nyakach County Hospital, Kisumu, Kenya were studied to determine the occurrence and effect of persistently low level viraemia on treatment outcome and as well as associated factors. The patients in this set up receive WHO aligned ART regimen; first-line ART consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI), and those switched to second-line ART receive regimen consisting of two NRTIs and a ritonavir-boosted protease inhibitor (PI). Virological monitoring during ART involves a first viral load measurement at 6 months and 12 months after initiation of treatment, and 12-monthly

measurements thereafter. Virological failure was defined as a viral load of >1000 copies/mL, confirmed by a second viral load within 3 months interval with adherence support in between. Most viral load results were generated by use of same assay that have quantification cut off of < 50 copies/mL. Data was collected from medical records and included demographic characteristics, ARV-treatment history (date positive, date initiated on ART, regimen, dates of regimen change if any), adherence. HIV stage at initiation based on WHO criteria, HIV-viral load measurements at occurrence of persistent low level viraemia and at the last test of persistent low-level viraemia. Adherence levels were determined using Morisky Medication Adherence Scale-4 records and information in the patients' medical records. Patients were included in the study at the date of first HIV RNA measurement after completing at least 12 months on ART and demonstrating low-level viraemia (HIV RNA level at detectable level of less than 1000 copies per mL) on at least two consecutive visits. All patients on ART who met the criteria for virologic failure (WHO definition), defined as two consecutive HIV RNA values >1000 copies per mL at this time point were excluded from the study. All patients were followed until the date of the last RNA measurement available. Because of the observational nature of this study using previously collected and anonymised data, individual informed consent was not required. Permission for the use of anonymised data was approved by Nyakach County Hospital Medical Superintendent. Approval to conduct this study was given by post graduate board of JOOUST and ethical approval by ethical review board of JOOUST. Confidentiality of information was maintained through use of anonymised data.

Stratification of Patients

During follow-up, patients were classified into four groups based on their peak viral load: 1) Permanently suppressed viremia (PSV), defined as one or more RNA values of

50 copies/mL (including cases with isolated HIV RNA measurements of 50 copies/mL followed by a continuously suppressed value without treatment change); 2) persistent LLV of 50-199 copies/mL (LLV1), defined as one or more consecutive RNA values between 50-199 copies/mL; and 3) persistent LLV 200-499 copies/mL (LLV2), defined as two or more consecutive HIV RNA values ≥50 copies per mL, with at least one value between 200 and 499 copies/mL; 4) persistent LLV 500-999 copies per mL (LLV3), defined as two or more consecutive HIV RNA values ≥50 copies per mL, with at least one value between 500 and 999 copies per mL. Further, patients were divided into two groups: those who had virologic failure and those who did not.

Data Processing and Analysis

The data was put into an Excel sheet and analysed using the SPSS 23.0 software (IBM SPSS, Inc., Chicago, IL, USA). Frequencies and percentages were used to represent baseline attributes and categorical data. For variables with or without normal distribution, continuous data were presented as means, standard deviations (SD), or medians and ranges, as indicated in table 1.

The major outcome measure for individuals receiving antiretroviral therapy (ART) was virological failure (defined as at least one viral load test of 1000 copies/mL). Secondary outcomes for patients receiving first-line ART were confirmed virological failure (defined as two or more consecutive viral load measurements of 1000 copies/mL within a three-month interval without re-suppression on first-line ART) and transition to second-line ART. Low-level viraemia was defined as the presence of at least two consecutive viral load readings of 51–999 copies/mL during antiretroviral therapy (ART). Low-level viraemia was classified as having a copy number of 50–199, 200–499, or 500–999.

The cumulative incidence of virologic failure was calculated using Cox regression, and statistically significant variations in the incidence of virologic failure across exposure statuses were determined using logistic regression. Patients related to two research outcomes: those who had virologic failure and those who did not. The duration of ART was determined from the time of commencement to the final follow-up in the non-failure group and from the time of virologic failure to the last follow-up in the failure group.

Univariate and multivariate Cox proportional hazard regression model analysis were used to determine predictors of virologic failure. Viral load data were analyzed as time-dependent variables in these models, allowing for independent evaluation of each interval between two viral loads, the first reflecting the predictor status and the second indicating the dichotomous outcome state. The results were presented in logistic regression as hazard ratios (HRs) indicating the relative probability of an outcome for each range of low-level viraemia compared to virological suppression of fewer than 50 copies per mL. Subjects were followed until virologic failure occurred or, in the case of censored observations, until the most recent visit with available viral load measurements. At the visit following the incidence of persistence as stated above, participants were enrolled into the survival analysis. Patients who had repeated episodes of low-level viraemia were categorised according to their highest result. Once a patient achieved a higher level of LLV persistence, he stayed at that level for the duration of the studies. Multivariate modeling was used to account for potential confounders, with the 10% change in estimate method (variables included that changed the hazard ratios [HRs] for the association between LLV and virologic failure by 10%) among the following variables: age, sex, residence, marital status, duration of ART, adherence, opportunistic infections, duration of persistence, and type of regimen. The significance threshold was set to P0.05 and the confidence interval at 95%.

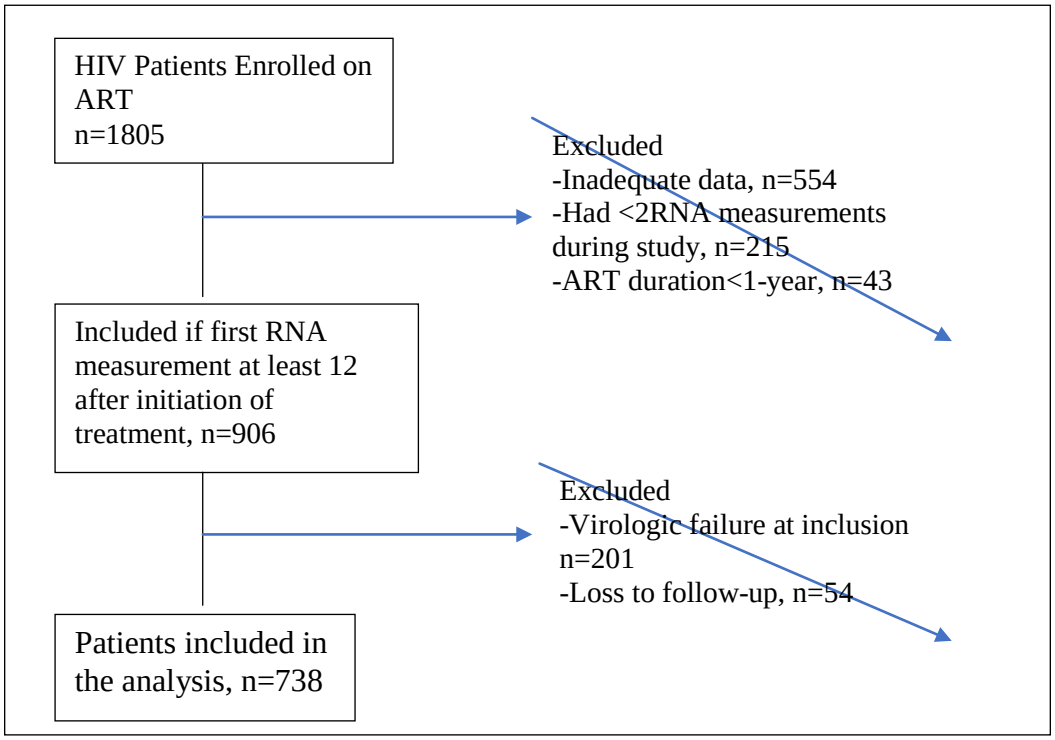

Figure 1: Flowchart of patients at Inclusion and Exclusion

RESULTS

Characteristics of Patients Included in The Study

Data from 738 HIV-1 infected patients attending Nyakach County Hospital comprehensive care clinic were obtained after application of inclusion criteria and included in the analysis. The Mean ± SD age of all patients was 37.8 ± 11.2 years and 27.4% of patients were male. The median duration of ART was 7.2 years (IQR 3.8-8.9). The patients were categorized in two groups, virologic failure and non-failure group. Of the 738 patients, 81 (10.9 %) developed virologic failure (one or more viral

load measurement of ≥1000 copies per mL) and 657 (89.1%) did not develop virologic failure. The patients' characteristics are described and compared as shown in Table 1.

Table 1: Characteristics and virologic outcomes of included patients

		Virologic Failure	
Characteristic	**All(n=738)**	**Yes(n=81)**	**No(n=657)**
Age(years)mean ±SD	37.8±11.2	37.8±11.6	37.8±11.2
Gender			
Male	202(27.4%)	22(27.2%)	180(27.4%)
Female	536(72.6%)	59(72.8%)	477(72.6%)
Previous history of opportunistic infection			
Yes	41(5.6%)	4(4.9%)	37(5.6%)
No	697(94.4%)	77(95.1%)	62(94.4%)
Marital status			
Married monogamous	369(50%)	38(46.9%)	331(30.4%)
Married polygamous	129(17.5%)	15(18.5%)	114(17.4%)
Widowed	180(24.4%)	21(25.9%)	159(24.2%)
Divorced	17(2.3%)	0	17(2.6%)
Single	43(5.8%)	7(8.6%)	36(5.5%)
Residence			
Central Nyakach	252(34.1%)	34(42%)	218(33.2%)
North Nyakach	207(28%)	15(18.5%)	192(29.2%)
West Nyakach	203(27.5%)	27(33.3%)	176(26.8%)
South West Nyakach	26(3.5%)	2(2.5%)	24(3.7%)
South East Nyakach	31(4.2%)	2(2.5%)	29(4.4%)
Other	19(2.6%)	1(1.2%)	18(2.7%)
Calendar year of start of ART			
<2010	249(33.7%)	41(50.6%)	208(31.7%)
2010-2013	231(31.3%)	19(23.5%)	212(32.3%)
>2013	258(35%)	21(25.9%)	237(36.1%)
Initial First line Regimen			
D4T/3TC/NVP	263(35.6%)	35(43.2%)	228(34.7%)
D4T/3TC/EFV	4(0.5%)	1(1.2%)	3(0.5%)
TDF/3TC/EFV	244(33.1%)	17(21%)	227(34.6%)
TDF/3TC/NVP	169(22.9%)	16(19.8%)	153(23.3%)
AZT/3TC/NVP	54(7.3%)	11(13.6%)	43(6.5%)
AZT/3TC/EFV	4(0.5%)	1(1.2%)	3(0.5%)
Viraemia on inclusion			
SV(<50cpm)	573(77.6%)	516(69.9%)	57(7.7%)
LLV1(50-199cpm)	90(12.2%)	83(11.2%)	7(0.9%)

LLV2(200-499cpm)	56(7.6%)	48(6.5%)	8(1.1%)
LLV3(500-999cpm)	19(2.6%)	10(1.4%)	9(1.2%)
Adherence			
Good (>95%)	719(97.4%)	73(90.1%)	646(98.3%)
Fair (81-94%)	14(1.9%)	5(6.2%)	9(1.4%)
Poor (<80%)	5(0.7%)	3(3.7%)	2(0.3%)
Duration of ART (years)median (IQR)	7.2(4.3-9.7)	6.6(3.7-9.5)	7.2 (4.4-9.7)

Abbreviations: IQR, interquartile range; SD, standard deviation, D4T,Stavudine;NVP;SV,suppressed viraemia Nevirapine;3TC, Lamivudine; TDF, Tenofovir, EFV, Efavirenz; AZT; Zidovudine, HIV, Human immunodeficiency virus; ART, antiretroviral therapy

Occurrence of Persistent Low Level Viraemia

Figure 2 represents how the patients changed viraemia profiles at inclusion and overtime during the study. The totals decreased overtime per year based on the years of follow up available for each individual patient because some patients entered the study at different time periods and duration. The occurrence of persistent low level viraemia overtime was determined by incidence rate per person years follow-up. Low level viraemia (HIV RNA 51-999cpm) was recorded in 165 (23%) of the patients at the time of study inclusion. Incidence of persistent low-level viraemia was 35.4 per 100 person-years of follow-up. The viraemia profiles changed over time per year as summarized in figure 2.

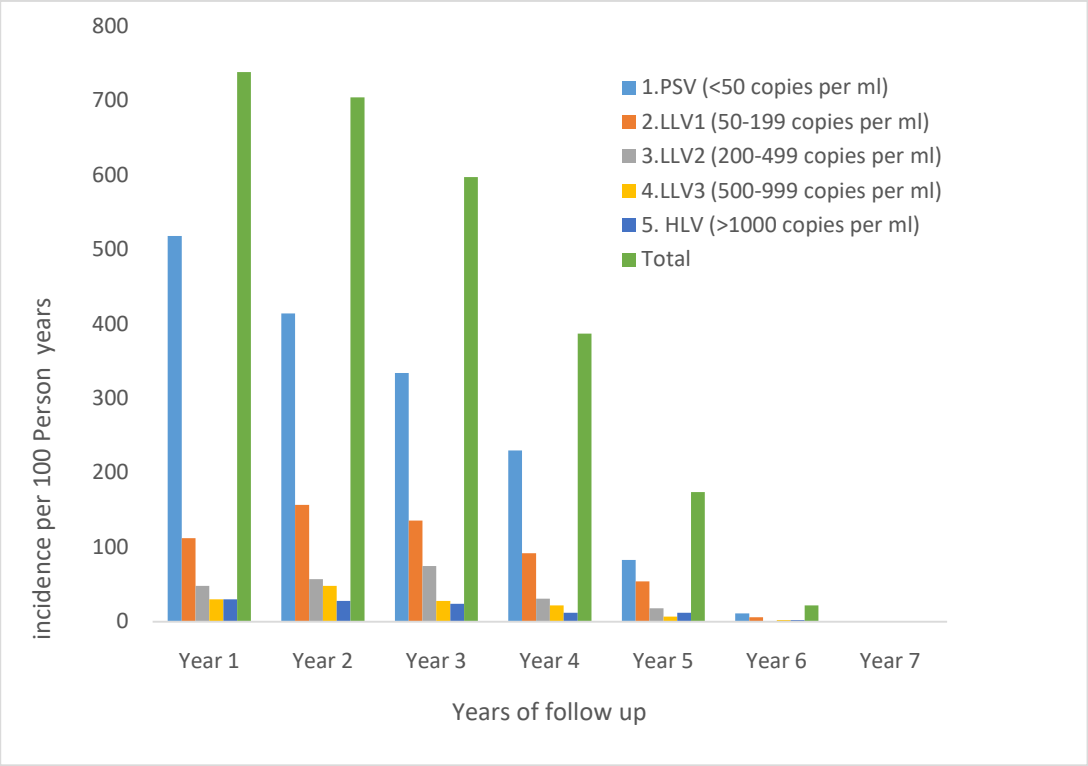

Figure 2: Incidence of low level viraemia and viraemia higher than1000 copies per mL

Abbreviations: PSV-persistently suppressed viremia; LLV1-low-level viremia 50-199 copies/mL; LLV2-low-level viremia 200-499 copies/mL; LLV3-low-level viremia 500-999 copies/mL HLV-high-level viremia.

Viremia Profiles at Inclusion and Proportion of Virologic Failure During Follow-up.

The 738 patients included in the study were in the following categories at the time of study inclusion; 573 (77.6%) had suppressed viraemia (HIV RNA levels <50 copies/mL). 90 (12.2%) had LLV1 (HIV RNA levels 50-199 copies/mL), 56 (7.6%) had LLV2 (HIV RNA levels 200-499 copies/mL) and 19 (2.6%) had LLV3 (HIV RNA levels 500-999 copies/mL). Thus, a total of 165(23%) had low level viraemia at study inclusion. During follow-up, the 573 patients with suppressed viraemia changed their viraemia categories as follows; 158 (27.6%) had suppressed viraemia, 181 (31.6%), changed to LLV1, 107 (18.7%) to LLV2, 70 (12.2%) changed to LLV3 and 57 (9.9%) experienced higher-level viremia. Progression of viremia strata during follow-up is shown in table 2. Proportions of subjects who progressed to virologic failure increased

further with higher ranges of persistent low level viraemia (14.3% for LLV2 and 47.4% for LLV3 versus 9.9% for suppressed viraemia). However, the proportion of subjects in LLV1 who progressed to virologic failure was low compared to supressed viraemia (7.8% versus 9.9%). During the study, of the 738 patients, 81 (10.9%) experienced virologic failure. Among the 81 patients with virologic failure, 39(48.1%) achieved virologic suppression (HIV RNA <50 copies/mL) without change of therapy and 7(8.6%) had confirmed virologic failure and a change of therapy to second line ART. This is as summarized in table 2.

Table 2. Viraemia Profiles and Virologic Failure during the Follow up

Baseline Category	**Maximum Category during Follow-up (copies/mL)**				
	<50	50-199	200-499	500-999	>1000
1. <50cpm 573 (77.6%)	158(27.6%)	181(31.6%)	107 (18.7%)	70 (12.2%)	57 (9.9%)
2. 50-199 cpm 90(12.2%)	19 (21.1%)	27 (30%)	18 (20%)	19 (21.1%)	7 (7.8%)
3.200-499 cpm 56 (7.6%)	14 (25%)	13 (23.2%)	12 (21.4%)	9 (16.1%)	8 (14.3%)
4. 500-999 cpm 19 (2.6%)	3 (15.8%)	3 (15.8%)	2 (10.5%)	2 (10.5%)	9 (47.4%)
Total	194 (26.3%)	224 (30.4%)	139 (18.8%)	100 (13.6%)	81 (11%)
Virologic failure	**Last available Viral Load Category (copies/mL)**				
	<50	**50-199**	**200-499**	**500-999**	**>1000**
5. >1000 cpm (81)	39 (48.1%)	24 (29.6%)	8 (9.9%)	3(3.7%)	7(8.6%)

Table 4.2: Table represents viremia categories at inclusion (baseline), change of categories during the follow-up time and the last available viral load. Reclassification was only made to higher viremia strata, and the reclassified subjects remained in that strata for the remaining follow-up period (unless progression to a higher viremia stratum occurred).

Association between Persistent viraemia category and Virologic Failure

The Cox model showed that LLV2 (HIV RNA levels 200-499 copies per mL) and LLV3 (HIV RNA levels 500-999 copies per mL) was associated with a significantly elevated risk of virological failure, hazard ratio (HR) 2.34 (95% CI 1.11-4.93, p=0.026) and hazard ratio (HR) 9.30 (95% CI 4.54-19.05, p<0.0001) respectively (Table 4.3). However, in multivariate analysis after adjusting for potential confounders, only LLV3 (HIV RNA levels 500-999 copies per mL) was associated with a significantly elevated

risk of virological failure, adjusted hazard ratio (HR) 9.87 (95% CI 4.32-22.54, p=0.001).

Table 3: Univariate and Multivariate analysis of factors associated with virologic failure among the patients included in the study

		Univariate			Multivariate		
Variables	N	HR	(95% CI)	P-value	HR	(95% CI)	P-value
Viremia Category							
1.<50RNA copies/mL	573	Ref			ref		
2.50-199 RNA copies/mL	90	1.17	(0.53-2.59)	0.692	1.28	(0.56-2.93)	0.553
3.200-499 RNA copies/mL	56	2.34	(1.11-4.93)	**0.026**	2.08	(0.94-4.59)	**0.070**
4.500-999RNA copies/mL	19	9.30	(4.54-19.05)	**<0.0001**	9.87	(4.32-22.54)	**<0.001**
Age		0.99	(0.98-1.02)	0.935	1.02	(0.99-1.04)	0.172
Gender							
1=Male	202	Ref					
2=Female	536	1.03	(0.63-1.69)	0.897	1.22	(0.68-2.18)	0.514
Marital Status							
1=Married monogamous	369	Ref			ref		
2=Married polygamous	129	1.43	(0.78-2.62)	0.247	1.29	(0.66-2.52)	0.461
3=Widowed	180	1.13	0.66-1.93)	0.651	0.99	(0.53-1.88)	0.994
4=Divorced	17	NA	(0.00---)	1.00	NA	(0.00--)	NA
5=Single	43	1.86	(0.83-4.16)	0.133	1.85	(0.75-4.55)	0.179
Residence							
1=Central Nyakach	252	Ref			ref		
2=North Nyakach	207	0.52	(0.28-0.95)	0.033	0.70	(0.37-1.32)	0.273
3=West Nyakach	203	1.01	(0.61-1.68)	0.959	1.02	(0.85-2.40)	0.184
4=South West Nyakach	26	0.56	(0.14-2.35)	0.431	0.50	(0.12-2.22)	0.365
5=South-East Nyakach	31	0.47	(0.11-1.97)	0.302	0.56	(0.13-2.42)	0.441
6=Other	19	0.43	(0.06-3.15)	0.401	0.53	(0.07-3.98)	0.534
WHO stage							
Stage I	232	Ref			ref		
Stage II	367	1.03	(0.59-1.78)	0.924	1.23	(0.68-2.25)	0.493
Stage III	135	1.29	(0.69-2.44)	0.423	2.57	(1.22-5.43)	0.013
Stage IV	4	9.33	(2.16-40.26)	**<0.003**	22.26	(4.19-107.90)	**<0.001**
BMI		0.99	(0.93-1.05)	0.787	0.99	(0.99-1.01)	0.681
Initial Regimen							
1=D4T3TCNVP	263	Ref			ref		
2=D4T3TCEFV	4	1.36	(0.19-9.99)	0.761	3.81	(0.45-31.93)	0.281
3=TDF3TCEFV	244	1.13	(0.62-2.09)	0.688	0.16	(0.04-0.58)	**0.005**
4=TDF3TCNVP	169	0.97	(0.53-1.78)	0.918	0.28	(0.13-0.59)	**0.001**
5=AZT3TCNVP	54	2.25	(1.23-4.50)	**0.022**	1.52	(0.60-3.82)	0.374
6=AZT3TCEFV	4	2.69	(0.37-19.74)	0.331	4.85	(0.29-79.89)	0.270
Adherence							
1=Good	719	Ref			ref		
2=Fair	14	3.79	(1.53-9.42)	**0.004**	1.76	(0.56-5.57)	0.383
3=Poor	5	7.12	(2.24-22.67)	**0.001**	0.92	(0.23-3.76)	0.912

Opportunistic Infections							
1=No	697	Ref			ref		
2=Yes	41	0.88	(0.32-2.44)	0.808	0.99	(0.32-3.07)	0.990
Duration of ART		0.78	(0.71-0.86)	**<0.0001**	0.62	(0.55-0.68)	**<0.001**

Factors Associated with Virologic Failure.

In Univariate and Multivariate analysis of factors associated with virologic failure among patients with persistent low level viraemia, there were no significant differences in age, gender, marital status, residence and history of opportunistic infections between the two groups as shown in Table 2. However, adherence, WHO classification, and duration of ART differed significantly between the two groups. The date of HIV diagnosis varied from 2005 to 2018.

In multivariate cox proportional hazard model analysis, after adjusting for potential confounders, viraemia category at the start of the study and adherence and WHO stage was significantly associated with virologic failure. Those in LLV3 (HIV RNA levels 500-999copies per mL) were 9.87 times more likely to experience virologic failure compared to those with PSV (HR 9.87 [95% CI 4.32-22.54]), P <0.0001]). Those in WHO stage 4 were 22.26 times more likely to experience virologic failure compared to WHO stage 1, (HR 22.26 [95% CI 4.19-107.90), P <0.001]). Adherence also played a key role in virologic failure. There was increased hazard of virologic failure among those who experienced fair and poor adherence, hazard ratio (HR) 3.79 [95% CI 1.53-9.42, p<0.004]) and hazard ratio (HR) 7.12 [95% CI 2.24-22.67, p=0.001]) respectively compared to those with good adherence. However, in multivariate analysis, after adjusting for potential confounders, adherence level was not significantly related with virologic failure. Duration of ART was also significantly related with virologic failure. (Table 4.3). The probability of having increased risk of virologic failure increased with

higher levels of persistent low level viraemia. The figures 4.2 and 4.3 shows graphical representations of cox regression analysis of virologic failure.

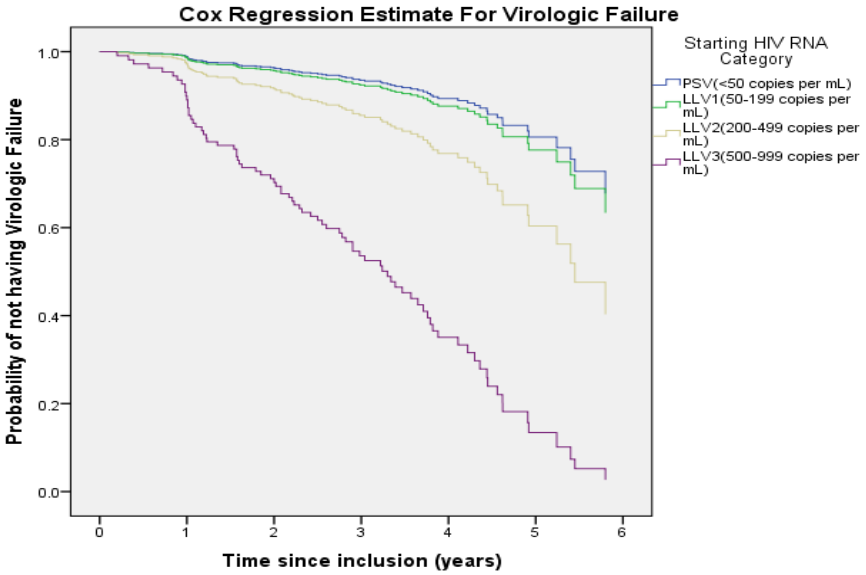

Figure 3: Cox estimate for virologic failure

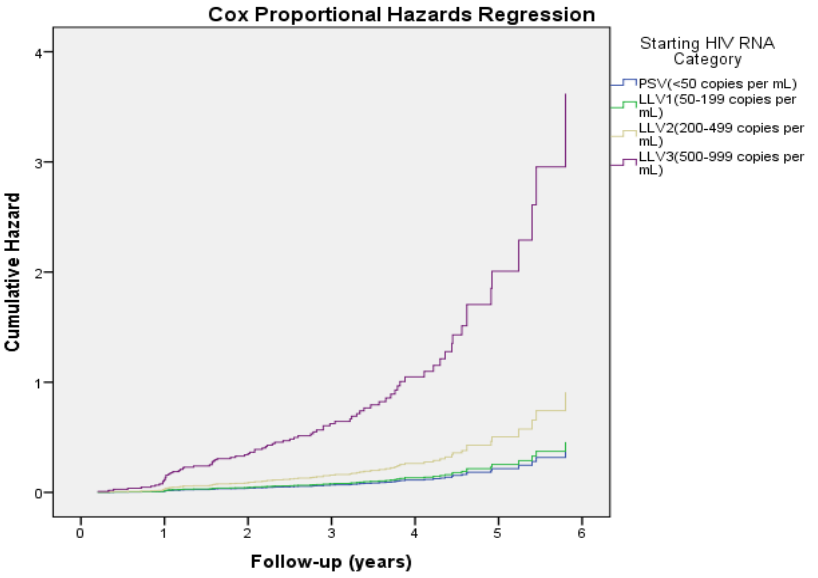

Figure 4: Cox proportional hazards regression analysis of virologic failure

DISCUSSION

This research examined the consequences of low-level viraemia in HIV-1-infected individuals receiving ART according to WHO recommendations in a resource-limited context. Overall, 10.9% of patients had virologic failure. This finding was consistent with that of prior research conducted in Uganda, which found that 11% of patients exhibited virologic non-suppression [8]. However, Herman et al discovered that virological failure occurred in 14380 (22%) of 69454 patients receiving first-line ART throughout follow-up. The percentage difference might be explained by changes in the research population, since this study employed a bigger cohort [13]. When compared to stably suppressed viraemia (HIV RNA 50 copies per mL), a baseline low-level viraemia of 500-999 copies per mL was observed to be linked with an increased likelihood of eventual virological failure during follow-up. The probability of virologic failure was substantially increased in univariate analysis but not in multivariate analysis for individuals with LLV2 in the lower range (200-499 copies per mL). This risk was not substantially increased in persons classified in the lower range of LLV1 (50-199 copies per mL). This is consistent with findings from a large cohort study in South Africa indicating the probability of virological failure rose further with increasing ranges and persistence of low-level viraemia [13]. A Swedish Cohort Study also discovered an increased risk of virologic failure in patients with viraemia greater than 200 copies per mL (LLV 200-999 copies per mL) (adjusted hazard ratio 3.14 [95% confidence interval 1.41-7.03, p0.01]), but not in those with viraemia between 50 and 199 copies per mL (1.01 [0.34-4.31, p = 0.99]; median follow-up 4.5 years [14]. Similarly, a prior research has shown that individuals with persistent low-level viremia of more than 400 copies per mL had a poorer prognosis than those with detectable viral replication at lower levels (HR 3.8, 95% CI 2.2-6.4; p0.001). [15]. In comparison, Laprise et al. identified an increased probability of viral failure in individuals with LLV levels of 50–200 copies

per mL. (HR 2.2, 95% CI 1.60-3.09) [16]. It is likely that the likelihood of virologic failure has decreased in recent years, probably as a result of improved patient monitoring and the use of more effective and better tolerated medication. Individuals with persistently detectable viral load, on the other hand, may be at a greater risk of virologic failure even if the absolute amount of viral load is in the low range, i.e. HIV RNA levels 50-200 copies/mL. Allowing persons living with HIV to have persistently low levels of viraemia increases the possibility of a gradual but steady build-up of resistance mutations that affect the patient's long-term ART administration and virus control. In this group, low-level viraemia occurred at a rate of 35.4 per 100 person years of follow-up. This was much more than what was found in prior research. [13]. This might be because the overall rate of virologic suppression was lower in this research setting and the South African study included a bigger cohort.

In a sub-analysis comparing people with virologic failure and their most recent available viral load, around fifty percent 39 (48.1%) managed resuppression to permanently suppressed viraemia without changing their regimen, whereas seven (8.6%) had confirmed virologic failure. This data corroborates recent research conducted in Uganda, which showed that 50% of people with apparent treatment failure achieved suppression after repeat testing without changing their medicine [8]. Gupta et al. discovered that one-quarter of persons with viral failure (>1000 copies per mL) at week 48 achieved resupression at 96 weeks [17] without changing their medication. This indicates that low-level viraemia is a common occurrence, is only a poor predictor of virologic failure, and that certain factors are involved. One probable reason is that this degree of viremia resulted in increased adherence to medication, which resulted in improved virological control. WHO recommendations [7] on antiretroviral therapy propose that patients who fail virologically be exposed to adherence support intervention, followed by a second viral load, prior to deciding on a regimen

modification. In this research context, people at risk of having virologic failure get facility-based intervention, including adherence assistance.

Low-level viraemia is becoming more widely recognized. However, its precise prognostic value on treatment outcomes is unknown due to methodological differences in the definitions of low level viraemia and virologic failure used across studies [5,16,18,] as well as differences in the plasma viral load assay used and patient population characteristics such as geographic location, type, and duration of ART. This complicates comparisons across research, posing a problem for appropriate therapy of individuals with low-level viraemia.

In multivariate analysis, the only significant predictors of risk of virologic failure throughout follow-up were a high baseline HIV RNA level of 500-999 copies per mL, HIV WHO stage III or IV, and duration of ART with persistent low-level viremia. Adherence was shown to be statistically significant only in univariate analysis. Age, gender, marital status, opportunistic infections, and residency did not seem to be associated with an increased risk of having virologic failure. Patients in WHO stage IV at the time of therapy initiation have a greater probability of treatment failure than those in stages I-III. An observational cohort research conducted in China [19] indicated that people who began treatment at an advanced stage of HIV were at a greater risk of virologic failure than those who received medication at stage 1. Given the well-established association between lapses in adherence and overt virologic failure, it is advisable to rule out inadequate adherence in all patients with chronic low level viraemia, while acknowledging that lapses in adherence may not be noticed in certain individuals.

As the use of plasma viral load tests with lower quantification limits increases, detection of extremely low level viraemia is increasing. However, the actual incidence rates of extremely low level viraemia are unknown owing to variations in the plasma viral load

test utilized and patient demographic factors such as the type and duration of antiretroviral therapy [20]. Thus, this research defined undetectable viraemia as HIV RNA levels fewer than 50 copies per mL. Management of undetectable viraemia (50 copies per mL) is a relatively recent recognition. There are no prospective studies to guide best practices, and there is no agreement over whether undetectable viraemia necessitates treatment intensification or a change in antiretroviral therapy, given the substantial inter-assay variability around the detection limits of real-time PCR systems. [20]. Low-level viraemia episodes during effective antiretroviral therapy appear to be the result of two distinct processes: clonal outgrowth from long-lived HIV-1-infected cells, presumably following their activation and proliferation, and ongoing viral replication, which includes the selection of new drug-resistant mutants [2,6]. However, Bozzi *et al*., 2019 [21] found no evidence of continuing HIV multiplication or compartmentalization in tissues during ART, but did identify clonal proliferation of already infected cells. Long-term persistence, rather than active replication, is the primary mechanism by which HIV is maintained. When antiretroviral therapy (ART) is commenced, HIV-infected cells present constitute the sole detectable source of persistence and are hence the ideal target for elimination [21]. These virions would not perpetuate infection in the presence of effective antiretroviral therapy (ART), as protease (PR) inhibitors interfere with the maturation of new viruses, rendering them defective and non-infectious, and reverse transcriptase (RT) inhibitors prevent infection of additional cells [6]. Despite this growing body of information, the mechanisms behind chronic low-level viraemia remain unknown.

Antiretroviral resistance is well documented and increases with persistent low-level viraemia (HIV RNA less than 1,000 copies per mL), though the reported prevalence varies according to ART experience, viral suppression/failure, duration of viremia, and treatment regimen [6,22,23.] Significant rates of re-suppression without modifying ART

have been reported in South Africa, even in patients with NNRTI resistance. The majority of cases in this study at risk of virologic failure were due to non-adherence issues. Due to the limitations of current assays, antiretroviral drug resistance in patients with very low viraemia has not been adequately characterized. This leaves those who continue to have persistently low levels of viraemia with the option of closer monitoring, which may include adherence counselling during follow-up. This is demonstrated by the fact that 48% of individuals with high-level viraemia (>1000 copies per mL) who faced confirmed virologic failure achieved viral suppression (HIV RNA 50 copies per mL) without changing their regimen. While the Kenyan ART guidelines [24] recognize that patients with persistent low-level viraemia are at an increased risk of treatment failure, development of ARV resistance, and death and thus require a similar case management approach as patients with VL 1,000 copies per mL, the guideline does not specify specific interventions for managing viraemia below this threshold, and thus any viral load below this threshold is considered treatment success. The study's findings indicate that low levels of viraemia, particularly those between 500 and 999 copies/mL, result in inferior treatment outcomes than effective virologic suppression at 50 copies/mL. As a result, strategies for monitoring those with low levels of viraemia are required.

This study offers both advantages and disadvantages. One of the study's advantages is that chronic low-level viraemia was divided into many groups, enabling the investigation of the impact of different viral load thresholds. This study also used the most recent known (most recent) viral load to determine the maximum viraemia reached during follow-up. This hasn't been properly characterized in previous studies. One limitation is that the participants came from a medical facility, which restricts the results' applicability to the broader public. Additional large-scale research exploring the treatment and therapeutic implications of low-level and undetectable viraemia is

encouraged in the battle against HIV to fill gaps in the knowledge base for patient care. Another limitation is that, because to the lack of pre-treatment viral load, CD4 cell count, and medication resistance test results, this study was unable to determine the mechanisms behind persistent low-level viraemia and their relationship to the likelihood of resistance mutations.

Conclusion

To conclude, the goal of this study was to learn more about the incidence of low-level viraemia and its impact on treatment outcomes, as well as other relevant characteristics. The rate of persistent low-level viraemia was found to be 35.4 per 100 person years of follow-up. Having a higher baseline low-level viraemia of 500-999 copies per mL was linked to a higher risk of virologic failure of >1000 copies per mL during follow-up, according to the findings. The likelihood of virologic failure is associated to adherence, WHO stage, and ART duration. Re-evaluation of the threshold for virologic failure, early identification, and therapeutic options for low-level viraemia should all be included in recommendations to improve treatment efficacy.

DECLARATIONS

Ethics

The study was approved by the Jaramogi Oginga Odinga University Ethics review Committee.

Consent for publication

Not applicable

Availability of data

The data sets used and analysed in this study are available on reasonable request from corresponding author

Competing interests

All authors have declared no competing interests in the submitted work

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Authors' contributions

JOO, DO and SO conceived and designed the study. JOO, DO and SO co-drafted the manuscript. CS critically revised the manuscript. All authors read and approved the final manuscript before submission.

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