Predictors and incidence of HIV viremia and Virologic Failure among HIV-infected Adults on First-Line Antiretroviral Therapy in Eswatini:

A Nested Case–Control Study

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Declaration

I declare that ***Predictors and incidence of HIV viremia and Virologic Failure among HIV-infected Adults on First-Line Antiretroviral Therapy in Eswatini: A Nested Case–Control Study*** is my own work. This work has not been submitted for any degree or examination in any other university and that all the sources and references I have used or quoted have been indicated and acknowledged by complete references.

Full name...**Altaye Habtegiorgis Kidane** ..... Date. September 8, 2021.

Signed.  .....

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Abstract

The global rapid scale up of access to antiretroviral treatment (ART) that has saved millions of lives of individuals infected with HIV through a durable and maximal viral suppression. However, achieving a 95% target of viral suppression to end the HIV epidemic demands close monitoring of clients on first line ART.

A nested case–control study design was used to investigate risk factors for viremia and virologic failure (VF) among HIV infected individuals (18 years and above) on first line ART for one or more years. All individuals initiated on first line ART from January 2015 to December 2019 were identified from the electronic medical records and clients with viremia and VF (cases) and without viremia (controls) were included in the study. The study protocol was reviewed and approved by the University of the Western Cape Biomedical Research Ethics Committee and Eswatini Health and Human Research Review Board. A secondary data from the electronic health information database was imported to an Excel database and exported to SPSS version 27 for analysis after data cleaning procedures.

A nested population of 6129 individuals above the age of 18 years initiated on first line ART regimen were identified; of these 374 had viremia (≥1000copies/mL) and 5755 had no viremia (<1000copies/mL). This sample size was more than the calculated for the study that provided a good statistical power and effect size. Among the study participants 63% were women, 94% were initiated on efavirenz based regimen and the mean and median age of participants were 38 and 36 years respectively and data skewed positively (statistic 0.843 SE0.031).

The baseline CD4 count was below 350cells/µL in 63.7% of participants suggestive of moderate to severe immune deficiency and 53% were on ART for over four years. The mean viral load was significantly lower among females compared to males (p-value=0.009; student t-test=1.3;61df), which also varied by CD4 count for both sexes with a statistically significant (p-value=0.014; F=3.3 df =3) variability for males compared to females. The viral load decreased as the CD4 count increased (p-value=0.006, df 3 F= 4); the adjusted odds ratio of viremia was statistically significant (P value<0.001; CI 0.279-0.826) for different categories of the CD4 count. The mean duration to develop viremia while on DTG, EFV and NVP based regimens were 59, 70 and 69 months respectively.

In conclusion, males had higher probability of developing viremia because of late presentation and the baseline CD4 count was a strong predictor of viremia and VF independent of sex. The BMI, WHO clinical staging, duration on ART and the ART regimen did not predict viremia and VF. The Eswatini HIV treatment program achieved a high virologic suppression although the proportion was less than the 95% target. These findings can be used to monitor progress of the national strategic plan to achieve HIV epidemic control and attainment of the 95–95-95 targets.

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Abbreviations

AOR	Adjusted odds ratio
ART	Antiretroviral therapy
BMI	Body mass index
CD	Cluster of Differentiation
CDC	Center Disease Control
DMT	Data management team
DTG	Dolutegravir
EFV	Efavirenze
FP	Family planning
HIV	Human immune deficiency virus
HMIS	Health management information system
ISTI	Integrase strand transfer inhibitor
MOH	Ministry of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitors
PLHIV	People Living with HIV
PMTCT:	Prevention of mother to child transmission
SD	Standard deviation
UNAIDS	United Nation Joint AIDS program
VF	Virologic failure
WHO	World Health Organization

Definitions of key terms

- **Viremia:** a plasma Viral Load L >1000 copies/mL suggestive of unsuppressed viral load
- **Virologic failure (VF):** Plasma viral load above 1000 copies/ ml based on two consecutive viral load measurements after 3 months, with adherence support. The patient must be taking ART for at least 6 months
- **Treatment failure**: the HIV virus is multiplying in the face of ARVs resulting in immunologic and clinical worsening.

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1. Study Background

This chapter describes ideas for the thesis, as well as the context of the study. The chapter also includes the justification for the study and how the research topic was developed to determine the main research problem that deserved investigation. The chapter clearly shows what research question the thesis hopes to answer or hypothesis it aims to investigate.

1:1 Introduction

Mortality and morbidity from infection with human immune deficiency virus (HIV) have reduced remarkably following the advent of antiretroviral treatment (ART). This reduction is mainly due to the rapid scale up of access to antiretroviral treatment (ART) globally that enrolled millions of individuals infected by HIV. By the end of 2019, 21.7 million individuals were on ART of which 59% were from sub-Saharan Africa (UNAIDS, 2019). This unprecedented public health program was a result of universal access to quality HIV clinical care and the attainment of durable and maximum viral suppression.

The Joint United Nations Program on HIV/AIDS (UNAIDS) and other key stakeholders revised the targets to end the HIV epidemic as a public health threat by 2030 (WHO, 2018). Initially, targets were set to diagnose 90% of HIV infected individuals accompanied by ART initiation for 90% of them and maintain 90% viral suppression by 2020. These targets were later revised to 95/95/95 by 2030 (UNAIDS, 2016).

Viral suppression is vital because HIV viremia can lead to depletion of the CD4 T-cells and development of opportunist conditions, which will be further complicated by regimen switching to second-line ART (Kumar et al., 2016). Viremia, using a public health approach, is defined as viral load results >1000copies/ml; likewise, virologic failure denotes two consecutive plasma viral load results >1000copies/ml after the patient is provided enhanced adherence counseling over 12 weeks. A first time viremia of >1000copies/ml may not necessarily be due to virologic failure (WHO, 2016). In resource rich settings, the definition of HIV viremia is viral load > 50 copies/ml while persistent viremia refers to two successive viral load test result > 50copies/ml (Revell A., et al., 2019).

According to the National ART program data in Eswatini, the proportion of individuals with viral load suppression while on first line ART is over 95% (PEPFAR, 2020). Likewise, the Swaziland's HIV Incidence Measurement Survey-2 (SHIMS-2) data showed a viral suppression rate of 93.3%

among study participants aged 25 years and older who self-reported taking ART (SHIMS, 2019). Routine viral load monitoring is recommended at six months, 12 months and every 12 months then after to assess poor adherence and early ART failure respectively (WHO, 2016). Since April 2017, viral load testing is routinely available for clients enrolled on ART across the country.

Eswatini started providing universal access to ART for all PLHIV in 2010 and the service was accessible in public and private health facilities. The eligibility criteria for ART initiations was revised several times until 2016 when ART initiation strategy called "Test and Start" was implemented regardless of CD4 count. The preferred first line ART regimen was comprised of tenofovir, emitristabine/lamivudine and efavirenz combination until October 2018 when dolutegravir was included as the preferred first line regimen (MOH, 2018).

1:2 Justification of the study

This study maximizes the benefits derived from routinely collected data overtime and using a nested case control research design to answer the research questions thus avoiding a complicated research design from a representative sample, which is usually the case in population based surveys. The routinely collected data from the Health management information system (HMIS) overcomes the need for control groups, which is a requirement in randomized control trails. The time-series nature of HMIS data allow comparison of the baseline findings with the subsequent outcomes across the interventions and produce opportunity to measure the correlations or causal relationships of the interventions with the health outcomes. Repeated measurements at defined time interval can provide trends and distributions of viremia and the high coverage of HMIS data can provide additional opportunity to investigate the casual relationships between interventions with health outcomes. Finally, the time series nature of data from HMIS can be useful for prediction of the future thus can be critical for resource mobilization and planning (McCleany and Hay, 1980).

Routine health information is defined as data that are continuously collected, stored, analyzed and disseminated to help decision making and improvement of services. Unlike data generated by population based periodic surveys and formal research, routine data is generated from a system deliberately designed to monitor the health service delivery. Surveys are considered to be the gold standards to generate data on determinants of health and to evaluate capacity of health systems and impacts of health services; however, data generated routinely from the HMIS are readily available at any time that could be used for decision-making, monitoring of quality of service delivery,

monitoring of programs and reporting progress towards achieving targets (Wagenaar BH., et al,). The repeated observations captured in real-time by the HMIS over extended period of time from all health facilities using key performance indicators of services can be exploited using robust research designs as proposed in this study to measure associations of risk factors for viremia and virlogical failure.

1.3 The Eswatini HMIS

Data on clinical services provided for clients on ART are recorded and stored in the health information system (HMIS) using an onsite electronic system. The HMIS routinely collects data on demographic characteristics and clinical variables at baseline. Although the routinely collected data could be of low quality, the use of real-time electronic data systems and the ongoing data quality assurance measures guarantee the data quality (Mphatswe W., et al. 2012).

The HMIS in Eswatini is organized (Figure1) in a way that essential data sets are captured and reported by the health facilities to the regional strategic information unit, which in turn transfers the regional aggregate data to the national data repository. The regional level information system routinely captures, stores and transmits data to the national level on indictors that are used to measure health services including laboratory results. Data on clinical services for clients on ART are also recorded and stored in the HMIS using an onsite electronic system. The HMIS routinely collects data on demographic characteristics and clinical variables at baseline including CD4 count, WHO clinical staging, ART regimen and treatment outcomes including viral load results over the duration of clients' follow-ups.

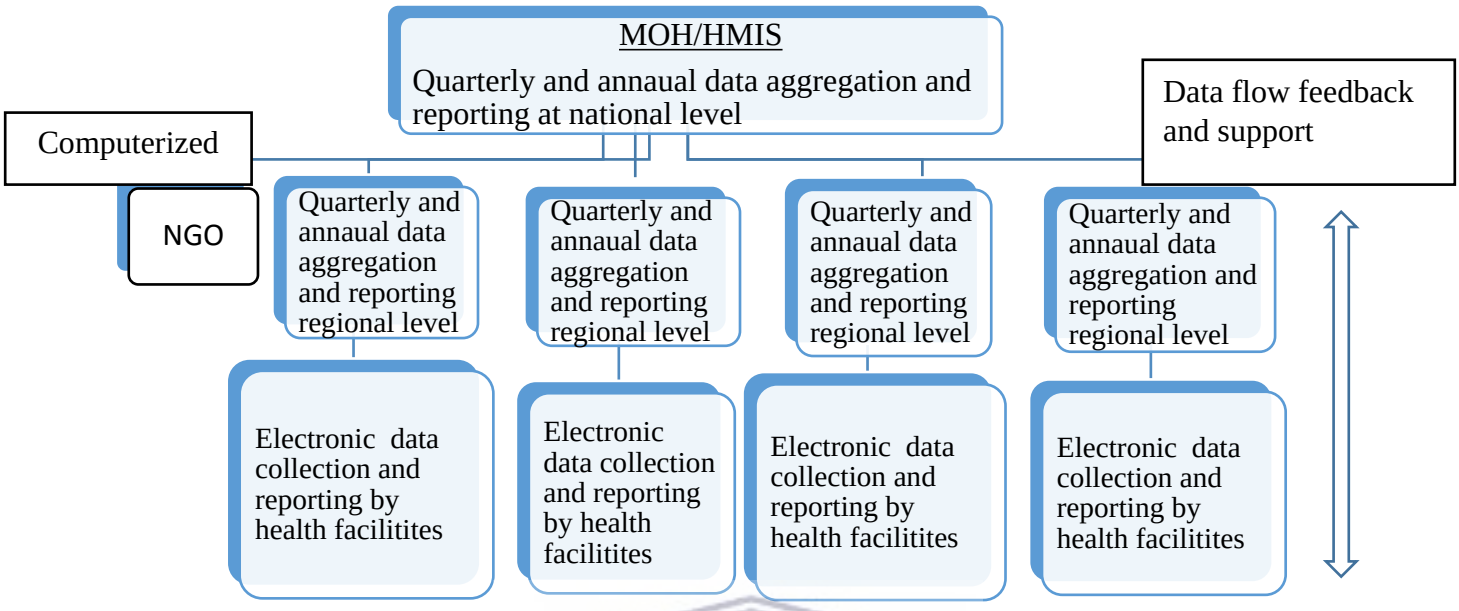

Figure 1: Description of the flow of information in the HMIS (source: MOH)

1.4 Research problem

The definition of viral suppression while on ART is variable based on availability of resources and public health approach. In resource rich settings, VF denotes a viral load greater than 50 copies/mL and this result suggests that the ART regimen has failed to suppress the multiplication of the virus (Bradley et al., 2016). In the United States, the Department of Health and Human Services (DHHS) guidelines define VF as the inability to maintain a VL below 200 copies/ mL with a recommendation to switch regimens based on a genotype of the virus (Cutrell J., et al; 2020). The European AIDS Clinical Society uses a stricter definition of VF when the viral load is > 50 copies/mL for 1 month and recommend changing therapy at that time. Several cohorts in high-income countries as well as recent clinical trials have reported viral suppression rates exceeding the 95% target (Gisslen et al., 2017). The definition of treatment success is revised as a decline in viral load to < 50 copies/mL within 6 months of commencing ART, and sustained thereafter (N. J. S. Dlamini, et al 2020). However, current WHO guidelines on the use of ARV drugs for HIV treatment recommend virologic monitoring using a higher threshold >1000copies/ml for viremia (WHO, 2016).

Eswatini has achieved the 95/95/95 target using program data (PEPFAR, 2020); however, treatment failure among HIV-positive clients on first-line antiretroviral therapy has not been assessed systematically. Specifically, predictors of viremia and VF while on first line ART requires investigation. This study sought to generate information to identify clients who have challenges adhering to first line ART regimen during their follow up. Such clients may require counselling for life long treatment before the initiation of ART and personalized follow up counselling. Further, the study sought to identify predictors of viremia and VF while on first line ART and guide the HIV program to develop interventions and implement prevention strategies.

2. Literature review

This chapter describes the key research concepts around which the study is organized. The findings from literature review are also structured to avoid repeating in order to make the review clear and succinct on what other authors found and how the various inputs contribute to the development of the research questions investigated by the current study. The chapter concludes with the main points that have emerged from the literature review and provides the basis for the development of the research.

2.1 Conceptual framework

WHO defines health as a complete physical, mental and spiritual wellbeing and not the absence of infirmity, which depends on underlying social, economic, and environmental determinants (Samba L.G and Kirigia J.M, 2014). However, these factors operate through proximate determinants in order to influence health. The proximate determinants of HIV infection that include behaviour factors related to HIV transmission, adherence to treatment of HIV and opportunistic infections can influence viral load and the health outcomes of PLHIV (Lopman B. et al; 2008). This is because the proximate determinants have direct links to the biological determinants that can affect the rate of new infections, determine the prevalence of HIV infection and progression to HIV disease and premature death.

Risk factors for viremia and VF can produce their effect at one of several causal levels, making interventions challenging. One suggested solution can be a proximate determinants framework, in which risk factors are grouped into ‘‘underlying’’, ‘‘proximate’’ and ‘‘biological’’ determinants.

The proximate determinates link the underlying risk factors with the biological factors for development of viremia and VF; however, the proximate determinants can be influenced by interventions that could have direct effect on biological determinants for development of viremia and VF. The proximate-determinants conceptual framework is therefore a useful outline for studies that include social and biological variables (Source: Boerma & Weir, 2005). The underlying and biological determinants of the framework operate through proximate determinants with the underlying factors affecting the biological determinants. In this particular study, the framework as shown in figure 2 suggest the pathway analysis as a tool for describing how interventions on

underlying and proximate variables could reduce the risk of viremia and VF by directly affecting the biological determinants that include the potency of ARVs and mutation of the virus.

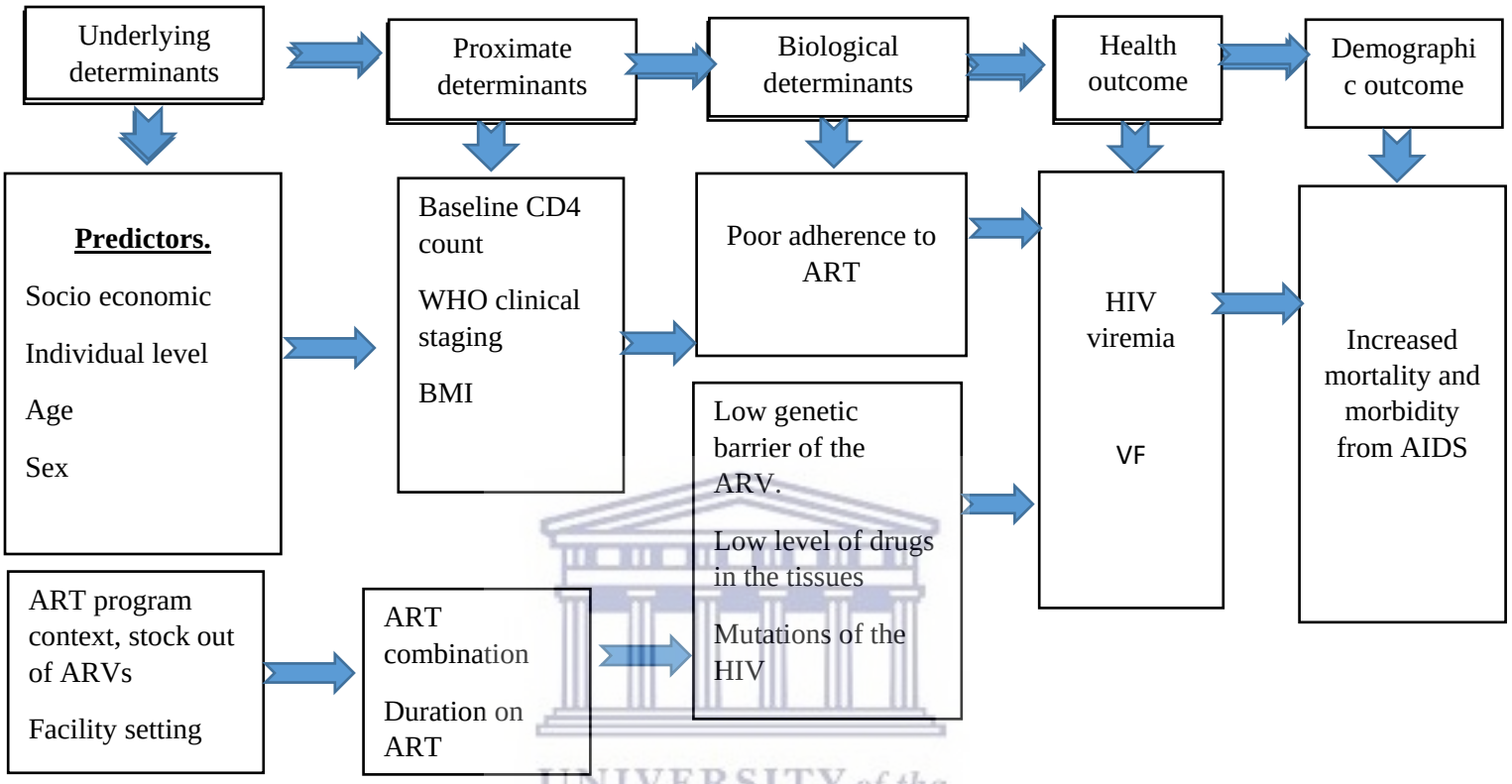

Figure 2: Conceptual framework for predictors of viremia and persistent viremia.

2.2 Measurements of viral load

Viral load monitoring consists of measurements of the virus copies/mL of plasma sample submitted at 6 and 12 months after initiation of ART and annual measurements are done thereafter in case of virologic suppression. The test is done in the national and regional molecular laboratories in Eswatini on plasma and dried blood spots of whole blood samples using the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, or Roche COBAS 960 system (Roche, CA, USA) and by Biocentric in a minority of cases.

2.3 Factors affecting optimal adherence to ART

A high VL result can be a product of primary resistance to the prescribed ART, inadequate ART drug levels because of altered pharmacokinetics, such as absorption difficulties, or drug–drug

interactions or most commonly inadequate patient adherence to treatment. However, a durable and maximum viral suppression depends on optimal adherence to ART influenced by several clinical and behaviour factors.

2.3.1 Behaviour factors

A meta-analysis based on 207 papers that reported on 103,836 patients showed that adherence to ART depended on patients' adherence self-efficacy, concerns about adverse effects of ART, and motivation to take ART for life, satisfaction with the HIV care provider and consequence of depressive symptoms, HIV stigma, and social support (Langebeek N. et al., 2014). A study done in Cameroon also showed that older subjects >40 years were less likely to be non-adherent ($p<0.0001$) compared to younger subjects ≤40 years (Fonsah J et al., 2017). A prospective study comprising a total of 961 HIV-1–infected women who initiated ART in USA showed white women were more likely than African American women to attain a virologic response (relative hazard [RH] = 1.34, P = 0.005), less likely to experience viral rebound (RH = 0.76, P = 0.051), and less likely to die (RH = 0.63, P = 0.040) over a median of 5.1 years of follow-up (Anastos K. et al., 2005). Furthermore, lower income, smoking, current drug use and depression were also associated with discontinuation of ART and inferior response after ART initiation (Belenky N. M. et al 2014.)

2.3.2 Baseline Clinical factors

The CD4 count at baseline can predict subsequent development of viremia and virologic failure. A retrospective cohort study in South Africa among non-pregnant adults who initiated ART at eight public clinics showed that the risk of viremia was lower among patients with CD4 counts >500 cells/μL at the time of ART initiation compared to those with CD4 ≤500 cells/μL (adjusted risk ratio 0.58, 95% CI 0.37 to 0.92) (Dorward J. et al., 2020). In contrast, a CD4 count below 200 cells/μL at base line among late presenters started on ART was associated with VF and development of resistant mutations (Stirrup O. et al., 2019). A case–control study conducted in Nigeria using data captured from an electronic medical record in a large treatment program showed older age; longer treatment duration and lower CD4 count were associated with more than twofold increase in VF (Ekong E. et al., 2020). A hospital based case–control study conducted in Ethiopia among adult patients on first-line antiretroviral treatment also showed current CD4 count below 200 cells/mm^3, low body mass index and poor adherence for ART treatment predicted VF (Ahmed M. et al., 2019). A cross-sectional study in Mozambique that included 334 adults on first-line ART for a median of 3 years showed younger age, WHO stage III/IV at ART initiation and low ART

adherence were associated with HIV-1 RNA ≥1000 copies/mL accompanied by HIV drug resistance mutations (Rupe´rez M. et al., 2015). A similar cross sectional study from Tanzania also showed the odds of having VF was independently associated with baseline CD4 counts of < 200 cells/µl (AOR = 8.6 (1.7 - 42.1), p = 0.008), poor adherence (p < 0.0001) and nevirapine based regimen (p = 0.003) (Gunda D. et al., 2019).

A cohort study among individuals started on protease inhibitor-containing regimen (n=1,281) found that HIV diagnosis for over 8 years, depression and advanced HIV disease at ART initiation and low CD4+ T-cell count were significantly associated with clinical progression or mortality (Villes V., et al., 2007). A study on the long-term (2–4 years) clinical and virologic outcome of an ART programme in rural South Africa using virologic suppression (HIV-RNA < 50 copies/mL) and failure (HIV-RNA > 1000 copies/mL) as primary and secondary endpoints respectively showed 63% of patients (466/735) had a fully suppressed HIV-RNA at a median of three years on ART. Male gender, low BMI and advanced immune deficiency at baseline were predictors of virologic failure (Barth R., *et al.*, 2010). A study in Nigeria also showed that older age, potency of ART regimen, lower CD4+ cell count, higher baseline viral load and suboptimal adherence predicted VF using a threshold of 400copies/ml (Meloni S., et al., 2016). Likewise, a low CD4 count, suboptimal adherence and history of fever in the past week were also predictors of VF among individuals on ART (n=2054) enrolled in the African Cohort Study (Kiweewa F., et al., 2019) using a threshold of >1000 copies/ml.

2.3.3 ART related factors

Inadequate potency of the ART regimen also contributes to viremia and virologic failure. Variable absorption, metabolism, poor penetration of the ART into HIV reservoirs and low genetic barriers to drug resistance can contribute to viremia and VF (Deeks S., 2000). A study conducted in USA has shown that the incorporation of the integrase strand inhibitors (INSTI) have high efficacy and tolerability, and may result in rapid and durable virologic suppression compared with regimens that contain protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Jacobson K. and Ogbuagu O., 2017). However, efavirenze containing regimen was less likely to lead to VF compared to nevirapine-based ART (Pillay P. et al., 2013).

In a prospective cohort study that included ART naïve patients aged ≥17 years-old who initiated TDF, d4T, or AZT in South Africa, TDF appeared to perform better than either d4T or AZT, with

less drug substitution and mortality though difference in virologic suppression was not found (Velen K., et al.,2013). In contrast, patients initiated on tenofovir in China had a significantly lower rate of VF than those on stavudine or zidovudine (6.7 vs. 11.9 failures per 100 person-years, P = 0.013) (Cheung C., et al., 2017).

An observation study in a real life setting in Europe among patients (n=2016) who started raltegravir, elvitegravir/cobicistat or dolutegravir with two NRTIs as first line ART regimen showed that, 167 patients experienced treatment failure (i.e. two consecutive plasma HIV RNAs 200 copies/mL) over a median of 11 months with a 6.5% one-year probability of treatment failure for raltegravir, 5.4% for dolutegravir and 6.7% for elvitegravir/cobicistat (P = 0.001). In addition, patients started on elvitegravir/cobicistat had a twofold risk of developing treatment failure compared to dolutegravir (Monforte A., et al., 2019). Another study from Brazil using real-time HIV program data showed that introduction of dolutegravir combined with tenoforvir and lamuvidine backbone reduced the viral load maximally compared with efavirenze (Pascom A., et al., 2019).

Therefore, it is imperative to investigate the diverse factors that may be associated with virologic failure dependent on the ART program setting and the local context (Huong D., et al., 2011). Firstly, the definitions of virologic failure and viremia were diverse across many countries and a threshold of viral load >1000copies/ml was mainly used in resource limited settings to fit in to the current WHO definition of virologic failure. Secondly, most of the studies on factors associated with HIV viremia were conducted in high income countries that used a lower threshold to determine viral load suppression (Parczewski M., et al., 2017). Thirdly, the composition of first line ART in resource rich setting included PIs, NRRTI or ISTI that make comparisons difficult with results from resource limited settings. Fourthly, the eligibility for ART initiation in Eswatini were revised repeatedly until 2016 when the test and start approach and optimization of ART using DTG were endorsed in 2018, which could affect the durability of viral load suppression. Lastly, baseline characteristics of patients initiated on ART could vary depending on the social and economic context including prevalence of advanced HIV disease, malnutrition and depression that could influence occurrence of VF (Khienprasit N., *et al.*,2017) making comparison problematic. In addition, patients' optimal adherence to ART and retention in care could vary (Bangsberg D., et al., 2001) by locality and country of residence hence the 95% target for optimal and durable

viral suppression may not be attainable demanding targeted interventions to optimize adherence to ART based on country specific risk profiles (Hermans L., et al., 2020).

3. Methodology

This chapter explains the research objectives, the research design used and the rational for selecting the design. Justifications for key concepts and variables are included as well as the data source and data collection tools. The sampling technique and the assumptions used to calculate the samples size are also described in the chapter including the details of data collection processes, how the data editing and data-coding procedures were carried out and measures used to avoid bias. The data analysis is also explained including the rationale behind the selection of analysis procedures as well as the actual procedures used.

3.1 Aim and objectives

The aim of this study was to determine the occurrence and predictors of viremia and VF among HIV-infected adults initiated on first line ART from January 2015 to December 2019 who were receiving regular follow-ups in hospitals and clinics in Eswatini. The specific objectives were;

1) To determine incidence of viremia among HIV-infected adults

2) To determine prevalence of VF among HIV-infected adults

3) To determine the association between viremia and ART combinations in the first line regimen and clients' clinical characteristics at the time of ART initiation

4) To determine the association between VF and ART combinations included in the first line regimen and clients' clinical characteristics at the time of ART initiation and duration on ART

This study provides answers to the following research questions;

- What is the incidence of viremia and VF among individuals on first line ART?
- What is the relationship between timing of ART initiation, duration on ART and use of different combinations of first line ART with development of viremia and treatment failure?
- What factors predict viremia and treatment failure?

3.2 The Research theoretical framework

The WHO recommends a public health approach for low and middle income countries (LMICs) to use a higher threshold of HIV-RNA>1000 copies (WHO, 2016). This definition of viral load ignores low level viremia and assumes it as suppression thus regimen change is not recommended for viral load results showing 200 to 1000 copies/ml. A prospective, multicenter, open cohort composed of HIV-positive individuals suggested that 1 in 5 study participants experienced VF (n = 383), and 207 (54.1%) subjects were on their first ART regimen when they failed (Joya C., et al, 2019). The median time to VF from ART initiation was 3.9 years (2.3–6.4). The proportion of subjects experiencing VF varied by exposure category and older age at ART initiation (aHR 0.71, per 10-year increase, 95% CI .61–.82) and use of a NNRTI (aHR 0.68, 95% CI .51–.90) or an INSTI-based regimen (aHR 0.26, 95% CI .13–.53) were protective. Furthermore, higher viral loads between 200 and 999 copies/mL, even on single determination, increased the risk of VF. However, there is a paucity of evidence about viral suppression and predictors of failure in resource limited settings. In South Africa, a 90% virologic suppression below the threshold of 1,000 copies/mL was observed on intention-to-treatment analysis but this target was not met at the 50-copies/mL (Herman L., et al., 2020). Even though a high virologic suppression was attained in this study, the presence of a large subset of long-term infectious patients within the treated population raised a risk for poor HIV treatment outcomes. In addition, the subset of long-term infectious patients with low level viremia could hinder efforts to control the epidemic demanding tools to establish the cause of viremia urgently for targeted adherence interventions.

As mentioned under section 2.1, the conceptual framework of the study was built using proximate determinants model. The conceptual framework to determine the incidence of viremia and virological failure with their predictors considers client related factors, duration on ART and the combinations of first line ART used.

3.3 Research design

The study used secondary data routinely collected through the Health Management Information System (HMIS) on variables related to HIV prevention and treatment to monitor and evaluate the quality of HIV health services, measure the attainment of goals and targets of the program and to influence practices and policies among others. These readily available data can also be used for clinical studies to assess treatment outcomes as anticipated in this study. The secondary data will

help to calculate person-years on ART, which may be demanding to collect from prospective cohorts. The major advantage associated with use of the data from HMIS is cost reduction. Moreover, the study can be conducted in a short period of time and the convenience it provides is enormous (Dale et al., 1988; Glaser, 1962; Smith, 2008). The data are already collected. In addition, a larger representative sample size of the target population permits greater validity and more generalizable findings to similar settings (Smith A., et al. 2011).

The study employed a nested case–control study design. A nested case-control study is a type of case-control study where cases and controls are drawn from a well-defined source population and the controls are sampled from that same cohort but consisting of people who did not develop the outcome (Biesheuvel C., et al; 2008). A nested case–control study provides control over cofounding factors because exposure information has been collected during the follow up of study participants. Hence, matching of basic confounding factors are already characterized in the large cohort that increases the statistical power to detect the influence of other clinically relevant factors (Friis R., 2017). Compared to a standard case-control study, a nested case control design offers the opportunity to differentiate risk factors from confounding factors because the cases and controls are 'nested' in an existing predefined source population with known sample size, which is not the situation in case control studies (Biesheuvel C., et al ;2008).

3.4 Study population and sampling

Adult HIV-infected patients on ART attending urban and rural public healthcare facilities in Eswatini were included in the study. ART was provided in the framework of the national ART program, which provides treatment and routine viral load monitoring free of charge. First-line ART regimen consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-NRTI (NNRTI) or an integrase strand transfer inhibitor (ISTI). Cohort patients were included in this analysis if they met inclusion criteria consisting of prescription of first-line ART and availability of first VL result performed 48 weeks after initiation of ART.

Clients initiated on first line ART from January 2015 to December 2019 were identified from the HMIS. Adults 18 years old or above on first line ART for one year or more were included while clients lost to follow-up, died and stopped ART were excluded. Exclusion criteria also included clients receiving mono- or dual ART or on regimens incompatible with first line ART. Clients under 18 years of age were excluded because adherence to ART and/or the combination of first

line regimen could be different from adults. Based on program and survey data, 10% of individuals were expected to have viremia > 1000copies/ml and 90% have viral suppression <1000copeies/ml making the ratio of 1:9. More controls were included for precise estimation of very large or small relative hazards (Gail M. H., et al. 2019). Exposure to first line ART among cases and controls was expected to be similar although different combinations of individual drugs were possible. The sample size calculation assumed an alpha level of 5%; statistical power of 90% set to achieve real differences in risk factors for VF; expected odds ratio associated with a meaningful difference of risk factors assumed at 2; a 10% probability of controls having similar exposure risk factors expected and a 20% probability of the risk factors among cases. Using EpiINfo, a sample size of 212 individuals with viremia or VF and 1906 clients with no viremia were adequate for the nested case control study. In addition, a separate sample of 212 individuals with VF who were switched to 2nd line ART (i.e. a confirmed first line regimen failure) was calculated to compare baseline characteristics with the cases and controls. The date of first line ART initiation was the cohort entry date. All clients were required to be on first line ART and under regular follow-up for one year or more after the cohort entry date. A flowchart showing the application of inclusion criteria to create the nest population is presented Figure 3 (Kathe N., et al., 2018).

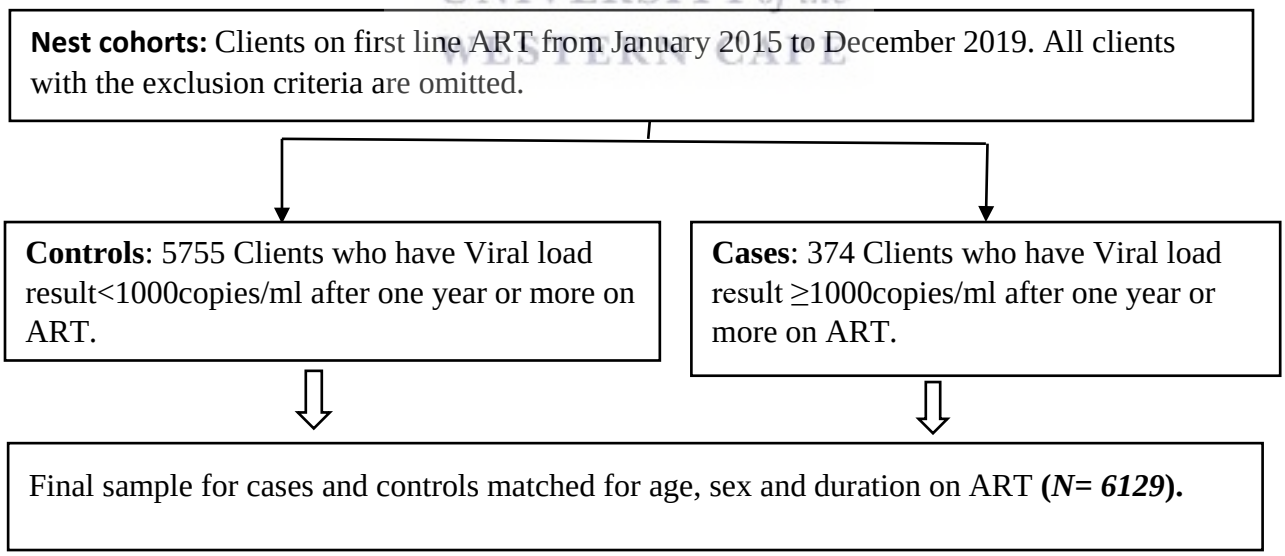

Figure 3: Inclusion criteria to create the Nest Population

3.5 Data abstraction and exporting

The health information system routinely collects data on demographic characteristics and clinical variables at baseline including CD4 count, WHO clinical staging, ART regimen and treatment outcomes over the duration of clients' follow-ups. These data were collected longitudinally to generate information on trends of the performance of the HIV program in the country. The data set was retrieved from the information system and exported to an Excel database. The variables in the data set included age and sex, body mass index (BMI) calculated as ratio of weight in Kgs to height squared in meters, obesity defined as a ratio ≥30; overweight 25-29 and normal 18.5-24 and underweight below 18.5. Additionally, the combinations of the first line ART regimen used, the clinical WHO staging at the time of ART initiation, viral load result a year or more after ART initiation, baseline CD4 count at the time of ART initiation, duration on ART and the viral load result were included in the data set.

The DMT was consulted to obtain the dataset as per the protocol. These data were transferred to an Excel database accompanied by data cleaning and deduplication to avoid double counting. Individuals that fulfil the exclusion criteria were discarded to establish the nested cohort. A data abstraction tool (Annex3), which guided the Excel database, was used after tested for its suitability. Data was abstracted in the same way for cases and control groups and data coding was done to transform the data.

3.6 Data management and analysis

Viremia and VF were the dependent variables; the independent variables included client related risk factors and composition of the ART regimen used throughout the course of the client follow up and duration on ART. Both numerical and categorical variables as predictors of viremia and VF were included based on evidence from previous studies. The characteristics of the cases and controls were reviewed using a univariate analysis. Variables and stratified frequencies across cases and controls were summarized together with confidence intervals for the prevalence estimates among cases and controls. The prevalence of risk factors among cases and controls with time to develop viremia was compared to measure the associations of the risk factors and the outcomes. The incidence rate of viremia and VF was calculated by the number of patients who developed these outcomes over the person-time from first line ART initiation to the development of the outcomes. Baseline characteristics between groups of patients with categorical variables was

compared using independent- t test and one-way ANOVA for the mean viral load test; a two-way ANOVA was used for categorical variables with significant relationship with the mean viral load result. Chi-square tests were used to compare categorical data as appropriate. Logistic regression models were used to identify covariates that were associated with viremia and VF. Factors that were significant at the p-value < 0.05 in the univariate analysis were evaluated in multivariable model (Khienprasit N. et al., 2011). Time to develop viremia was estimated using Kaplan-Meier time to event analyses (Campbell M. and Machin D., 2007).

The HMIS captures real-time data from the health facilities electronically during the clients' clinic encounters. Nearly all facilities that provide comprehensive HIV prevention and treatment services in Eswatini are using electronic medical records in real time. The electronic system captures longitudinally the demographic characteristics of the client, clinical findings, laboratory results, the type of service provided (e.g. ART, FP, PMTCT etc.), pharmacy dispensing, date of client visit and the next appointment date. All clinical data were captured in real-time and the data were synchronized through a computerized network connected to the central server at national level. Each client had a unique identifier that was used to retrieve individual medical records.

The data were de-duplicated using the clients' unique identifier, sex, age, date of ART initiation and the first line ART regimen used. All clients who started ART from January 1, 2015 to December 31, 2019 were filtered using the date of ART initiation and included in the data set. Clients that fulfilled the exclusion criteria were omitted as mentioned under section 3.4. The viral load result and viral load result authorization date were filtered corresponding to the baseline variables for each client. The duration on ART was calculated using the last day of December 2020 assuming the clients who started ART in December 2019 were on ART for at least a year hence satisfying the inclusion criteria. Similarly, the time to first viral load test was calculated in days by subtracting the ART initiation date from the viral load result authorization date.

After completing data cleaning in the Excel database and establishing the nested cohorts with complete baseline variables of interest and the viral load results, the data were exported to SPSS version 27 for analysis. Some numerical continuous variables (e.g. BMI, viral load result and CD4 count) were recoded to categorical variables. A descriptive analysis was done using frequency, mean, medial and standard deviation as appropriate. Similarly, a bi-variate analysis was conducted, using different tests, to identify associations between the dependent and independent variables.

Crude odds ratios were computed to assess presence of significant associations; correlation and regression with appropriate statistical tests were also used to measure the degree of associations.

Multivariate analysis and Cox hazard ratio were conducted to control the effect of confounding variables and determine the true effect of the predictors on the dependent variables using adjusted odds ratio hazard ratio respectively. Time to viremia was also determined using a log rank test and a Kaplan Meir curve to compare the proportion of clients that develop high viral load with the time to develop high viral load for the different ARVs used in the first line regimen combinations.

4. Ethical considerations

The study protocol was submitted to University of the Western Cape Biomedical Research Ethics Committee and Eswatini Health and Human Research Review Board for review and approval (Annex 1&2). The study used secondary data that were not linked to individual patient identifiers. The research relied on publicly available information that was legally accessible to the public so long us privacy and confidentiality of clients were protected (MOH, 2019). The study was conducted after incorporating the feedbacks from the respective IRBs and obtaining final approval from the relevant IRBs (Annex 1& 2). The data were analysed in a password protected computer and stored as per the requirement specified in the protocol.

5: Results

This chapter describes the actual sample for both cases and controls and summarizes the main results. The key trends, patterns and associations of the predictors of viremia and VF are illustrated. Finally, the chapter concludes by summarizing the key findings reflecting the correlations of predictors with viremia and VF.

5.1 Characteristics of participants with and without Viremia

A total of 6,129 individuals above the age of 18 years on first line ART regimen who started the treatment from January 1, 2015 to December 31, 2019 were identified for the nested cohort. The nested cohort comprised 374 cases with viremia and 5,755 controls without viremia, which was more than the sample size calculated for the study that provided a good statistical power for the study and a robust effect size. A robust effect size means that the independent variables are very good predictors of the dependent variable (Serdar C; et al. 2020).

A descriptive analysis of baseline characteristics of clients on first line ART regimen showed that majority (63%) of the study participants were female with a mean and median age of 38 and 36 years respectively and a SD of 10 (Table 1). Majority (93%) of the study participants were initiated on efavirenz based first line regimen while six percent were initiated on dolutegravir based regimen; nevirapine was used in 70 individuals accounting for one percent of the total first line ART initiations. The proportion of individuals on dolutegravir based regimen increased to 73.0% by end of December 2019 while 23% were on efavirenz based regimen; the remaining four percent of the clients were on other regimens including protease inhibitors.

All individuals had their first viral load test done after a median duration of 483 days post-ART initiation of which 5,755 (94%) had viral load result<1000copies/mL while the remaining 374 (6%) had viral load result ≥1000copies/mL. Using the CDC classification of viral load results, 88.3% of individuals had below 50copies/mL, 92.2% had viral load below 200copeies/mL and 1.8% had between 200 and 1000copies/mL. The cumulative incidence of first time high viral load using a cutoff 1000copies/mL was close to 8%, which is slightly higher than the prevalence of 6%. The majority (93%) of the study participants had their first viral load test after a year or more post ART initiations while only seven percent of the participants had their viral load done less than 6

months after ART initiation. The majority (63.7%) of the participants had a CD4 count below 350cells/µL at baseline suggestive of moderate to severe immune deficiency; however less than 18% of the participants presented with WHO clinical stage III or above while 63.7% were classified as WHO clinical stage I.

The mean BMI was 24 and the median was 25; 41.3% of the participants were classified as overweight or obese while 13% had BMI below 18.5 suggesting undernutrition. The majority of the study participants (53%) were on ART for over four years while the remaining 37% and 10% were on ART for one to two and two to four years respectively.

Table 1: Baseline characteristics of clients on first line ART January 2015-December 2019.

Variable (N=6129)	N	%
Sex		
Female	3881	63.3
Male	2248	36.7
Age	*Median age =36 yrs Mean 38 yrs [range :74 yrs] SD =10*	
<20	30	0.5
20-29	1237	20.2
30-39	2579	42.1
40-49	1487	24.3
50+	796	13.0
1st line ART regimen at initiation		
TDF+3TC+EFV	5600	91.4
TDF+3TC+DTG	374	6.1
Others	245	2.5
Final ART regimen including 2nd line		
TDF+3TC+DTG	4373	71.3
TDF+3TC+EFV	1407	23.0
Others	349	5.7
VL load result: WHO classification		
Suppressed	5755	93.9
Unsuppressed	374	6.1
VL load result copies/mL: CDC classification		
Below 200 copies/ml	5648	92.2
200 to 1000copies/mL	107	1.8
Over 1000 copies/mL	374	6.1
Days to 1st VL result; Median day: 483 ; range 1113 days , min=81days, Max =1194 days		
<180	432	7.0
180-732	3959	64.6
>732	1738	28.4
Baseline CD4 count cells/µL	Median= 264; Mean= 314; SD= 237	
<200	2347	38.3
200-500	2689	43.9
>500	1093	17.8
WHO clinical stage		
Stage I	3903	63.7
Stage II	1138	18.6
Stage III	948	15.5
Stage IV	140	2.3
BMI at baseline	Mean 24; median= 25 range =63; Min15 Max= 78; SD= 6	
<18.5	800	13.1
18.5-25	2797	45.6
25-30	1484	24.2
>30	1048	17.1
Duration on ART in months	mean =48; median =49 ;SD=12; Min=12; Max 73	
12-24months	624	10.2
24-48months	2251	36.7
>48months	3254	53.1

5.2 Predicators of viremia: bivariate analysis

A Chi-square test was computed looking at the association between the binary viral load variables and the baseline variables that were recoded to categorical variables. The Chi-Square result showed a statistically significant association between the viral load result and the baseline variables included in the study ($p<0.001$) as shown in Table 2. The *p*-value for the Chi-square test is smaller than the alpha value hence rejecting the null hypothesis that the categorical baseline variables are independent of viremia or no viremia. Nonetheless, these findings needed validation using a multivariate analysis to find out the relationship as shown in section 5.3

Table 2: Association of viral load result with baseline characteristics of clients on first line ART:

	1st VL result	CD4 Baseline count	BMI baseline	Months on ART	Age grouped	ART regimen
Chi-Square	4724.3	664.7	1548.5	1724.6	4212.5	9800.8
df	1	3	3	2	5	2
P-value.	0.000	0.000	0.000	0.000	0.000	0.000

The mean of viral load results in copies/mL was compared between categorical variables using independent t-test and a one-way ANOVA as appropriate. The mean viral load was significantly lower among females compared to males (p-value=0.009; student t-test=1.3;61df). The mean comparison of viral load between categories of CD4 count was significantly lower as the CD4 count categories increase (p-value=0.006, df 3 F= 4) while the mean comparison with the remaining baseline categorical variables did not show statistically significant relationships. There was an inverse relationship between the CD4 count categories and the mean viral load showing that as the immune deficiency advances, the viral load increased suggesting severity of HIV infection (figure 4).

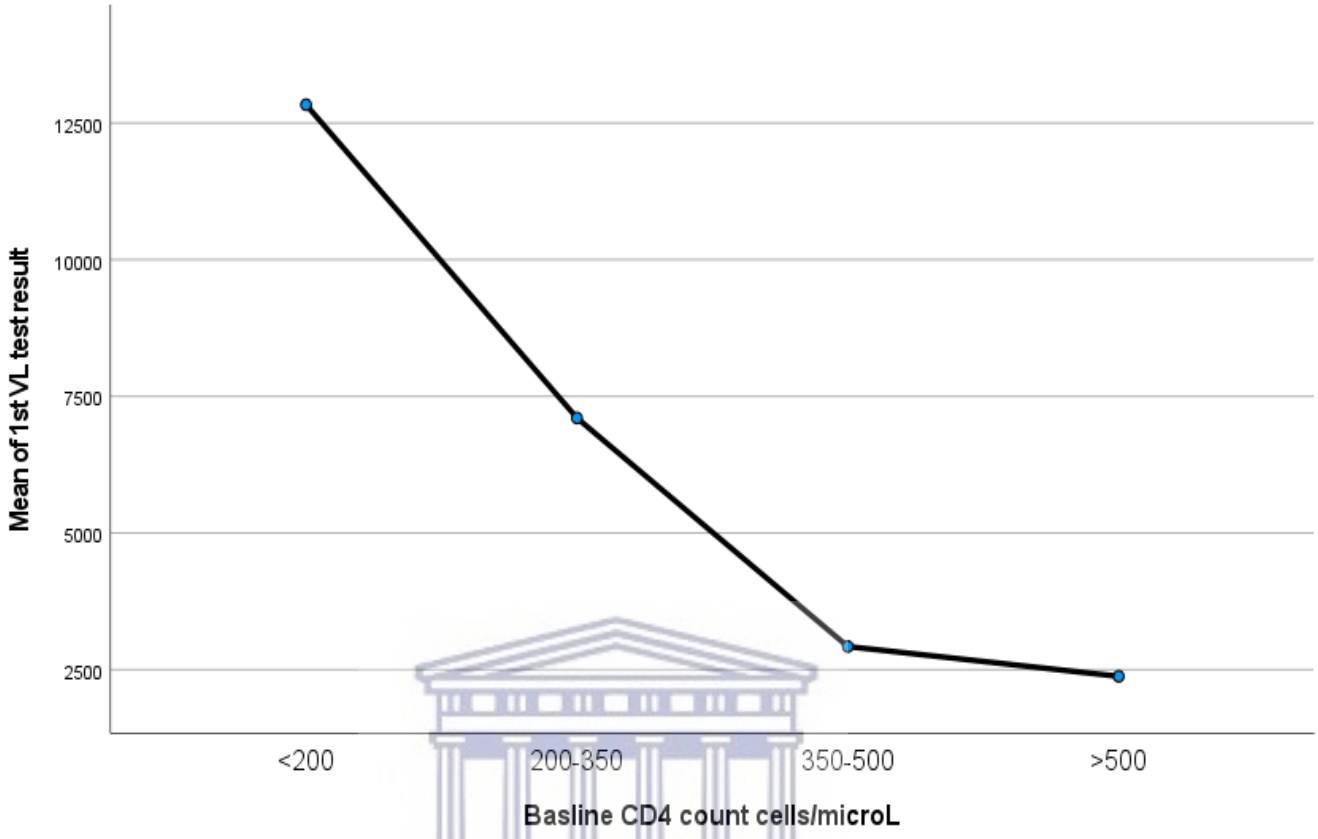

Figure 4: Mean viral load by category of CD4count cells/µL.

A mean viral load comparison was done using a two-way ANOVA test for those risk factors with statistically significant differences. The variability of mean viral load by CD4 count as the main effect with sex was not equal for males and females and the difference was statistically significant (p-value=0.014; F=3.3 df =3) although there was no statistically significant interaction between CD4 count category and sex (p-value=0.174). The mean variability of viral load by CD4 count and sex is shown in figure 5. However, the Levin test of equality of variance was significant for the two-way ANOVA test of variance for viral load result with CD4 count categories and sex.

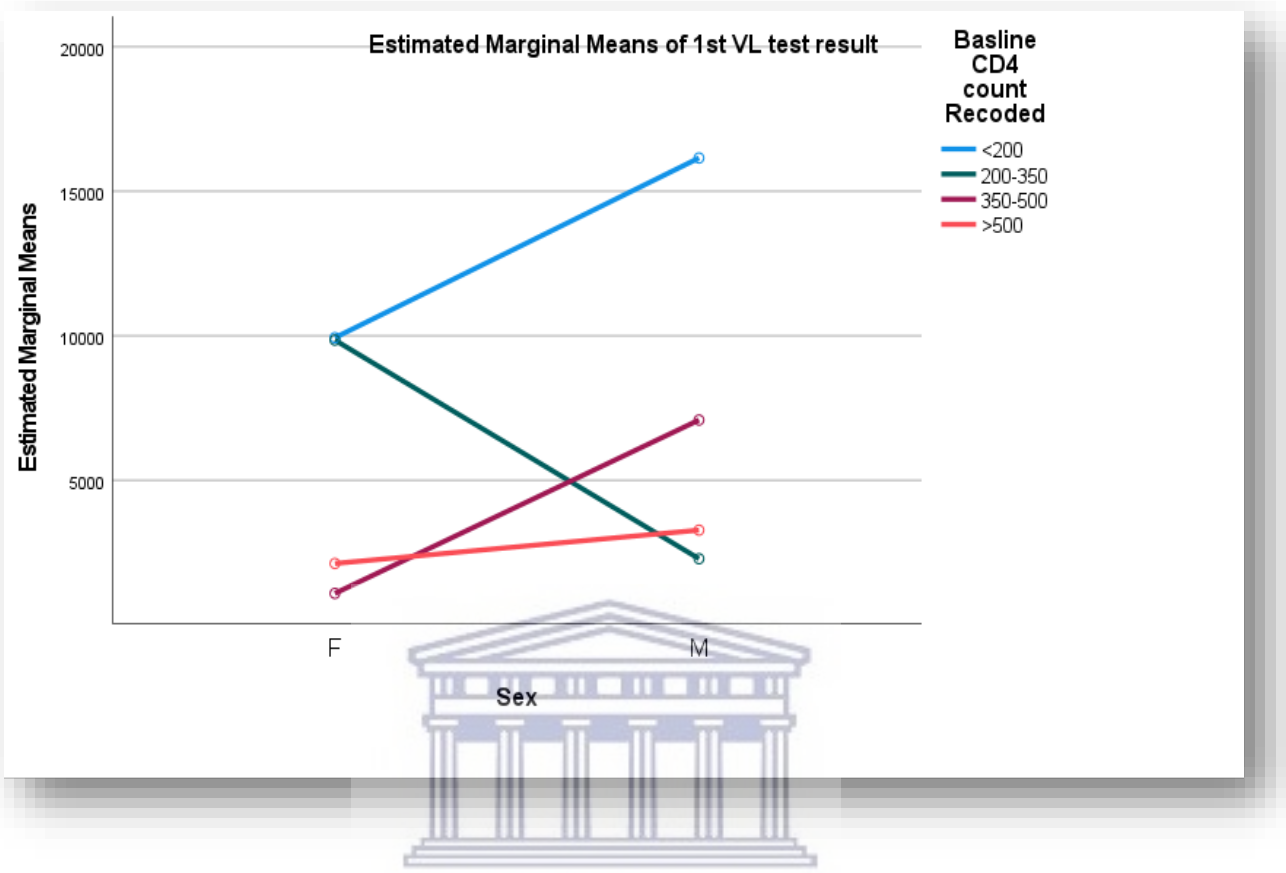

Figure 5: Interactions of CD4 count categories and sex with mean viral load result.

To understand the mean variability difference of viral load by sex, a two-way AVOVA was done looking at difference of CD4 count by sex. The mean CD4 count for female clients was 348 cells/µL compared to 256 cells/µL for male clients, which was statistically significant (p-value < 0.001; F=222; df=1). In addition, cross tabulation of sex and CD4 count categories was done to compare the difference of percentage of CD4 count for each category by sex as shown in Table 3. The difference in proportion of CD4 count for each category for male was significantly lower than female (Pearson Chi-Square test 219; df=3 p-value<0.001).

Table 3: relationship of CD4 count category by sex.

		Baseline CD4 count cells/µL			
		<200	200-350	350-500	>500
F	Count	1252	992	785	852
	% within Baseline CD4 count	53%	64%	69%	78%
M	Count	1095	566	346	241
	% within Baseline CD4 count	47%	36%	31%	22%

A bivariate data analysis was conducted to measure the odds of viremia and no viremia using baseline variables and the results are shown in Table 4. The odds of viremia were significantly (P value <0.05; OR=0.356; 95%CI=0.132-0.961) lower among clients aged 24 and above compared to clients below the age of 20. ART regimen and WHO clinical staging had no statistically significant association with viremia (P value>0.05; OR=0.899; 95%CI=0.383-2.11).

The baseline CD4 count had statistically significant association with viremia; as the CD4 count increased above 200cells/mL, the odds of viremia reduced (p-value=0.01; OR=0.64; 95%CI=0.49-0.83). BMI at baseline also had statistically significant association with viremia. That is overweight and obesity had lower odds of developing viremia compared to those with undernutrition (p-value<0.05; OR=0.61;95%CI=0.41-0.61). Duration on ART had statistically significant association with viremia; Clients on ART for two or more years had lower odds to develop viremia than compared to those on ART for less than two years (p-value<0.05; OR 0.66;95%CL=0.48-0.91).

Table 4: Correlation of baseline characteristics of the nested population on first line ART.

Variables (n=6129)	Frequency	Crude odds ratio (COR)	P value	95%CI	
Sex					
Male	2248	1			
Female	3881	0.826	0.093	0.661	1.03
Age group					
<20	30	1			
20-24	230	0.838	0.736	0.299	2.343
24-30	1007	0.356	0.041	0.132	0.961
30-40	2579	0.337	0.029	0.127	0.893
40-50	1487	0.273	0.01	0.102	0.733
50+	796	0.189	0.002	0.068	0.529
ART regimen					
DTG based regimen	377	1			
EFV based regimen	5692	0.899	0.899	0.383	2.11
NVP based regimen	60	0.461	0.461	0.208	1.023
WHO staging					
Stage I	3903	0.731	0.33	0.389	1.373
Stage II	1138	0.596	0.131	0.304	1.167
Stage III	948	1.066	0.849	0.553	2.057
Stage IV	140	1			
CD4 count					
<200	2347	1			
200-350	1558	0.640	0.001	0.491	0.831
350-500	1131	0.444	0.000	0.316	0.622
>500	1093	0.595	0.001	0.437	0.810
BMI					
<18.5	800	1			
18.5-25	2797	0.971	0.853	0.715	1.320
25-30	1484	0.704	0.052	0.493	1.003
>30	1048	0.612	0.016	0.411	0.911
Months on ART					
12-24 moths	624	1			
24-48 months	2251	0.636	0.007	0.457	0.886
>48 months	3254	0.662	0.01	0.483	0.907

5.3 Predictors of viremia: multivariate analysis

A multivariate analysis was conducted using logistic regression with viremia as the dependent variable. Sex, age, CD4 count at baseline, ART regimen at initiation, duration on ART and BMI at baseline were included in the model as independent variables [Table 5]. Sex had significant association with viremia after controlling all possible confounders. Females were 0.72 times less likely to develop viremia compared to male counter parts (**AOR** .716; p-value 0.009; 95% CI=0.558-0.919).

Age was also found to be statistically significant during the multivariate analysis after adjusting for possible confounders. Generally, young participants [20-30 years of age] were 4 times more likely to develop viremia compared to those aged less than 20 years. Similarly, those in age group of 30-50 years were two times more likely to develop viremia compared to those below 20 years of age [**AOR** 2 ;p-value< .004; 95% CI =1.66-13.68] [Table 5].

Baseline CD4 count had statistically significant association with viremia. Patients with CD4 count of 200-350 cells/µL were 0.627 times less likely to develop viremia compared to those with CD4 count below 200cells /µL [**AOR**=0.627, P-value =0.001; 95% CI=0.746-0.826]. Similarly, patients with CD4 count of 350-500cells/µL were 0.4 times less likely to develop viremia compared to those with CD4 count of less than 200cells /µL [A**OR**=0.398; P-value=0.000; 95% CI=0.279-0.567]. Clients with CD4 count of greater than 500 were 0.5 less likely to develop viremia compared to their counter parts with CD4 below 200cells /µL (**AOR**=0.508; P-value =0.000 95% CI=0.365-0.708]

WHO clinical staging, BMI at baseline and duration on ART had statistical significance with viremia during the bi-variate analysis. However, the statistical significance was not observed during the multivariate analysis [Table 5].

Table 5: Multivariate analysis of the baseline characteristics of clients on first line ART with high viral load.

Variables	N	AOR	P value	CI (95%)	
Sex					
Male	2248	1			
female	3881	.716	0.009	0.558	0.919
Age group					
<20	30	1			
20-24	230	4.772	0.004	1.665	13.675
24-30	1007	4.682	0.000	2.704	8.109
30-40	2579	1.967	0.004	1.236	3.130
40-50	1487	1.771	0.006	1.176	2.667
50+	796	1.378	0.153	0.888	2.137
ART regimen					
DTG based regimen	377	1			
EFV based regimen	5692	1.181	0.730	0.459	3.040
NVP based regimen	60	0.544	0.144	0.240	1.231
WHO clinical staging					
Stage I	3903	1			
Stage II	1138	0.935	0.839	0.488	1.792
Stage III	948	0.718	0.345	0.362	1.427
Stage IV	140	1.184	0.620	0.697	2.309
CD4 count (cells/µL)					
<200	2347	1			
200-350	1558	0.627	0.001	0.746	0.826
350-500	1131	0.398	0.000	0.279	0.567
>500	1093	0.508	0.000	0.365	0.708
BMI					
<18.5	800	1			
18.5-25	2797	1.006	0.754	0.736	1.736
25-30	1484	0.754	0.126	0.525	1.083
>30	1048	0.698	0.086	0.463	1.052
Months on ART					
12-24 moths	624	1			
24-48 months	2251	0.992	0.971	0.634	1.550
>48 monthn	3254	1.149	0.547	0.731	1.804

5.4 Time to develop viremia by regimen type

The time to develop viremia was compared among clients that were started on dolutegravir, efavirenz and nevirapine containing first line regime using a time to event analysis (Table 6).

Table 6: Summary of viremia by first line ART regimen type.

	Nested Cohort (N=6129)	Cases with viremia (N=374)	Controls without viremia (N=5755)	
DTG based regimen	377	40	337	89.4%
EFV based regimen	5692	327	5365	94.3%
NVP based regimen	60	7	53	88.3%

The mean duration to develop viremia for clients on DTG, EFV and NVP based regimens were 59, 70 and 69 months respectively. The overall comparison using the Log Rank (Mantel-Cox) test of equality of high viral load distributions for the different levels of ART regimens showed a statistically significant difference for the different first line ART regimens used (p-value 0.000; Chi-Square 510.3 with df=2). The time to develop high viral load by regimen type is illustrated in figure 6; the Kaplan Meir graph shows early development of high viral load among clients started on DTG followed by NVP and EFV in that order.

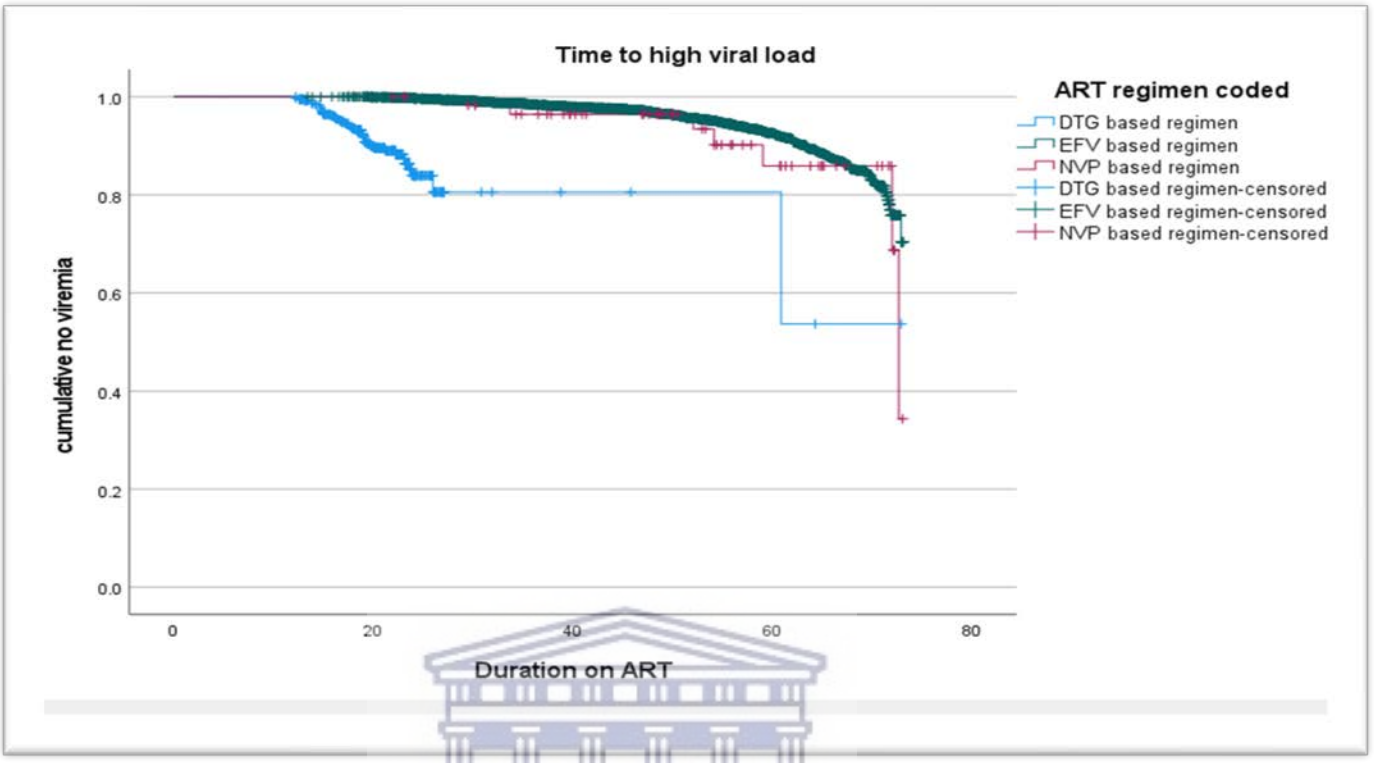

Figure 6: Time to develop high viral load by regimen type.

5.5 Characteristics of clients transitioned to second line ART regimen

Clients that were started on first line ART between January 2015 to December 2019 but transitioned to second line ART after confirmed first line regimen failure were filtered from the data set. A total of 113 clients were switched to second line regimen after first line ART failure and their baseline characteristics are shown in table 7. The majority (56%) of the clients were female and the mean age was 37 years with SD of eight. All clients had high viral load with a median of 49,200copies/mL (range 1020-4,606,493 copies/mL). The mean BMI and duration on ART were 23 and 51 months respectively while the median CD4 count was 103 cells/µL (range 1 to 791 cells/µL). The prevalence of treatment failure was 1.8% and 63% of the clients developed treatment failure after a median of 53 months on ART. About 77% of the clients had count below 200cells/µL indicating advanced HIV disease while close to 63% of the clients had WHO clinical stages I and II conditions. Nearly, 97% of the clients failed while on efavirnze based regimen that was the preferred first line ART in the country at the time of ART initiation.

Table 7: Clients transitioned to second line after treatment failure.

Variables (N=113)	N	%
Sex		
F	63	55.8
M	50	44.2
Age group		
< 20	1	0.9
20-25	8	7.1
25-30	14	12.4
30-40	52	46.0
40-50	31	27.4
above 50	7	6.2
First line ART regimen		
ABC+3TC+EFV	2	1.8
TDF+3TC+EFV	109	96.5
TDF+3TC+NVP	2	1.8
Second line ART regimen		
ABC+3TC+ATV/r	6	5.3
AZT+3TC+ATV/r	62	54.9
AZT+3TC+DTG	30	26.5
AZT+3TC+LPV/r	14	12.4
TDF+3TC+DRV/r+ DTG	1	0.9
WHO staging		
I	48	42.5
II	23	20.4
III	39	34.5
IV	3	2.7
CD4 count cells/µL		
<200	87	77.0
200-350	18	15.9
350-500	4	3.5
>500	4	3.5
Duration on ART in months		
12 - 24	1	0.9
4 - 48	41	36.3
>48	71	62.8
BMI at baseline		
<18.5	24	21.2
18.5-25	52	46.0
25-30	29	25.7
>30	8	7.1

5.6 Viral load results category using low level Viremia detection limit.

The viral load result was stratified using the low detection limit of 50copies/ml to compare VF with the definition of failure in resource rich setting as shown in figure 5. Considering the recent definition of virologic failure, only 88.3% of the clients who started first line ART were able to achieve viral load below 50copies/mL.

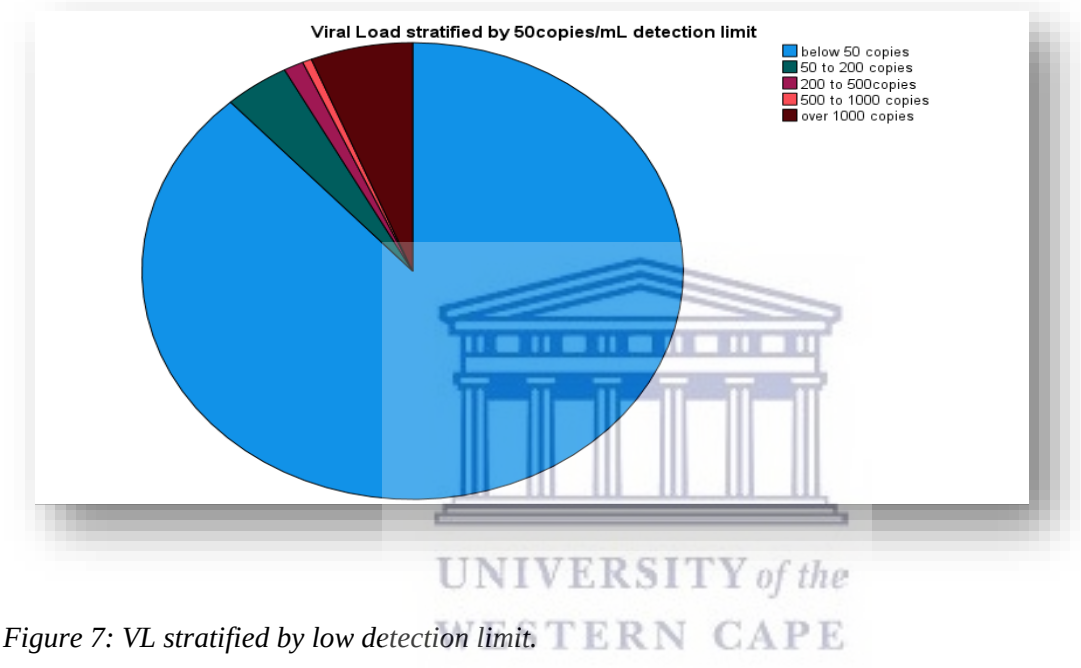

Figure 7: VL stratified by low detection limit.

5.7 Predicted viremia for Specific Covariate Patterns

A multivariate logistic regression may not be suitable for the analysis of predictors of viremia in this study because the duration of follow up of clients after initiation of first line ART was not fixed for the study participants. Accordingly, a Cox proportional hazards regression model was used as it is a flexible tool for assessing the relationship of multiple predictors to time-to-event outcome and has much in common with linear and logistic models (Vittinghoff E. et al; 2012). In this study with multiple categorical variables as predictors of viremia, sequences of categorical variables were created to represent group membership with one group serving as the reference to measure the hazard ratio(HR) of developing viremia as shown in Table 8. Male study participants had 38% more chance of developing viremia (P values 0.01; HR=1.4; 95% CI= 1.08-1.76) and

participants with a baseline CD4 count below 350cells/µL had a statistically significant risk of developing viremia (P-value 0.01; HR=1.5; 95% CI=1.12-2.1) while high category of CD4 count was protective even though the p value was insignificant. The hazards of developing viremia increased from 15 to 25 fold as the duration on ART was extended for two or four years respectively and the hazard ratio was statistically significant (P value= 0; HR≥ 15; 95% CI=9-24). The risk of viremia had inverse relationship with age; participates below the age of 30 years were likely to have five to six fold of developing viremia (P value= 0; HR= 6; 95%CI= 5-15) while those between 30 and 50 had close to two-fold risk of viremia, which was statistically significant (P- value <0.01; HR=1.6; 95% CI =1.1-2.5). Clients on DTG based regimen had 80% more chance of developing viremia compared to efavirenz although the hazard ratio was not statistically significant. BMI and WHO clinical staging at baseline did not predict the hazards of viremia using the Cox model.

Table 8: Cox model for effect of various predictors on hazards of developing viremia.

Variables in the Equation								
Predictors	Variation Coefficient	SE	Wald	df	Sig.	Hazard ratio(HR)	95.0% CI for HR	
							Lower	Upper
Male sex	.319	.124	6.624	1	0.010	1.376	1.079	1.755
Base line CD4 count			24.029	3	0.000			
200-350cells/mm3	.425	.160	7.027	1	0.008	1.529	1.117	2.094
350-500cells/mm3	-.011	.178	.004	1	0.949	0.989	.698	1.401
>500cells/mm3	-.283	.206	1.898	1	0.168	.753	.503	1.127
BMI at baseline			11.800	3	0.008			
BMI 18-25	.301	.202	2.226	1	0.136	1.351	.910	2.007
BMI 25-30	.388	.170	5.197	1	0.023	1.475	1.056	2.059
BMI>30	-.033	.188	.030	1	0.863	.968	.669	1.400
Months on ART			307.683	2	0.000			
12-24months	2.696	.252	114.359	1	0.000	14.821	9.042	24.292
24-48months	3.248	.185	307.444	1	0.000	25.747	17.908	37.019
Age grouped			46.723	5	0.000			
20-24years	1.739	.494	12.409	1	0.000	5.694	2.163	14.987
24-30years	1.511	.263	32.894	1	0.000	4.530	2.703	7.591
30-40years	.605	.229	6.961	1	0.008	1.831	1.168	2.869
40-50years	.511	.203	6.307	1	0.012	1.667	1.119	2.483
50+years	.256	.219	1.375	1	.241	1.292	.842	1.984
ART regimen			12.067	2	.002			
DTG based	.614	.446	1.893	1	.169	1.848	.771	4.431
EFV based	-.192	.391	.241	1	.623	.825	.383	1.776

6. Discussion

This chapter gives a summary of the key findings and makes comparison with findings from the literature. It also discusses nonconformities from what has been known before in relation to viremia and VF in literature from resource limited settings and provides possible explanations or suggestions to resolve the nonconformities. The chapter also shows the implications and relevance of the findings of the study to influence clinical practice and policy changes together with suggestions of specific questions that need further research.

6.1 Discussion of results

The revised UNAIDS targets for HIV diagnosis, ART initiation and viral suppression denoted as 95/95/95 respectively were endorsed by many countries including Eswatini and these targets were aligned with the of HIV diagnosis and treatment program goals. This study showed that the 3rd 95% target for viral suppression was not achieved using both the 1000copies/mL or 50copies/mL cutoff point for viral suppression. However, this finding was higher compared to other studies done in the region including Eswatini. A large multicenter cohort of PLHIV on first line ART in South Africa revealed a 90% virological suppression rate using 1000copies/mL cutoff and the suppression rate in this cohort dropped to 80% using a 50copies/mL limit (Lucas E. Hermans L., et al .2020). Another population-based nationally representative surveys conducted in five countries in southern Africa including Eswatini showed that 88.8% of adults on ART achieved viral suppression (VL < 1000 copies/mL), while 11.2% had viremia (VL ≥ 1000 copies/mL) including 8.2% who experienced VF (Haas A.D., et al; 2020). The higher VL suppression difference in this study compared to other studies could be due to ART optimization using DTG which is known to produce higher viral load suppression compared to NNRTI based regimen, which were used in the above mentioned studies.

The low viral suppression rate is noteworthy in relation to not achieving the 3rd 95 target when compared with a recent analysis of some European cohorts that have reported on treatment suppression rates around 95% at the 50-copies/mL threshold. The use of integrase inhibitors in these settings was the explanation for the observed difference even though it was not observed in this study (P. Kohler; et al. 2015).

The median duration for a viral load test after ART initiation was 16 months, which was delayed by 4 months from the recommended time. The mean viral load result was significantly associated with baseline characteristics of clients like sex, age, CD4 count, BMI, ART regimen and duration on ART. The majority of clients presented with a CD4count of 350celles/µL suggesting moderate to severe immune deficiency, which was significantly associated with viremia and VF that was comparable with findings from other studies (Stirrup O., et al 2019). The odds of VF were higher among those who had a CD4 cell count of ≤ 200cells/mm3, which was comparable to results from other studies (Lailulo Y., et al. 2020).

The mean viremia was lower among female study participants compared to male and the mean CD4 count of female clients was significantly higher than male participants. To understand the mean variability of viral load by sex, the two-way ANOVA test using CD4 count as the main effect showed a significant difference between female and male without significant interactions between CD4 count category and sex. The study therefore showed that female sex was a predictor of low likelihood of viremia compared to male participants. A study in Uganda (Bulage L., et al.2017) showed comparable findings that males had a slightly higher proportion of non-suppressed patients compared to females. Likewise, a multi centered study in South Africa (Hermans L., et al .2020) and a multi-country study in Southern Africa (Haas A. D., et al; 2020) including Eswatini showed that female sex and higher CD4count had lower odds to develop VF. The participants in the South African study developed viremia after a median follow-up of 152 (IQR: 61–265) weeks and being male and below 35 years of age and having a CD4 count below 200 cells/µL prior to start of ART were risk factors for viremia. The risk factors for development of viremia in this study were similar with the findings from the study in South Africa. Delayed diagnosis of HIV among men could be a possible explanation for increased odds to develop viremia and thus HIV services should improve early diagnosis and treatment of HIV before depletion of the CD4 count.

The odds of viremia were 2- 4 times more among those participants 20-50 years of age compared to those below 20. This is a divergent finding because one would have expected viremia in the extreme age groups due to poor adherence or age related defect in CD4 immune recovery respectively (Ekong E., et al; 2020; Palmer DB.2013*).* Nevertheless, the odds of viremia were not

significantly related with BMI, duration on ART, WHO clinical staging and the ART regimen used.

The mean duration to develop viremia for clients on DTG based regimen was shorter compared to NNRTI based regimens and the difference was statistically significant. This study finding was contrary to large study findings on the effect of DTG based regimen. Starting treatment naive patients on dolutegravir containing ART has an increased likelihood of achieving viral suppression in comparison to NNRTI containing ART and the average benefit is particularly evident in those with high viral load at baseline (Crucian M., et al; 2019). The explanation for low viral suppression while on DTG in this study could be due to the short duration of follow up and the smaller sample size and ART optimization that started late in 2018. In addition, perhaps those on NNRTI based regimen with viremia were selectively missed early and not included in the study sample. However, this finding merits further investigation because viremia and VF could happen while on DTG based regimen underscoring the importance of adherence to treatment (Monforte A., et al 2019).

The hazards ratio of developing viremia using the Cox proportional hard model for different covariates at baseline was consistent with the findings of predictors identified by the logistic regression model. Male sex, low CD4 count and younger age were independent predictors of viremia using both models. Furthermore, the Cox proportional model was able to identify duration on ART as a predictor of viremia and the proportional hazard ratio for viremia increased as the duration of ART also increased. Although this result was not supported by the logistic regression model the finding could be explained by accumulation of drug resistant virus overtime resulting in viremia among ART experienced clients (Fleming J. et al; 2019).

The prevalence of VF among those who started ART from January 2015 to December 2019 and required transition to second line ART was 1.8% after 53 months of follow up. The majority of the clients with VF had advanced immune deficiency state at baseline consistent with previous studies (Lailulo Y., et al. 2020). More female patients developed VF and the mean viremia level was high compared to the mean of first-time viremia. All clients developed VF while on NNRTI based regimen. However, there was no significant association found between the baseline characteristics of those patients with VF on bivariate and multivariate analysis.

The underlying and proximate determinants influenced the development of viremia and VF in this study; these factors like age, sex, baseline CD4 count and regimen used are variables that can be influenced by interventions through tailored adherence preparation and counseling to directly affect the development of viremia and VF.

6.2 Scope and Limitation of the study

The study had some limitations; Other client related factors like income, drug use, patients urban versus rural residence, educational and marital status, mental health and smoking that could have contributed to poor adherence to ART and occurrence of viremia and VF were not evaluated because these variables were not included in the MOH HMIS and yet could have operated as unadjusted confounders. Estimates of virologic suppression in this study may not be representative of actual program conditions because it only included clients started on ART over a five years' period. This may therefore overestimate the mean frequency of virologic suppression in the broader population on ART.

The study also used data abstracted from the health information system that could have limitations of potential missing clinical and laboratory data. Missing data may originate from lack of adequate capturing of electronic data by the clinicians but also from patient movement between clinics. Nonetheless, given these limitations, cross-referencing of electronic medical record data to laboratory information system and the fact that the missing data happen at random for both cases with viremia and controls without viremia can provide data quality assurance and ensure the validity of study findings. Some degree of survival bias can also be expected in longitudinal analysis of virologic suppression, as patients who reach adverse treatment outcomes such as switching to second-line ART or lost to follow up are excluded from the analyses indicating additional limitations (Herman L., et al 2020). Finally, factors related to the HIV program and the contextual factors in Eswatini may restrict the generalizability of the findings from this study even though the results can provide unique insights on treatment outcomes to guide current clinical practice under programmatic conditions in LMIC.

6.3 Reliability and validity

The quality of data from HMIS was checked regularly through onsite mentorship and supervision of healthcare workers who recorded the clients' data in the electronic system. The validity of this study depended on the suitability of the design and the representativeness of the survey sample together with the adequate sample size that was used in the study. Investigator bias was prevented as transfer of data from the information system was done by the DMT and confounding factors were dealt during analysis. In addition, outcome measure (viral load results) was defined and the data abstraction tool incorporated known baseline risk factors for viremia. The generalizability of the study findings was also preserved by the selection of cases and controls from the same cohort with similar exposure risk factors; similarly, precision of the data was ensured by the adequate sample size for cases and controls. In addition, the design of the study also controlled the effects of confounding factors because exposure data have been collected during the clients follow up and appropriate statistical methods were used during analysis to remove effects of confounders thus giving additional guarantee for external validity. Although data from electronic medical record (EMR) often contain missing values, this study expects missing data to occur at random (Chen D. et al., 2019). Viral load measurements were taken on similar schedules and exposure risk factors were comparable for both cases and controls and thus missing data were unlikely to introduce bias.

7: Conclusions and Recommendations

Using routinely collected data, it was possible to successfully assess the incidence and prevalence of viremia and VF among clients on first line ART in Eswatini. Furthermore, the study was able to determine the association between baseline characteristics as predictors of future viremia and virologic failure.

The study found that males have higher probability of developing viremia, which could be because of late presentation as measured by the significantly lower CD4 count in males compared to females. The ART program therefore need to improve health service seeking behavior of men for HIV services so that they are diagnosed before depletion of the CD4 count and put of treatment early and mitigate the occurrence of viremia and VF. The baseline CD4 count below 350cells/µL was a significant predictor of viremia and VF independent of sex underscoring the need for early diagnosis of HIV before depletion of the CD4count. Younger age below 30 years of age and duration on ART also predicted viremia that could be related to poor adherence to treatment. Nevertheless, the BMI, WHO clinical staging and the combination of first line ART regimen initiated were not predictors of viremia and virologic failure. Therefore, these predictors need attention during clinical practice to prevent development of viremia and treatment failure.

Despite the limitations of the study, the results from a large sample of clients on first line ART revealed unique insights into treatment outcomes that can influence clinical practice under programmatic conditions in Eswatini. A high virologic suppression rates were attained in the Eswatini treatment program; however, it was far less than the desired rates of suppression to reach the 3rd 95 target. Clinical practice should consider the low baseline CD4 count, male sex, younger age and the regimen used as predictors of viremia and virological failure and hence specific interventions targeting these subset of patients should be in place during ART initiation to mitigate their impacts on outcome of ART. Differences other than baseline clinical characteristics among cases and controls were not evaluated in this study; however, these factors should be investigated for their effect on viremia and VF. Further study may be also required to elucidate the long-term benefits of ISTI regimen for viral suppression as DTG is the preferred first line ART regimen and NNRTI based regimens are phased out. The findings on HIV viremia and virologic failure from

secondary data can be used to monitor the progress of the national action plan to achieve the goal of HIV epidemic control and attainment of the 95–95-95 targets.

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9 Appendices

1. Research authorization

UNIVERSITY of the WESTERN CAPE

YEARS of hope, action & knowledge

09 November 2020

Dr A Kidane
School of Public Health
Faculty of Community and Health Sciences

Ethics Reference Number: BM20/9/3

Project Title: Predictors and incidence of HIV viremia and virological failure among HIV infected Adults on First-Line Antiretroviral Therapy in Ethiopia: A Nested Case–Control Study

Approval Period: 09 November 2020 – 09 November 2023

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for the duration of the project.

Permission to conduct the study must be submitted to BMREC for record-keeping.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

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NHREC Registration Number: BMREC-130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE.

2. RESEARCH PROTOCOL CLEARANCE CERTIFICATE

RESEARCH PROTOCOL CLEARANCE CERTIFICATE

BOARD REGISTRATION NUMBER	FWA 00026661/IRB 00011253						
PROTOCOL REFERENCE NUMBER	SHR324/2020						
Type of review	Expedited	X		Full Board			
Name of Organization	Master' student						
Title of study	Predictors and incidence of HIV viremia and virological failure among HIV-infected Adults on First-Line Antiretroviral Therapy in Eswatini: A Nested Case–Control Study.						
Protocol version	1.0						
Nature of protocol	New	X	Amendment		Renewal		Extension
List of study sites	CMIS						
Name of Principal Investigator	Dr. Altaye Kidane						
Names of Co- Investigators	N/A						
Names of steering committee members in the case of clinical trials	N/A						
Names of Data and Safety Committee members in the case of clinical trials	N/A						
Level of risk (Tick appropriate box)	Minimal X		More than minimal		High		
Clearance status (Tick appropriate box)	Approved	X		Disapproved			
Study approval validity period	Start date 21/01/2021		End date 21/01/2022				
Secondary approval validity end dates	Renewal end date		End date				
Signature of Chairperson							
Signing date	21/01/2021						
Secretariat Contact Details	Name of contact officers	Babazile Shongwe					
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21 JAN 2021

List of study sites	**CMIS**
Name of Principal Investigator	**Dr. Altaye Kidane**
Names of Co- Investigators	**N/A**
Names of steering committee members in the	**N/A**
case of clinical trials	
Names of Data and Safety Committee members in the case of clinical trials	**N/A**
Level of risk (Tick appropriate box)	Minimal
	X

3. HIV viremia and virological failure among Adults on first line ART in Swaziland

Electronic Medical Record Abstraction Form

Study ID number: ☐☐☐

A. Client Baseline information: To determine the relative risk of viremia and virological failure in relation to client's characteristics.

1.	Date of Birth (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __ ☐ Missing
2.	Sex	☐ M ☐ Missing ☐ F
3.	Date Tested HIV Positive (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __ ☐ Missing
4.	HIV clinic enrollment date (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __ ☐ Missing
5.	Age (years) at enrollment into HIV care	__ __ years ☐ Missing
6.	Weight (Kg) at enrollment into HIV care	__ __ __ Kg ☐ Missing
7.	Height (cm) at enrollment into HIV care	__ __ __ cm ☐ Missing
8.	BMI at enrollment	☐ >30 ☐ 25-29 ☐18-24 ☐< 18 ☐ Not recorded
9.	Date of ART initiation (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __ ☐ Missing
10.	Weight (Kg) at ART initiation	__ __ __ Kg ☐ Missing
11.	Height (cm) at ART initiation	

		__ __ __ cm ☐ Missing
12.	BMI at ART initiation	☐ >30 ☐ 25-29 ☐18-24 ☐< 18 ☐ Not recorded
13.	CD4+ results at ART initiation	_____cells/mm3 ☐ Missing
14.	WHO clinical stage at ART initiation	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ Missing

B. Client Follow up information: 1) To determine incidence of viremia among HIV-infected adults; 2) To determine incidence of virological failure among HIV-infected adults**.**

15.	HIV clinic last visit date (DD/MM/YYYY)	__ __/ __ __/ __ __ __ __ ☐ Missing
16.	Weight (Kg) at last visit date	__ __ __ Kg ☐ Missing
17.	Height (cm) at ART initiation	__ __ __ cm ☐ Missing
18.	BMI at ART initiation	☐ >30 ☐ 25-29 ☐18-24 ☐< 18 ☐ Not recorded
19.	CD4+ results at last visit date	_____cells/mm3 ☐ Missing
20.	WHO stage at last visit date	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ Missing
21.	Viral load results at last visit date	_____ copies/mL ☐ Missing
22.	Last Viral load result date	_____ copies/mL ☐ Missing

23.	Viral load results and previous dates	_____ copies/mL; __ __ / __ __ / __ __ __ __ _____ copies/mL; __ __ / __ __ / __ __ __ __ _____ copies/mL; __ __ / __ __ / __ __ __ __

C. Pharmacy medication pick-up data: To determine the relative risk of viremia and virological failure in relation to ART combinations in the first line regimen, clients' characteristics including clinical variables at the time of ART initiation and duration on ART.

24.	Date first line ART medicines first collected	__ __ / __ __ / __ __ __ __ ☐ Missing
25.	First line ART regimen	☐ TDF + 3TC + EFV ☐ Missing ☐ TDF + 3TC + NVP ☐ AZT + 3TC + EFV ☐ AZT + 3TC + NVP ☐ ABC + 3TC + EFV ☐ ABC + 3TC + NVP ☐ TDF + 3TC + DTG ☐ AZT + 3TC + DTG
26.	Most recent 1st line ART medicine collection date	__ __ / __ __ / __ __ __ __ ☐ Missing
27.	Last first line ART regimen	☐ TDF + 3TC + EFV ☐ Missing ☐ TDF + 3TC + NVP ☐ AZT + 3TC + EFV ☐ AZT + 3TC + NVP ☐ ABC + 3TC + EFV ☐ ABC + 3TC + NVP ☐ TDF + 3TC + DTG ☐ AZT + 3TC + DTG ☐ Other:_____
28.	Date second line ART medicines first collected	__ __ / __ __ / __ __ __ __ ☐ Missing

29.	Current second line ART regimen	☐ TDF + 3TC + LPV/r ☐ Missing ☐ TDF + 3TC + ATV/r ☐ AZT + 3TC + LPV/r ☐ AZT + 3TC + ATV/r ☐ ABC + 3TC + LPV/r ☐ ABC + 3TC + ATV/r ☐ Other_____ _____