CHARACTERISTICS AND OUTCOMES OF HIV-INFECTED CHILDREN ON ANTIRETROVIRAL THERAPY REFERRED FOR VIROLOGICAL FAILURE

Investigator

Xitshembiso Confidence Magomani

Student number: 0600404G

Department of paediatrics and child health

University of the Witwatersrand, Johannesburg, South Africa

Supervisors

Dr Nosisa Sipambo

Department of Paediatrics and Child Health

University of the Witwatersrand

Dr Fikile Cynthia Mabena

Department of Paediatrics and Child Health

University of the Witwatersrand

May 2024

Research report to be submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, as part of the requirements for the degree of Master of Medicine in Paediatrics.

Declaration

I, Xitshembiso Confidence Magomani declare that this research report is my own original work. It is being submitted for the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. The report has not been submitted before for any degree or examination at this or any other institution.

Signature of the candidate

04th day of June 2024 in Johannesburg

Acknowledgements

I wish to express my utmost gratitude to my supervisors, Dr Nosisa Sipambo and Dr Fikile Mabena, for their expert guidance, patience, and steady support during this entire process. I would like to thank the Harriet Shezi Children's Clinic staff for assisting me with patient records retrieval. I am grateful to my husband Vukosi Maluleke and my parents for their continuous support.

Dedication

I dedicate this work to my children; may they always continue learning.

Table of Contents

1. Abbreviations

ART: Antiretroviral Therapy

AZT: Zidovudine

CLHIV: Children Living with HIV

D4T: Stavudine

EFV: Efavirenz

FTC: Emtricitabine

HIV: Human Immunodeficiency Virus

HSCC: Harriet Shezi Children's Clinic

LPV/RTV: Lopinavir/Ritonavir

NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors

NVP: Nevirapine

PI: Protease Inhibitors

TDF: Tenofovir

TB: Tuberculosis

UNAIDS: The Joint United Nations Programme on HIV/AIDS

VF: Virological Failure

VL: Viral Load

WHO: World Health Organization

2. List of Tables

3. List of Figures

4. Submissible paper

Characteristics and outcomes of Children Living with HIV on antiretroviral therapy referred to a tertiary site for virological failure.

X C Magomani[1] MBBCh; **F C Mabena**[1] MBChB, MMed Pead; **N Sipambo**[1] MBBCh, MMed Pead

[1] Department of Paediatrics and Child Health, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg, South Africa.[1]

Corresponding Author: XC Magomani (xitshembisomagomani@gmail.com)

4.1 Abstract

Background: The advent of Anti-Retroviral Therapy (ART) has substantially improved virological outcomes for individuals globally living with Human Immunodeficiency Virus. However, the rates of virological suppression in children and adolescents continue to lag behind those observed in adults.

Objectives: This study aims to describe the characteristics and outcomes of children on ART referred to Hariet Shezi Children's Clinic, Chris Hani Baragwanath Academic Hospital for virological failure. Additionally, the research seeks to identify factors associated with virological failure in this specific population.

Methods: Conducted as a retrospective review, this study examined the records of children living with HIV aged 0-14 years, referred to Hariet Shezi Children's Clinic, for management of virological failure between 01 January 2010 and 31 December 2019.

Results: Among the 105 patients meeting the inclusion criteria, the median age at referral was 11 years (IQR 6.7-13 years), with 51.4% being male. Significant proportions (37.3%) of patients above the age of 8 were unaware of their HIV status. At referral, 35.2% were on a Non-nucleoside reverse transcriptase inhibitor-based regimen, and 82% had a VL log 10 exceeding 4. Notably, 86.5% of those on NNRTI were switched to a second-line regimen, compared to 4.4% on Protease inhibitor-based regimens. Despite differences in interventions, virological outcomes were similar at 6 ±3 months. Approximately 56.2% of patients received adherence counselling in conjunction with other interventions, indicating that virological failure in the majority resulted from sub-optimal adherence and additional factors. A significant reduction in median viral load was observed at 6 ±3 months and 12±3 months post-intervention ($P < 0.001$).

Conclusion: Viral suppression remains a challenge in the paediatric and adolescent populations. Addressing this challenge necessitates a multifaceted approach involving various interventions. Greater resource allocation towards the optimization of viral suppression rates in children and adolescents living with HIV is imperative.

KEYWORDS: Children living with HIV, Anti-Retroviral Therapy, Virological Failure, Virological Outcomes

4.2 Introduction

As of the end of 2022, South Africa recorded an estimated 7.6 million individuals living with Human Immunodeficiency Virus (HIV), with 230,000 being children aged 0-14 years [1]. The absence of Antiretroviral Therapy (ART) results in significant mortality for paediatric patients, as half of those born with HIV die by the age of two years, and 80% succumb by the age of five years [2]

In alignment with the third Sustainable development goal to eradicate HIV/AIDS by 2030, the Joint United Nations Programme on HIV/AIDS (UNAIDS) has revised the 90-90-90 targets which were not met in 2020, aiming for 95-95-95 testing and treatment outcomes by 2025. [3] This entails ensuring that 95% of people living with HIV are aware of their status, 95% of those aware are on ART, and 95% of those on ART achieve viral suppression. While Vertical Transmission Prevention initiatives have resulted in a reduced vertical transmission rate of 3% and an improved early infant diagnosis rate of 93% in South Africa in 2022, [1] children aged 0-14 lag behind adults in achieving the 95-95-95 targets. [4] In 2022, the adult population reached a 94%-80%-92% adherence to these targets, while children fell short at 82%-65%-67%. [1]

Virological failure (VF) in the paediatric population poses a substantial challenge, with varied definitions globally. The World Health Organization (WHO) defines VF as a plasma Viral Load exceeding 1000 RNA copies/ml on two consecutive blood samples, taken at least three months apart, despite adequate adherence support after a minimum of six months on ART [5] South African HIV guidelines have adopted this definition, [6] recommending Viral load (VL) testing at 6 months, 12months, and at least annually thereafter for virally suppressed patients. VF rates are considerably high in low middle-income countries, with a South African study reporting a cumulative VF incidence of 26% one-year post ART initiation, escalating to 36% five years later. [7] Similar trends were observed in other African paediatric studies. [8,9]

Children living with HIV (CLHIV) often exhibit high viral loads and rapid disease progression, making early virological suppression crucial. [10,11] Demographic and clinical factors contribute to increased VF rates, with poor adherence and drug resistance identified as key culprits in some paediatric cohorts. [12,13] Factors affecting adherence in this population include regimen complexity, drug side effects, large pills, unpalatable syrups, stigma, disclosure, caregiver type, and changes related to adolescence. [14]

Understanding the characteristics and outcomes of children experiencing VF is paramount for improving virological outcomes in the context of South African CLHIV. The study objectives were to describe the demographic and clinical

characteristics and outcomes of patients referred to Harriet Shezi Children's Clinic (HSCC) for virological failure and determine the factors associated with virological failure.

4.3 Methods

This retrospective descriptive study focused on CLHIV undergoing antiretroviral therapy referred for the management of virological failure at the HSCC, which is located at Chris Hani Baragwanath Academic Hospital, a tertiary level hospital in Soweto, Johannesburg, South Africa. The clinic provides a service to children and adolescents with advanced HIV disease, including those with VF and resistance to ART requiring second- or third-line ART. The study sample comprised of CLHIV aged 0 to 14 years who were referred to HSCC from surrounding clinics and hospitals for VF management between 01 January 2010 and 31 December 2019. Patients were considered to have VF if they had at least 2 viral load measurements of >1000 copies/ml taken at least 3months (+/-2) apart with the first VL taken at least 6 months after ART initiation. [6] Hospital records of all children aged 0-14 years who were managed at HSCC for VF during the study period were retrieved from the HSCC records using the database-generated list. Records of referred patients with sufficient routinely collected data required for the analysis were selected and those of children who developed VF while at HSCC were excluded. Selected data was entered into a Microsoft excel prepared datasheet and included the participants baseline and referral information, intervention strategies provided and VL results. Baseline information described parameters relating to the time at or just before ART initiation and included gender, age at initiation, baseline CD4 count, WHO disease stage, Tuberculosis (TB) status, Prevention of mother to child transmission (PMTCT) exposure status (maternal or infant), and initial ART regimen. Referral information described parameters the first time the patient was seen at HSCC after a referral from surrounding clinic/hospital and included current age, type of caregiver, disclosure status, TB status, duration on ART, duration of VF, recent VL and current ART regimen. ART duration was described as the time from ART initiation until referral to HSCC and duration of VF as the period from the first VL of more than 1000 after at least 6 months on ART to time of referral.

During the study period, significant changes occurred in the South African HIV and PMTCT management guidelines. Initially, in the early ART era under the 2004 guidelines. eligible pregnant women received Lamivudine(3TC) +Stavudine(D4T) +NVP as a preferred regimen. Single dose Nevirapine(NVP) was still the backbone for PMTCT, with a stringent criteria for lifelong ART eligibility for both pregnant women and children.[15] First line regimen for children less than 3 years was D4T+3TC+Lopinavir/Ritonavir(LPV/RTV) and 3TC+D4T+Efiverenz(EFV) for those older than 3 years.[15]Under the 2010 South African PMTCT and HIV management guidelines , pregnant woman with CD4 counts of =/< 350 and those with WHO clinical stages 3 and 4 regardless of CD4 counts were eligible for lifelong ART and were started on Tenofovir(TDF)+3TC or Emtricitabine(FTC) +NVP while those not eligible were started on Zidovudine(AZT) from 14 weeks of gestation and given single dose NVP and AZT 3 hourly during labour and a single dose of TDF and FTC intrapartum.[16] Infant prophylaxis comprised of NVP for 6 weeks or for the duration of breast feeding. Eligibility for children included all infants less than 1 year of age, Children 1 – 5 years with clinical stage 3 or 4 or CD4 ≤ 25 % or absolute CD4 count < 750 cells/µl and Children >

5 years to 15 years with clinical stage 3 or 4 or CD4 < 350 cells/µl.[17] From 2015 to 2019, all pregnant women were eligible for lifelong ART from the first antenatal care visit regardless of CD4 count and WHO clinical stage, receiving a fixed dose combination of TDF+3TC /FTC + EFV if no contraindications to the preferred regimen or would receive intrapartum prophylaxis as before if not on lifelong ART.[18] In these guidelines, exposed infants were stratified into low or high-risk categories and received either NVP alone for 6 or 12 weeks or NVP plus AZT for 6 weeks. All infants and children under the age of 5 years were eligible for lifelong ART and those 5 to 15 years were eligible if they were WHO stages 3 or 4 or had CD4 counts less than 500. Guided by evidence supporting the universal test and treat principle a directive was issued in 2016 removing all age, WHO clinical staging and CD 4 count eligibility criteria. For the duration of the study period, first line ART regimens for infants less than 3 years were PI-based and NNRTI-based for children older than 3 years. [16,17-18] Children failing first line NNRTI-based regimens were changed to a PI-based regimen and those failing PI-based regimens were managed in consultation with an expert in paediatric ART management.

Intervention strategies which were provided at HSCC were collected from the records and included one or a combination of the following: regimen switch, regimen modification, adherence counselling and HIV status disclosure facilitation. Regimen switch entails switching a patient who is failing either first or second-line ART regimen to a different class of antiretroviral drugs while modification simplifies a regimen using same class drug substitution, fixed dose combinations or daily dosing. Disclosure status was classified as none if the patient had not been given any form of information regarding their HIV status, partial if the disclosure process was not yet completed and full If the patient had been informed and was fully aware of their HIV status. Adherence counselling was conducted by a team of experienced adherence counsellors in HSCC. The documented VL result at 6 +/- 3 months and at 12 +/- 3 months following an intervention at HSCC was used for the description of virological outcomes.

4.3.1 Statistical Analysis

Collected data was imported from excel and analysed in Stata version 15. Frequencies and percentages were used to describe categorical variables. Continuous variables were described using median and interquartile range. Pearson's chi squared tests were used to compare proportions, and where data was sparse, Fisher's exact tests were used instead. For comparison of medians for repeated measures of viral load in the same child at different time points, signed rank tests (referral vs. 6± months and referral vs. 12± months) and Friedman's nonparametric two-way analysis of variance, Kendall's Coefficient of Concordance (across all three VL tests – at referral, 6 and 12 months) were used to compare medians. Statistical significance was set at 5%.

4.3.2 Ethics

The research protocol was approved by the University of the Witwatersrand Human Research Ethics Committee with the ethics clearance certificate number M200722.

4.4 Results

A total of 982 patients were managed for VF at HSCC during the study period and 105 of these patients fulfilled the study inclusion criteria (Figure 1).

Figure 1: Flow diagram indicating selection of study participants referred for management of VF at HSCC, Jan 2010-Dec 2019.

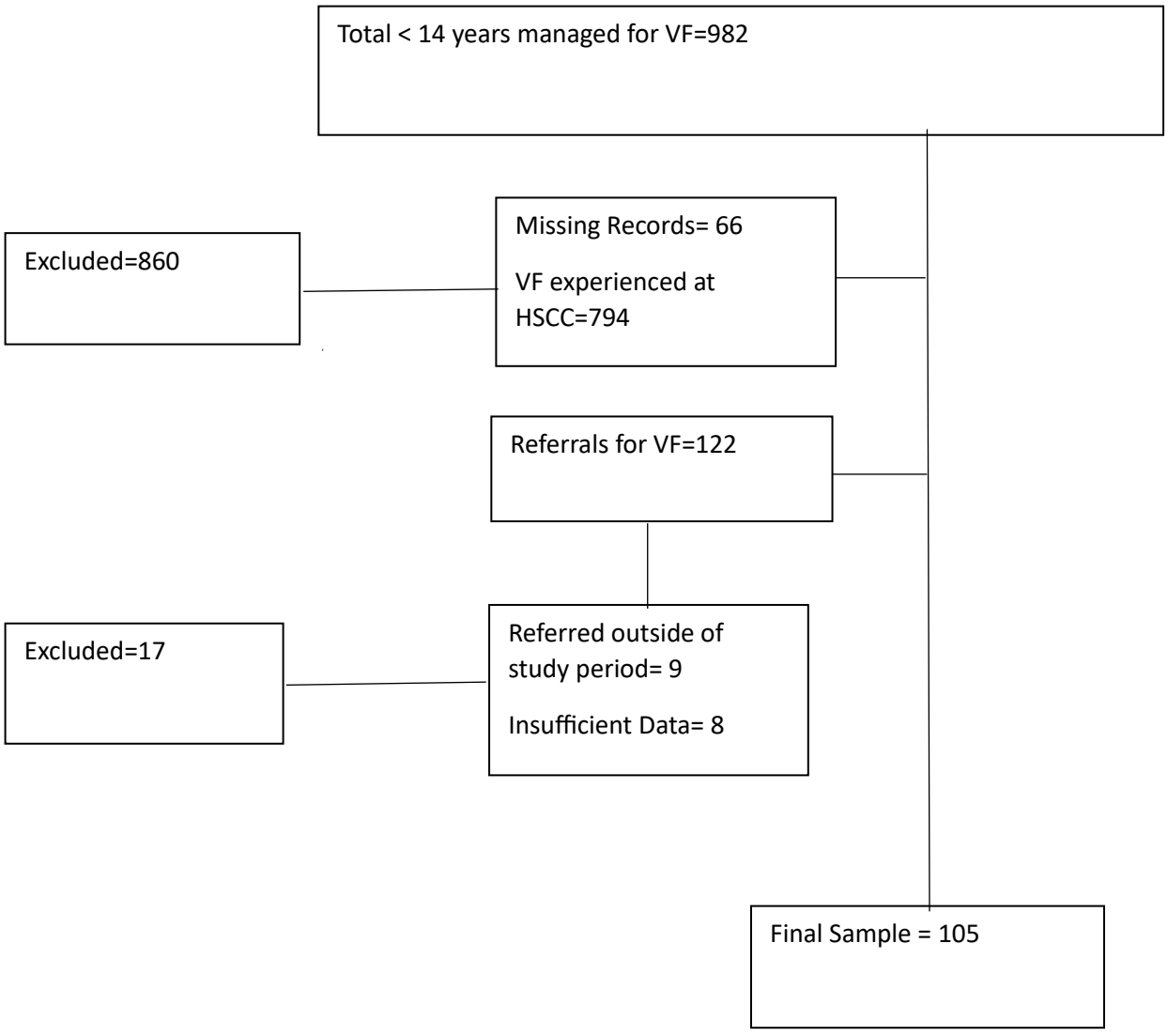


Table 1: Baseline characteristics amongst Children Living with HIV referred for virological failure at Harriet Shezi Children's Clinic, Jan 2010-Dec 2019.

Variables at initiation			
Age at ART initiation	N =105	***Gender***	N=105
Median (IQR)	***3 (1-6) years***	Female	51 (48.6%)
0 - 3 years	47 (44.8%)	Male	54 (51.4%)
4--10 years	53 (50.4%)	***TB co-infection***	N= 105
11-14 years	5 (4.8%)	No	72 (68.6%)
CD4 counts children < 5 years	N-=38	Yes	33 (31.4%)
Median CD4 count percentage (IQR)	**11.6 (6.34-18.8) %**	*Initial ART regimen*	N=105
0-15%	26(67.6%)	**NNRTI-based**	**56 (53.3%)**
16-25%	6(16.2%)	3TC+D4T+EFV	20
>25%	6(16.2%)	3TC+ABC+EFV	35
CD4 counts children > 5 years	N=23	3TC+AZT+EFV	1
Median CD4 absolute count (IQR)	**171(27-305)**	**PI –based**	**49 (46.7%)**
0-200	14 (60.9%)	3TC+ABC+LPV/RTV	29
200-500	6 (26.1%)	3TC+D4T+LPV/RTV	20
>500	3 (13.0%)	Duration on ART	N=105
WHO Clinical stages	N=99	**Median (IQR) years**	**6 (2-8)**
I	14(13.21%)	</= 2	29 (27.6%)
II	6(5.55%)	>/= 2	76 (72.4%)

III	56(52.83%)		
IV	18(16.98%)		

Abbreviation(s): ART antiretroviral therapy, IQR interquartile range, TB tuberculosis, NNRTI non-nucleoside reverse transcriptase inhibitor, NRTI nucleoside reverse transcriptase inhibitor, PI protease inhibitor, N number. NVP Nevirapine AZT Zidovudine LPV/RTV lopinavir/Ritonavir 3TC lamivudine, EFV Efavirenz d4t Stavudine ABC Abacavir

Table 2: Referral characteristics amongst Children Living with HIV referred for virological failure at Harriet Shezi Children's Clinic, Jan 2010-Dec 2019.

Variables at referral			
Age	N=105	Type of current regimen	N=105
Median (IQR)	***11 (*6.7-13) years**	**NNRTI-based**	37 (35.2%)
0 - 3 years	11 (10.5%)	3TC+D4T+EFV	12
4--10 years	31 (29.5%)	3TC+ABC+EFV	21
11-14 years	63 (60.0%)	3TC+AZT+EFV	4
Viral load at referral (Log10)	**N=105**	**PI –based**	68 (64.8%)
Median (IQR)/Log_{10}	**4.8 (4.0-5.3)**	3TC+ABC+LPV/Ritonavir	34
<4	23 (21.9%)	3TC+D4T+LPV/Ritonavir	22
≥4	82 (78.1%)	*3TC+AZT+LPV/Ritonavir*	8
		AZT+ABC+ LPV/Ritonavir	4
Current ART regimen	N=105	TB co-infection	N=105
First line	80 (76.2%)	Yes	4 (3.8%)
Second line	25 (23.8)	No	101 (96.2%)
Caregiver type	N=105	***HIV status disclosure to children* ≥8 years**	**N=74**
Both parents	11(10.5%)	**None**	**38(51.4%)**
One parent	57 (54.3%)	**Partial**	**7(9.4%)**
Non biological caregiver	37 (35.2%)	**Full**	**29(39.2%)**

Abbreviation(s): ART antiretroviral therapy, IQR interquartile range, TB tuberculosis, NNRTI non-nucleoside reverse transcriptase inhibitor, NRTI nucleoside reverse transcriptase inhibitor, PI protease inhibitor, N number. NVP Nevirapine AZT Zidovudine LPV/RTV lopinavir/Ritonavir 3TC lamivudine, EFV Efavirenz d4t Stavudine ABC Abacavir

Table 1 and Table 2 summarize the characteristics of patients at baseline and referral respectively. Out of the 105 patients, 54(51.4%) were male. The analysis revealed a median age of 3 years (IQR 1-6) at initiation, with more than half of the study participants initiated between the ages 4-10 years. More than 80% of patients under the age of 5 years exhibited baseline CD4 counts of less than 25 %. Among children aged over 5 years, 14/23 (60.9%) had CD4 counts below 200. Most patients commenced ART while clinically presenting at WHO HIV stages III and IV (76/99, 76.7%). The median duration of VF before referral to HSCC was 12 months (IQR 6-24). Patients had been on ART for a median duration of 6 years (IQR 2-8) at the time of referral.

The median age at referral was 11 years (IQR 6.7-13 years), with 60% of study participants being in the age group 11-14 years at referral. Majority of children were referred with extremely high viral loads with almost 80% exhibiting a viral load log of greater or equal to 4. In terms of regimen specifics, the predominant starting regimen was NNRTI-based. Most patients on a PI-based regimen at referral had initially been initiated on it as part of first-line therapy (47/68, 69.1%). More than one-third of patients were still on a failing NNRTI-based regimen at the time of referral. PMTCT status was documented for 69 individuals, with 38 (55%) having ART exposure through either maternal or infant PMTCT. In instances of co-morbid TB disease, 33 children had TB at ART initiation and 4 developed TB infection while on ART after developing VF. 21/33 patients (63.6%) of those with co- morbid TB at initiation were on a PI-based regimen while receiving TB therapy and all 4 patients with TB at referral were on a PI-based ART regimen, reflecting considerations for comorbidity management.

Figure 2: disclosure status patterns for children amongst children 8 years and older Living with HIV referred for virological failure at Harriet Shezi Children's Clinic, Jan 2010-Dec 2019.

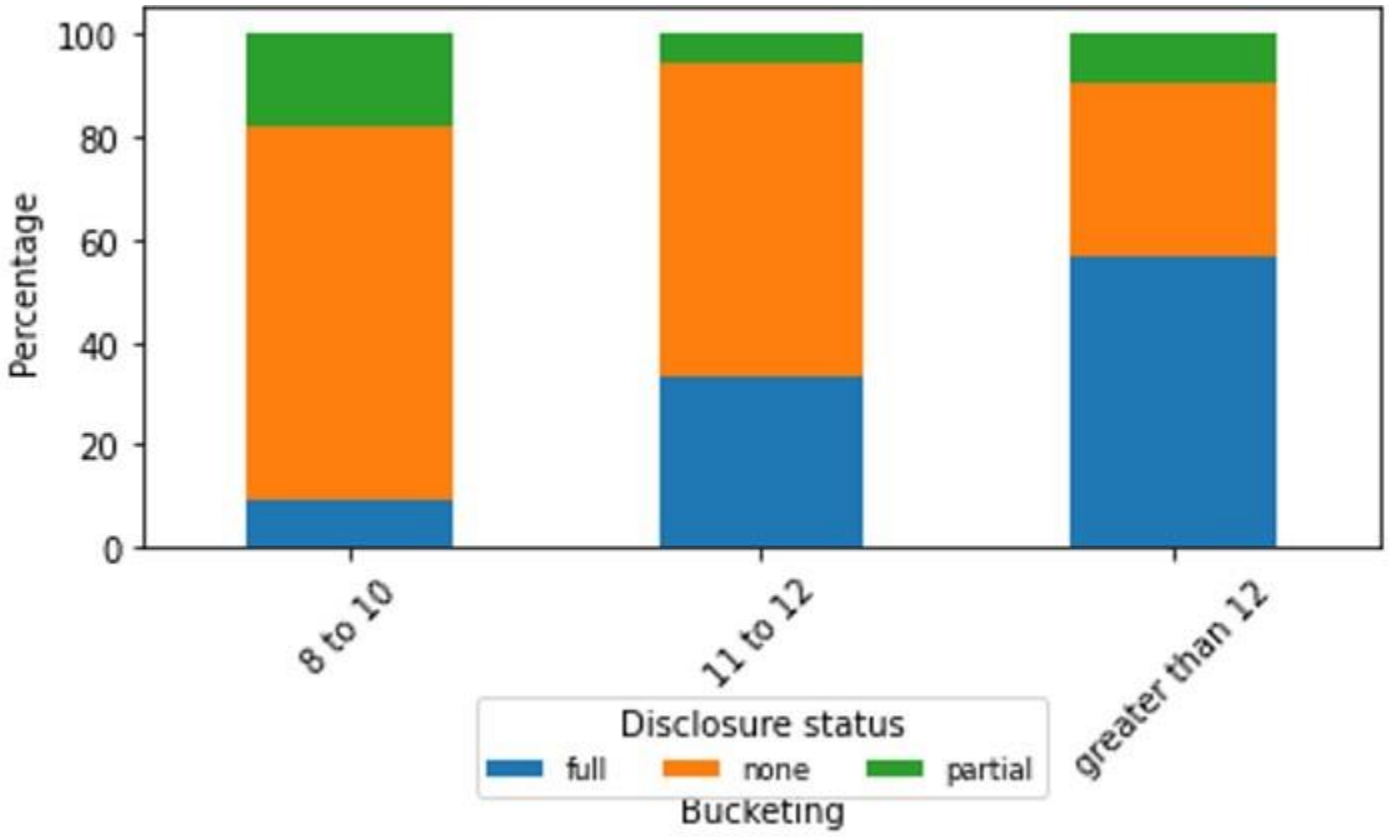
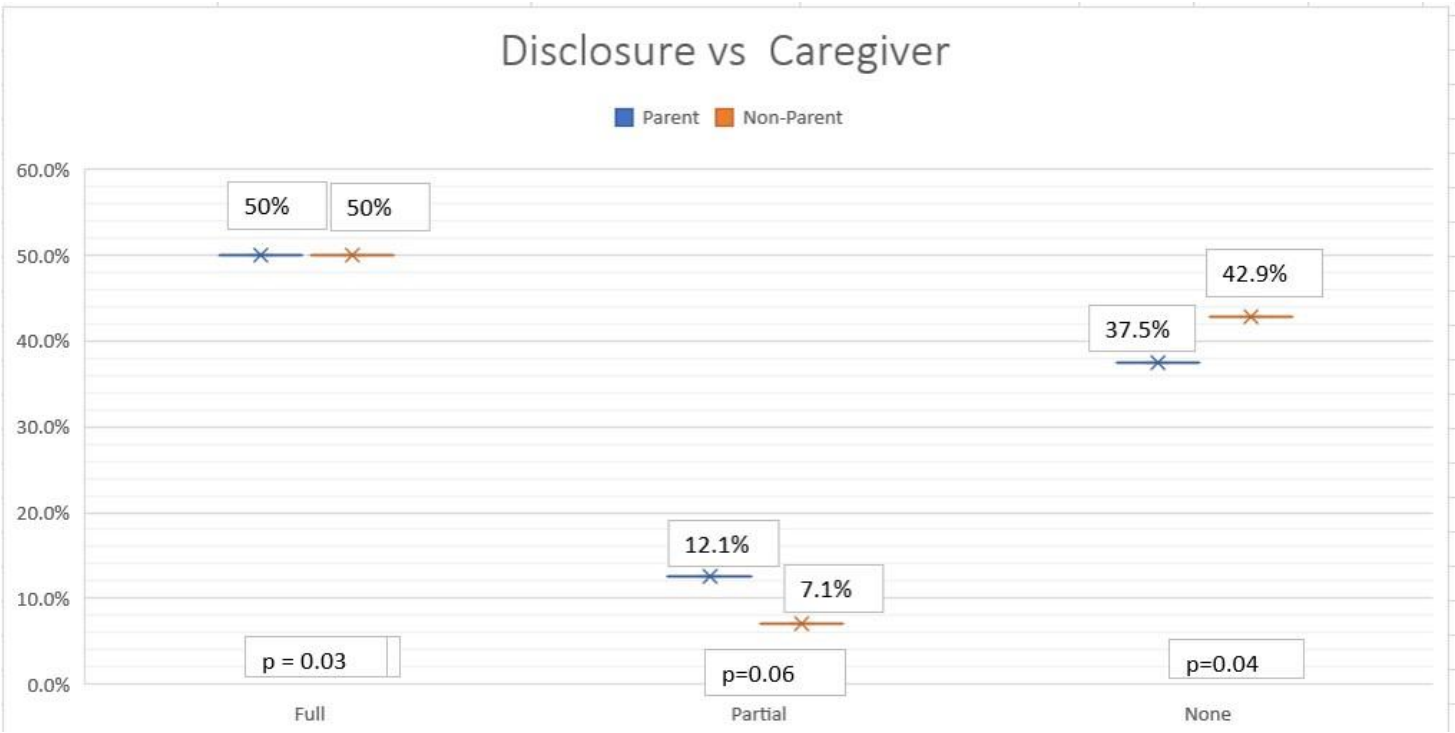


Figure 3:Comparison of HIV status disclosure patterns in those with biological parent and non-biological parent caregivers in children 8 years and older.



Regarding familial support, almost two-thirds of the patients were cared for by a biological parent (68/105, 64.8%), with 11/68 (16.2%) living with both biological parents. The disclosure of HIV status was analyzed specifically for children aged 8 years and older. The findings unveiled that among this subgroup, 29/74 (39.2) had undergone full disclosure, 7/74(9.4%) had received partial disclosure, and 38/74(51.4%) had received no disclosure at all. Figure 2 compares disclosure status in age subgroups 8-10, 11-12 and more than 12 years and reveals that disclosure increased with age as there were more full disclosures in those more than 12 years compared to other younger age groups. A comparative analysis of disclosure status was conducted between parent and non-parent caregivers within the same age group. The results, depicted in Figure 3, indicate no significant difference in the proportions of disclosure among the various caregivers.

Table 3: Intervention strategies amongst children living with HIV managed for virological failure at Harriet Shezi Children's Clinic, Jan 2010-Dec 2019.

Intervention	**N=**105
Regimen switch	35 (33.3%)
Regimen modification	35 (33.3%)
Adherence counselling	94 (89.5%)
Disclosure facilitation	26 (24.8%)

Table 3 delineates the frequencies of employed intervention strategies for all patients under consideration. Adherence counselling emerged as a primary intervention for a considerable proportion of patients, provided as the sole strategy for 35/105 (33.3%) of individuals. Majority of patients, 59/105 (56.2%) received adherence counselling along with another intervention, which included regimen switch/modification or disclosure facilitation. Additionally, 11/105 (10.5%) of patients underwent alternative interventions, such as regimen adjustments or disclosure facilitation, without adherence counselling. Upon closer examination, a noteworthy disparity in the utilization of adherence counselling was observed between two distinct age groups. Specifically, higher proportions of adherence counselling were administered to children older than 10 years, in contrast to those aged less than 10 years ($P<0.001$).

Figure 4: Viral load outcomes following intervention amongst children living with HIV referred to Harriet Shezi Children's Clinic for virological failure Jan 2010-Dec 2019.

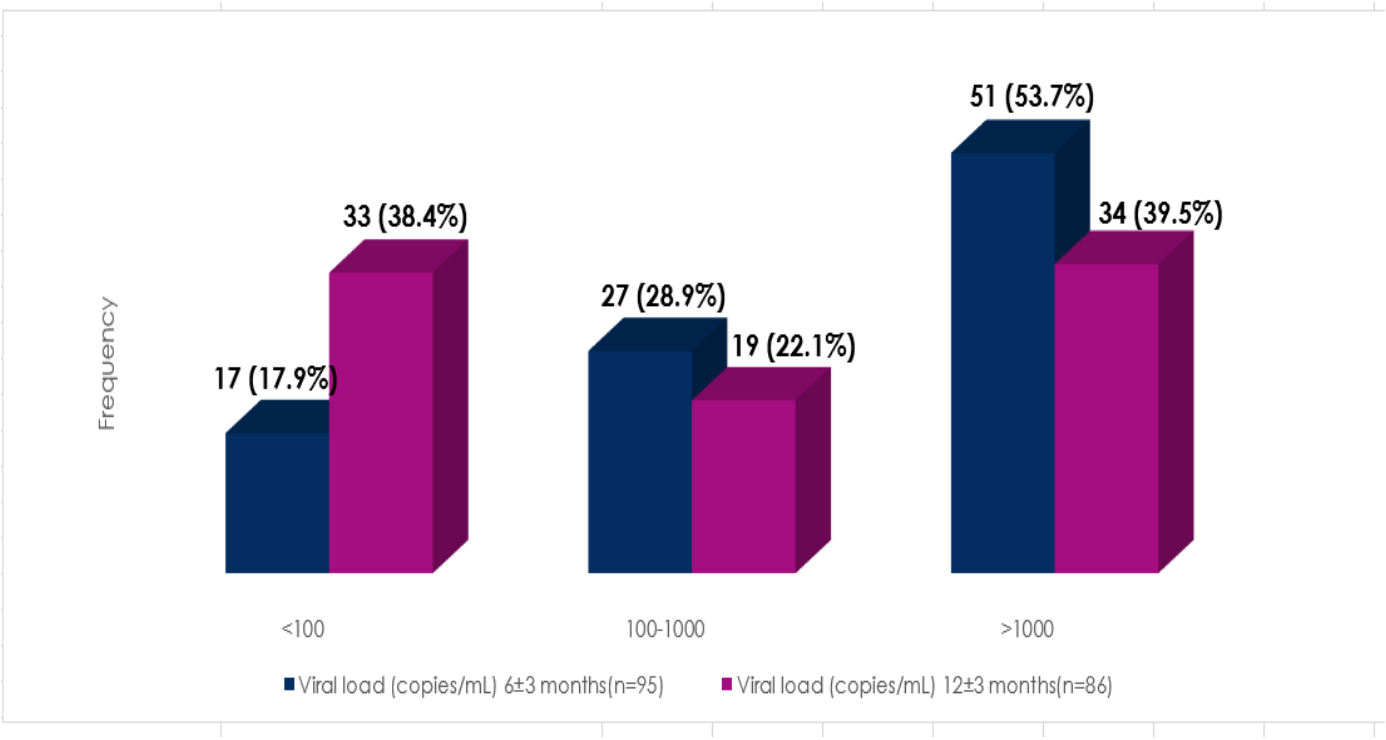


Table 4: Comparison of Viral Load (log10) at referral vs. at 6±3months and at 12±3months following intervention

Viral Load (log10)	**Median (IQR)**	**Signrank test** **VL referral vs. 6±3 months**	**Signrank test** **VL referral vs. 12±3 months**	**Friedman test** **VL referral vs. 6±3 months vs. 12±3 months**
VL at referral	4.8 (4.0-5.3)	p<0.001	p<0.001	P=0.004
6 months	3.2 (2.3 – 4.5)			
12 months	2.8 (1.8 – 4.3)			

Figure 4 provides a comprehensive illustration of patient outcomes based on Viral Load (VL) measurements obtained at 6±3 months and 12±3 months post-intervention and shows the number of patients who successfully suppressed to below 100 and 1000 copies/mL, juxtaposed against those who failed to suppress, exhibiting VL above 1000 copies/mL after the intervention. Out of the 95 participants who had a documented VL at 6±3 months, 44(46.3%) achieved virological suppression with VL below 1000 copies/ML. There was more improvement on VL suppression rates at 12±3 months with 52 out of 86 (60.5%) of those with a documented VL achieving viral suppression with majority attaining VL levels of less than 100. Table 4 presents the median VL at referral, 6±3 months, and 12±3 months, offering a comparative analysis of the median VL across these three crucial time points. The median VL at 6±3 months and 12±3 months were significantly lower than at referral ($p<0.001$) when compared using a Signrank test. A comparison of median VL across the three time points (referral, 6±3 months and 12±3 months) using the Friedman test showed a significant reduction in VL ($P<0.004$).

Table 5: Comparison of intervention strategies and Viral Load suppression amongst children on NNRTI and PI based regimens managed for virological failure at Harriet Shezi Children's Clinic, Jan 2010-Dec 2019.

Interventions N= 105	**NNRTI based regimen n= 37**	**PI based regimen n=68**
Regimen switch	32/37(86.5%)	3/68(4.4%)
Adherence counselling	28/37(75.5%)	66/68(97.1%)
Regimen Modification	4/37(10.8%)	31/68(45.6%)
Disclosure facilitation	12/37(32.4%)	14/68(20.5%)
Viral suppression (VL<1000)	**NNRTI based regimen**	**PI based regimen**
6± 3months n=44 P value 0.46	21/44(44.7%)	23/44(52.2%)
12± 3months n=52 P value **0.043**	16/52(30.8%)	36/52(69.2%)

In-depth comparisons of interventions and viral suppression rates in patients on NNRTI and PI based regimens are shown in Table 5, shedding light on potential regimen-specific variations in response to interventions. While those referred on NNRTI-based regimens showed higher regimen switch rates (86.5%), PI-based regimens exhibited lower switch rates (4.4%) but received more adherence counselling 66/68(97.1%) and regimen modification 31/68(45.6%). Despite significant differences in interventions between the two regimens, the VL suppression proportions at 6± 3months were noticeably similar. There was a statistically significant higher VL suppression rate at 12±3 months (69.2%) for those referred on PI-based regimens compared to those referred on NNRTI-based regimen (30.8%). p=0.043

Figure 5: Comparison of Viral Load outcomes per intervention at 6±3 months.

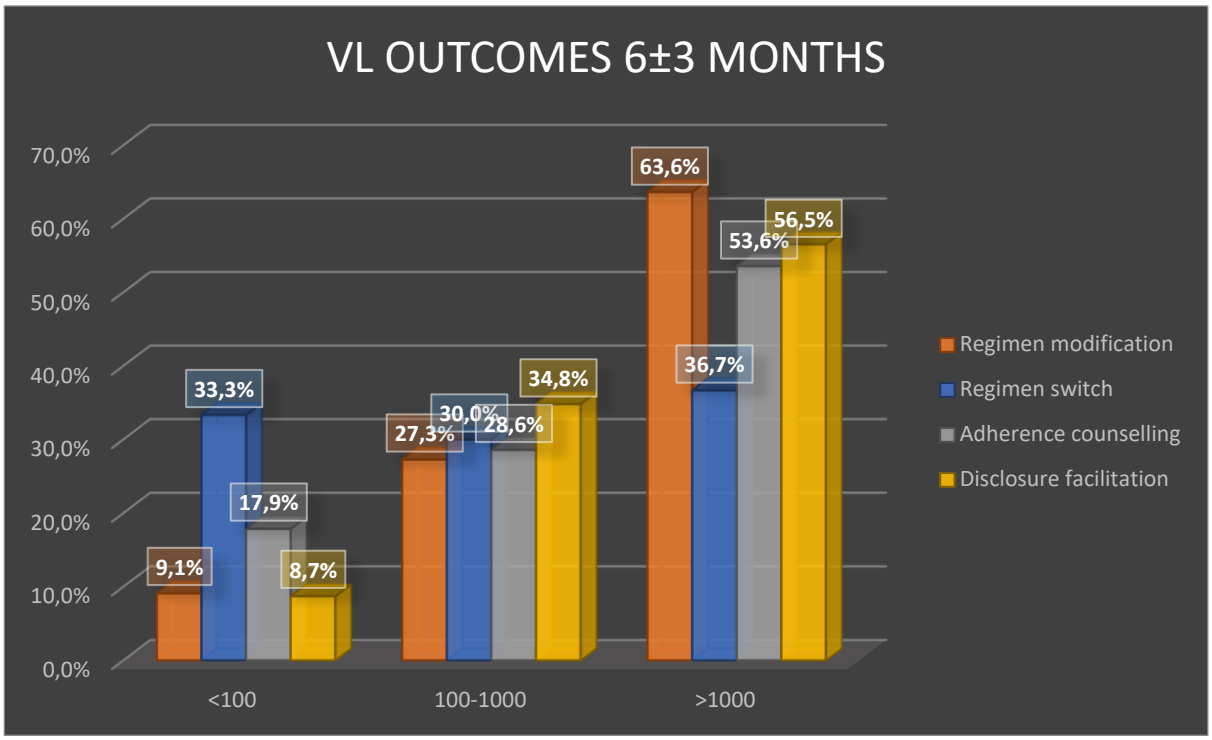
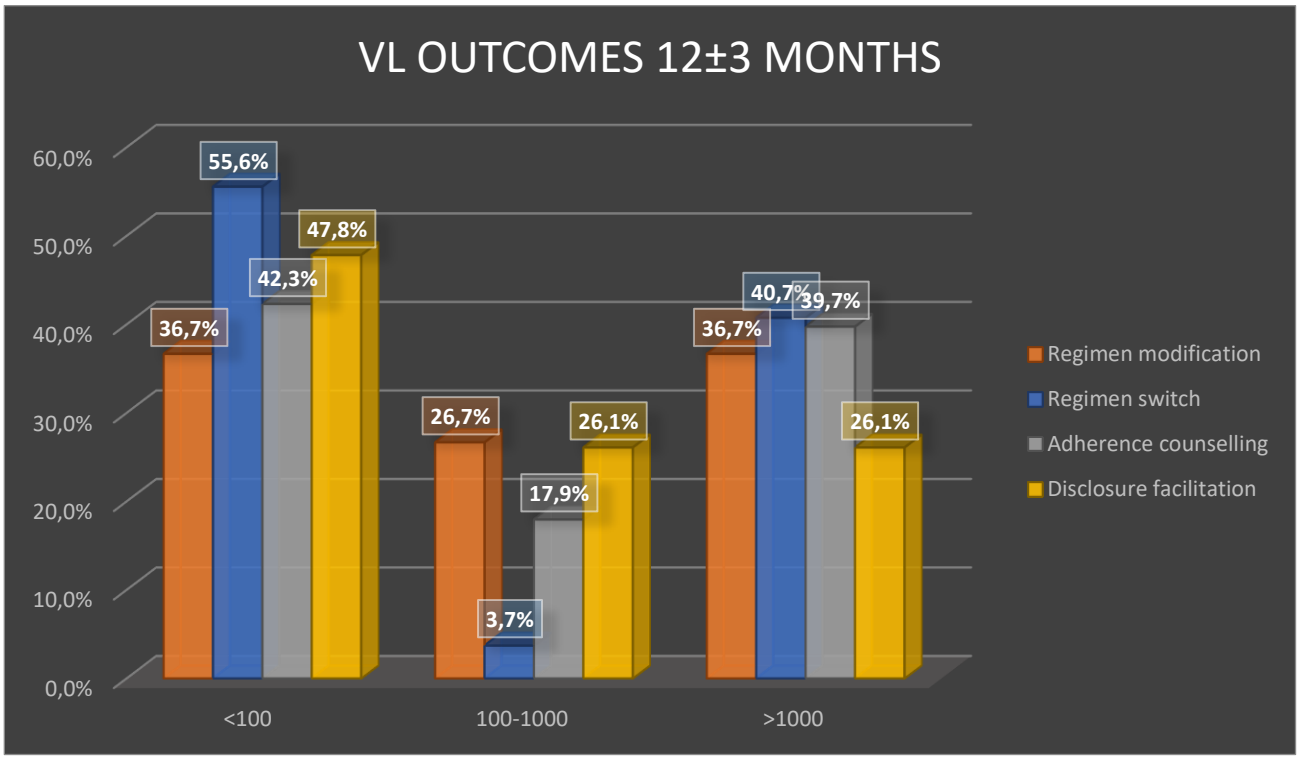


Figure 6: Comparison of Viral Load outcomes per intervention at 12±3 months.



Further insights into VL outcomes at 6±3 months and 12±3 months are delineated for each specific intervention in Figures 5 and 6 respectively. These figures offer a granular view of the impact of individual interventions on VL suppression over time. Regimen switch intervention resulted in more VL suppressions at 6±3 months compared to other interventions, with 63.3% of patients managed with regimen switch attaining VL of less than 1000 copies/mL. Adherence counselling, regimen modification and disclosure facilitation demonstrated improved viral suppression rates at 12±3 months. There was an observed slight decrease in the number of suppressed patients in those managed with regimen switch at 12±3 months. The majority of virologic suppressions at 12±3 months comprised of VL below 100 copies/ mL for all interventions.

4.5 Discussion

CLHIV often present with a high VL burden and early immunologic compromise, emphasizing the critical role of early ART initiation in improving viral suppression and reducing mortality and morbidity.[19] However, in our study, many patients were initiated on ART at advanced WHO clinical stages III and IV, with a median age of 3 years at ART initiation. Furthermore, approximately one-third of study participants had TB at the time of ART initiation, potentially exacerbating weakened immunity and contributing to increased risk of VF as observed in some cohorts. [20,21] The recent South African HIV management guidelines advocate for a test and treat all approach enabling all persons diagnosed with HIV to access ART regardless of age, CD4 counts and clinical stage. [22] With the impressive scale up in ART and the improved early infant testing rate, early ART initiation during infancy can result in rapid virological suppression and improve virologic outcomes in CLHIV.

There was no statistically significant gender difference in our study population. A South African study conducted in CLHIV revealed no difference in the risk of VF between males and females. [23] A contrasting finding from a paediatric cohort in Cameroon linked male gender to increased risk of VF. [24] Female gender was an independent risk factor for VF in a paediatric study conducted in Tanzania. [25] The contrasting studies underscore the need for further research to elucidate the complex relationship between gender and VF.

At the time of referral for VF management, over half of the study participants were older than 10 years, indicating a concerning trend given the challenges associated with puberty and adolescence. This period initiates physical and metabolic changes and potential substance misuse which may affect plasma ART levels, and therefore increasing susceptibility to VF. [26] Notably, a multivariate analysis of a Thai cohort identified age ranges between 10 and 16 years as predictive of VF. [27]

Our data reveals a significantly high utilization of NNRTI-based regimens at initiation. Considering the late age at initiation observed in our study, this is consistent with the South African HIV management guidelines during the period of the study which initiated children older than 3 years on an NNRTI-based regimen. [15,16-17] NNRTI-based regimens pose an increased risk of VF in the light of their associated low genetic barrier to resistance. [28,29-30] Additionally, a significant proportion of those failing NNRTI-based regimens had not been switched to a PI-based second line regimen as per the South African guidelines at a time of referral. [16,17] This finding is consistent with low switching rates previously observed in the South African context. Possible reasons for poor switching. [31] Reasons for delayed switching were not explored in this study but a South African study exploring barriers of switching to second line therapy in children and found that health care workers would delay switching to try preserve future ART drug options in cases where a PI-based regimen is the only other future therapy option, [32]as was the case during our study period. Other observed barriers included poor VL monitoring leading to delays in detecting VF and barriers related to the need to achieve optimal caregiver adherence prior to switching. The new

South African guidelines favour the exclusion of NNRTI-based first line regimens and introduced the use of Dolutegravir -based regimens for use starting from early infancy with the aim of improving virologic outcomes in a paediatric population. [22]

Comparisons between NNRTI and PI-based regimens revealed distinct patterns in interventions and outcomes. Despite the significant differences in interventions, The VL suppression proportions at 6± 3months were similar for both regimens but statistically significant differences at 12±3 months were observed between the two regimens. The dynamics of VF particularly in the context of PI-based regimens merit specific attention. Compared to NNRTIs, major PI mutations occur infrequently. An earlier investigation conducted in our setting revealed that 10.7% of patients failing first line PI -based regimen exhibited major PI mutations. [33] Sub-optimal adherence was suggested as a plausible explanation for VF as half of the patients in the cohort, including some of those who had major PI mutations continued the failing PI-based regimen and still later attained virological suppression. Similarly, VF in children who failed a PI-based regimen was primarily attributed to sub-optimal adherence in a paediatric trial carried out in Asia. [34] Notably, our research identified that majority of the study participants were on a PI-based regimen while undergoing TB therapy. The substantial prevalence of PI-based regimens during TB therapy raises pertinent questions about the potential implications for virological outcomes in our study participants. Rifampicin, which is one of the backbone drugs for TB therapy is a potent stimulator of the cytochrome P450 liver enzyme, which in turn metabolizes Lopinavir and NVP, decreasing their serum concentrations and effectiveness.[35] HIV infection and TB guidelines at the period of our study recommended Ritonavir-boosted PI regimen with adjusted Ritonavir dosage or double PI dosing in older children in order to overcome this effect as Ritonavir inhibits CP450 enzyme.[15,36] Failure to adjust dosing in these instances decreases LPV serum levels thereby increasing the risk of VF. Observations from previous cohorts have hinted at the potential role of drug interactions, drug adverse events, and regimen complexity in contributing to VF in individuals with co-morbid TB. [37, 38] The consistency across cohorts strengthens the validity of our results and emphasizes the importance of tailored interventions for different regimens and the need for a holistic approach to patient care.

Despite these challenges, positive virological outcomes were observed in our study, with a significant decline in the median VL at 6±3 months and 12±3 months post-interventions. Additionally, there was a substantial decline in VL across all three points when comparing VL at referral, at 6±3 months and at 12±3 months. Adherence counselling, often combined with other interventions, emerged as a predominant strategy, suggesting that VF in our cohort may stem from a combination of sub-optimal adherence and additional factors like regimen failure and non-disclosure. Sub-optimal adherence has been consistently linked to increased rates of VF in several cohorts. [20, 26, 39]

Regimen switch intervention yielded favourable outcomes, especially in patients on NNRTI-based regimens more noticeably within the first 6±3months.This is not surprising because of the low genetic barrier of NNRTIs where improved adherence is not expected to result in virological suppression on the same regimen but rather benefits the newer susceptible regimen. However, a modest decline in VL suppression at 12±3 months was observed with regimen switch, comparative with findings of a Latin American study which highlighted the challenges in sustaining positive Virological outcomes over an extended period. [40] This observation emphasizes the need for ongoing vigilance and continual adherence support over time.

Regimen modification, HIV status disclosure, and adherence counselling interventions demonstrated improved virological outcomes at 12±3 months compared to 6±3 months. The positive impact of these interventions on virological suppression reaffirms the importance of comprehensive and sustained support strategies for CLHIV.

Contrary to previous studies where the presence of a biological caregiver predicted favourable adherence, [41, 42] our findings suggest a disconnect between biological caregiver presence and VF rates as most of our patients (64.8%) experienced VF while under the care of at least one biological parent. This unexpected discrepancy prompts further investigation into adherence patterns and caregiver dynamics in our setting.

HIV status disclosure is a critical aspect of adherence. [43] None-disclosure has been identified as a risk factor for VF in previous studies. [44, 45] HIV status disclosure emerged as a concerning issue, with a significant proportion of children 8 years and older not receiving disclosure. Our findings suggest that disclosure increases with age, and this is expected as disclosure is expected to be done in a systematic manner suitable for the age and developmental maturity of the child. [46] Our study additionally revealed uniformity in disclosure practices, irrespective of the caregiver status. Low disclosure rates by biological caregivers were previously revealed in a meta-analysis study. [47] The unexpected different finding in our study could be influenced by our small study sample size. The urgency for improved disclosure practices by all caregivers needs to be emphasized to enhance virological outcomes.

4.6 Study Limitations

While our study provides valuable insights, the retrospective nature of the study introduces inherent limitations that warrant careful consideration. The reliance on historical records inherently carries the risk of missing data and undocumented information, emphasizing the need for cautious interpretation into the study findings. The study sample's relatively small size further limits the generalizability of the findings. The delicate nature of VF may necessitate a larger cohort for comprehensive generalization. Another critical limitation arises from the composition

of our study sample, which exclusively included patients referred with a documented VF and without a comparison group, this restricted the ability to comprehensively assess factors associated with VF. Additionally, the absence of a detailed adherence pattern assessment may limit the depth of our understanding of the factors contributing to VF in our population.

4.7 Conclusion

Viral suppression remains a challenge in children and adolescents. This study highlights the complexities of managing VF in this population. Tailored interventions such as adherence counselling, regimen switch, regimen modification, and disclosure interventions can be employed to address this challenge. Based on the findings of this study, it is evident that more substantial resources need to be allocated to optimize the effectiveness of interventions and strategies aimed at achieving and maintaining viral suppression specifically towards children and adolescents living with HIV.

5. References

1. Joint United Nations Programme on HIV/AIDS. 2023 UNAIDS Data. Geneva: Joint United Nations Programme on HIV/AIDS; October 2023. Available from: https://www.unaids.org/en/resources/documents/2023/2023_unaids_data.(Accessed 31 December 2023).

2. World Health Organization. Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access. Recommendations for a public health approach. Geneva: World Health Organization; 2006. Available from: https://iris.who.int/handle/10665/164255. (Accessed January 10, 2020.)

3. UNAIDS. Global aids strategy 2021–2026: end inequalities. end aids. 2021. Available from: https://www.childrenandaids.org/sites/default/files/2023-11/231130%20UNCEF_HIV_Global_Snapshot_2023UPDATED_0.pdf. (Accessed 02 January 2024).

4. UNICEF. UNICEF HIV Global Snapshot 2023. 2023. Available from: https://www.childrenandaids.org/sites/default/files/2023-11/231130%20UNCEF_HIV_Global_Snapshot_2023UPDATED_0.pdf. (Accessed 02 January 2024.)

5. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2nd ed. Geneva: World Health Organization; 1 June 2016. Available from: https://www.who.int/publications-detail-redirect/9789241549684. (Accessed 10 January 2020).

6. National Department of Health, South Africa. National Consolidated Guidelines for the Management of HIV in Adults, Adolescents, Children, and Infants. February 2020. Available from: https://knowledgehub.health.gov.za/elibrary/national-consolidated-guidelines-management-hiv-adults-adolescents-children-and-infants. (Accessed 15 July 2023).

7. Makatini ZN, Blackard JT, Mda S, Miles P, Towobola O. High virological failure rates in HIV-1 perinatally infected children in South Africa: A retrospective cohort study. S Afr Med J. 2021;111(3):255-9. http://dx.doi.org/10.7196/samj.2021.v111i3.15221

8. Kadima J, Patterson E, Mburu M, Blat C, Nyanduko M, Bukusi EA, et al. Correction: Adoption of routine virologic testing and predictors of virologic failure among HIV-infected children on antiretroviral treatment in western Kenya. PloS one. 2019;14(1).http://dx.doi.org/10.1371/journal.pone.021090899. Huibers MHW, Kityo C, Boerma RS, Kaudha E, Sigaloff KCE, Balinda SN, et al. Long-term virological outcomes, failure and acquired resistance in a large cohort of Ugandan children. The Journal of antimicrobial chemotherapy. 2019;74(10):3035-43. http://dx.doi.org/10.1093/jac/dkz266

10. Schröter J, de Boer RJ. What explains the poor contraction of the viral load during paediatric HIV infection? Journal of Theoretical Biology.2023 Aug;570:111521. http://dx.doi.org/10.1016/j.jtbi.2023.111521

11. Tiemessen CT, Kuhn L. Immune pathogenesis of pediatric HIV-1 infection. Current HIV/AIDS Reports. 2006;3(1):13–9. http://dx.doi.org/10.1007/s11904-006-0003-4

12. Sebunya R, Musiime V, Kitaka SB, Ndeezi G. Incidence and risk factors for first line anti retroviral treatment failure among Ugandan children attending an urban HIV clinic. AIDS research and therapy. 2013;10(1):25. http://dx.doi.org/10.1186/1742-6405-10-25

13. Kityo C, Boerma RS, Sigaloff KCE, Kaudha E, Calis JCJ, Musiime V, et al. Pretreatment HIV drug resistance results in virological failure and accumulation of additional resistance mutations in Ugandan children. The Journal of antimicrobial chemotherapy. 2017;72(9):2587-95. http://dx.doi.org/10.1093/jac/dkx188

14. Simoni JM, Montgomery A, Martin E, New M, Demas PA, Rana S. Adherence to antiretroviral therapy for pediatric HIV infection: a qualitative systematic review with recommendations for research and clinical management. Pediatrics. 2007;119(6):e1371-83. http://dx.doi.org/10.1542/peds.2006-1232

15. National Department of Health, South Africa. National Antiretroviral Treatment Guidelines. 2004. Available from:https://www.gov.za/sites/default/files/gcis_document/201409/artguidelines0.pdf. Accessed (24 May 2024).

16. National Department of Health, South Africa; South African National AIDS Council. Clinical Guidelines: Prevention of Mother-to-Child Transmission (PMTCT). 2nd Edition. Pretoria: National Department of Health; 2010. 9Accessed 24 May 2024).

17. National Department of Health, South Africa. Guidelines for the Management of HIV in Children. 2nd Edition. Pretoria: National Department of Health; 2010. Available from: https://sahivsoc.org/Files/Guidelines_for_Management_of_HIV_in_Children_2010.pdf. (Accessed 25 May 2024).

18. National Department of Health, Republic of South Africa. National Consolidated Guidelines for the Management of HIV in Adults, Adolescents, Children, and Infants. April 2015. Available from : https://knowledgehub.health.gov.za/elibrary/national-consolidated-guidelines-management-hiv-adults-adolescents-children-and-infants (Accessed: 25 May 2024).

19. Cotton MF, Violari A, Otwombe K, Panchia R, Dobbels E, Rabie H, et al. Early time-limited antiretroviral therapy versus deferred therapy in South African infants infected with HIV: results from the children with HIV early antiretroviral (CHER) randomised trial. Lancet (London, England). 2013;382(9904):1555-63. http://dx.doi.org/10.1016/s0140-6736(13)61409-9

20. Yassin S, Gebretekle GB. Magnitude and predictors of antiretroviral treatment failure among HIV-infected children in Fiche and Kuyu hospitals, Oromia region, Ethiopia: a retrospective cohort study. Pharmacology research & perspectives. 2017;5(1):e00296. http://dx.doi.org/10.1002/prp2.296

21. Barennes H, Virak K, Rouet F, Buisson Y, Strobel M, Vibol U. Factors associated with the failure of first and second-line antiretroviral therapies therapy, a case control study in Cambodian HIV-1 infected children. BMC research notes. 2016;9:69. http://dx.doi.org/10.1186/s13104-016-1884-y

22. Republic of South Africa National Department of Health. 2023 ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy and Breastfeeding, Adolescents, Children, Infants and Neonates. June 2023, Version 4. Available from: https://knowledgehub.health.gov.za/elibrary/2023-art-clinical-guidelines-management-hiv-adults-pregnancy-and-breastfeeding-adolescents (Accessed 24 May 2024)

23. Shiau S, Kuhn L, Strehlau R, Martens L, McIlleron H, Meredith S, et al. Sex differences in responses to antiretroviral treatment in South African HIV-infected children on ritonavir-boosted lopinavir- and nevirapine-based treatment.BMC Pediatrics.2014;14(1). http://dx.doi.org/10.1186/1471-2431-14-39

24. Njom Nlend AE, Motaze AN, Ndiang ST, Fokam J. Predictors of Virologic Failure on First-line Antiretroviral Therapy Among Children in a Referral Pediatric Center in Cameroon. The Pediatric infectious disease journal. 2017;36(11):1067-72. http://dx.doi.org/10.1097/inf.0000000000001672

25. Muri L, Gamell A, Ntamatungiro AJ, Glass TR, Luwanda LB, Battegay M, et al. Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. AIDS (London, England). 2017;31(1):61-70. http://dx.doi.org/10.1097/qad.0000000000001273

26. Sithole Z, Mbizvo E, Chonzi P, Mungati M, Juru TP, Shambira G, et al. Virological failure among adolescents on ART, Harare City, 2017- a case-control study. BMC infectious diseases. 2018;18(1):469. http://dx.doi.org/10.1186/s12879-018-3372-6

27. Bunupuradah T, Sricharoenchai S, Hansudewechakul R, Klinbuayaem V, Teeraananchai S, Wittawatmongkol O, et al. Risk of first-line antiretroviral therapy failure in HIV-infected Thai children and adolescents. The Pediatric infectious disease journal. 2015;34(3):e58-62. http://dx.doi.org/10.1097/inf.0000000000000584

28. Crowell CS, Maiga AI, Sylla M, Taiwo B, Kone N, Oron AP, et al. High Rates of Baseline Drug Resistance and Virologic Failure Among ART-naive HIV-infected Children in Mali. The Pediatric infectious disease journal. 2017;36(11):e258-e63. http://dx.doi.org/10.1097/inf.0000000000001575

29. Boerma RS, Boender TS, Sigaloff KC, Rinke de Wit TF, van Hensbroek MB, Ndembi N, et al. High levels of pre-treatment HIV drug resistance and treatment failure in Nigerian children. Journal of the International AIDS Society. 2016;19(1):21140. http://dx.doi.org/10.7448/ias.19.1.21140

30. Vaz P, Augusto O, Bila D, Macassa E, Vubil A, Jani IV, et al. Surveillance of HIV drug resistance in children receiving antiretroviral therapy: a pilot study of the World Health Organization's generic protocol in Maputo, Mozambique. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54 Suppl 4:S369-74. http://dx.doi.org/10.1093/cid/cis006

31. Davies MA, Moultrie H, Eley B, Rabie H, Van Cutsem G, Giddy J, et al. Virologic failure and second-line antiretroviral therapy in children in South Africa--the IeDEA Southern Africa collaboration. Journal of acquired immune deficiency syndromes (1999). 2011;56(3):270-8. http://dx.doi.org/10.1097/qai.0b013e3182060610

32. Kerr SJ, Puthanakit T. Barriers to paediatric switching to second-line ART. The Lancet HIV [Internet]. 2019 Feb;6(2):e71–2. Available from: http://dx.doi.org/10.1016/s2352-3018(18)30334-5 (Accessed 25 May 2024)

33. Meyers T, Sawry S, Wong JY, Moultrie H, Pinillos F, Fairlie L, et al. Virologic failure among children taking lopinavir/ritonavir-containing first-line antiretroviral therapy in South Africa. The Pediatric infectious disease journal. 2015;34(2):175-9. http://dx.doi.org/10.1097/inf.0000000000000544

34. Sudjaritruk T, Aurpibul L, Ly PS, Le TPK, Bunupuradah T, Hansudewechakul R, et al. Incidence of Postsuppression Virologic Rebound in Perinatally HIV-Infected Asian Adolescents on Stable Combination Antiretroviral Therapy. The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2017;61(1):91-8.
http://dx.doi.org/10.1016/j.jadohealth.2017.01.014

35. Harries A, Maher D, Graham S. TB/HIV: A Clinical Manual. Geneva: World Health Organization; 2004. Stop TB Department, World Health Organization, Geneva, Switzerland; Stephen Graham, Wellcome Trust Research Laboratories, Blantyre, Malawi and Liverpool School of Tropical Medicine, Liverpool, England. ISBN 92 4 154634 4. Available from: https://www.who.int/publications-detail-redirect/9241546344]. (Accessed 25 May 2025).

36. National Department of Health, Republic of South Africa. Guidelines for the Management of Tuberculosis in Children. Pretoria: National Department of Health, Republic of South Africa; April 2013. https://knowledgehub.health.gov.za/elibrary/national-guidelines-management-tuberculosis-children . ISBN: 978-1-920031-81-7. (Accessed May 25, 2024).

37. Costenaro P, Penazzato M, Lundin R, Rossi G, Massavon W, Patel D, et al. Predictors of Treatment Failure in HIV-Positive Children Receiving Combination Antiretroviral Therapy: Cohort Data from Mozambique and Uganda. Journal of the Pediatric Infectious Diseases Society. 2015;4(1):39-48.
http://dx.doi.org/10.1093/jpids/piu032

38. Mu W, Bartlett AW, Bunupuradah T, Chokephaibulkit K, Kumarasamy N, Ly PS, et al. Early and Late Virologic Failure After Virologic Suppression in HIV-Infected Asian Children and Adolescents. Journal of acquired immune deficiency syndromes (1999). 2019;80(3):308-15. http://dx.doi.org/10.1097/qai.0000000000001921

39. Yihun BA, Kibret GD, Leshargie CT. Correction: Incidence and predictors of treatment failure among children on first-line antiretroviral therapy in Amhara Region Referral Hospitals, northwest Ethiopia 2018: A retrospective study. PloS one. 2019;14(6):e0217901. http://dx.doi.org/10.1371/journal.pone.0215300

40. Somerville K, Jenkins CA, Carlucci JG, Person AK, Machado DM, Luque MT, et al. Outcomes After Second-Line Antiretroviral Therapy in Children Living with HIV in Latin America. Journal of acquired immune deficiency syndromes (1999). 2021;87(3):993-1001. http://dx.doi.org/10.1097/qai.0000000000002678

41. Nyogea D, Mtenga S, Henning L, Franzeck FC, Glass TR, Letang E, et al. Determinants of antiretroviral adherence among HIV positive children and teenagers in rural Tanzania: a mixed methods study. BMC infectious diseases. 2015;15:28. http://dx.doi.org/10.1186/s12879-015-0753-y

42. Tran CT, Pham TH, Tran KT, Nguyen TKC, Larsson M. Caretakers' barriers to pediatric antiretroviral therapy adherence in Vietnam - A qualitative and quantitative study. Applied nursing research: ANR. 2017; 35:1-5. http://dx.doi.org/10.1016/j.apnr.2017.02.016

43. Nichols J, Steinmetz A, Paintsil E. Impact of HIV-Status Disclosure on Adherence to Antiretroviral Therapy Among HIV-Infected Children in Resource-Limited Settings: A Systematic Review. AIDS and behavior. 2017;21(1):59-69. http://dx.doi.org/10.1007/s10461-016-1481-z

44. Nichols JS, Kyriakides TC, Antwi S, Renner L, Lartey M, Seaneke OA, et al. High prevalence of non-adherence to antiretroviral therapy among undisclosed HIV-infected children in Ghana. AIDS care. 2019;31(1):25-34.
http://dx.doi.org/10.1080/09540121.2018.1524113

45. Sisay MM, Ayele TA, Gelaw YA, Tsegaye AT, Gelaye KA, Melak MF. Incidence and risk factors of first-line antiretroviral treatment failure among human immunodeficiency virus-infected children in Amhara regional state, Ethiopia: a retrospective follow-up study. BMJ open. 2018;8(4): e019181. http://dx.doi.org/10.1136/bmjopen-2017-019181

46. Lesch A, Swartz L, Kagee A, Moodley K, Kafaar Z, Myer L, et al. Paediatric HIV/AIDS disclosure: towards a developmental and process-oriented approach. AIDS Care [Internet]. 2007 Jun 14;19(6):811–6. Available from: http://dx.doi.org/10.1080/09540120601129301

47. Armoon B, Fleury MJ, Higgs P, Bayat AH, Bayani A, Mohammadi R, et al. Prevalence, socio-demographics and service use determinants associated with disclosure of HIV/AIDS status to infected children: a systematic review and meta-analysis by 1985-2021. 2022;80(1):154. http://dx.doi.org/10.1186/s13690-022-00910-6

6. Appendices

Appendix A

Approved Research Protocol

Characteristics and outcomes of HIV -infected children on antiretroviral therapy referred for virological failure.

Student

Dr Xitshembiso Confidence Magomani

MBBCH (Wits), DCH

Staff number: A0020632

Student number: 0600404G

Degree: MMed paediatrics

Contact details

Email: malulekeveronica@gmail.com

Cell number: 0793077190

Hospital: Chris Hani Baragwanath Academic Hospital (CHBAH)

Setting: Harriet Shezi Children's Clinic (HSCC)

Supervisors

1. Dr Nosisa Sipambo

MB BCH (Wits), MMed (Wits), FCPaed (SA)

Department of Paediatrics and Child Health,

Faculty of Health Sciences,

University of the Witwatersrand

2. Dr Fikile Cynthia Mabena

MBChB (UP), MMed (Wits), FCPaed (SA), Cert ID Paed (SA), DipHIVMan (SA), DTM&H (Wits)

Department of Paediatrics and Child Health,

Faculty of Health Sciences,

University of the Witwatersrand

Contents

1. Literature review

Children generally have very high viral loads and early immunologic damage compared to adults, therefore obtaining rapid virological suppression is paramount in this age group.[1] Early age at ART initiation in children has been shown to favour virologic suppression and lower viral burden.[2,3-4] In addition; early ART initiation reduces mortality and morbidity and improves growth and neuro cognitive outcomes.[5,6-7] ART guidelines have therefore shifted over the years to now initiate all infants, children and adolescents on ART regardless of CD4 count and clinical staging.[8]

On the contrary, Age of less than 3 years has been found to be a risk factor for virological failure in other cohorts.[9,10] Caregiver dependency for medication administration, complex pharmacokinetics and poor palatability of paediatric ART formulations, [11,12] together with associated weaker immunity, high malnutrition rates and diarrheal diseases in younger children put them at increased risk of poor adherence [1,10] and continue to threaten the third arm of the 90-90-90 objective. Another vulnerable age group is adolescents as they are generally responsible for administering their own treatment. Coupled with issues surrounding adherence and stigma, the physical and metabolic changes associated with puberty and substance abuse also affect plasma drug levels, putting them at a high risk of VF. [13] A cohort study conducted on Thai children and adolescents to assess the risk of ART failure showed that being 10-16 years of age predicted VF in a multivariate analysis.[14] European and Ethiopian cohorts also found that initiating ART between the ages of 10-15 years of age was associated with increased risk of VF. [9,15]

Studies have investigated the association between the gender of a child and VF and most of them found that male gender was linked to poor viral suppression. [1, 16-17] In contrast, a Tanzanian cohort study found that female gender was independently associated with an increased risk of VF. [11] The reasons for the difference in the risk of VF by gender are not well understood. Postulated reasons suggest biological differences between male and female and the different levels of care and supervision provided by parents for a child's gender. [16] A SA study exploring gender differences in response to ART in SA children on PI-based regimen did not find any differences in the risk of VF between males and females. [18] The association between VF and gender of the child is poorly understood and requires further research.

The median time it takes to develop VF once a child is on ART differs amongst patients. Early viral load monitoring has enabled the detection of VF in children as early as 6 months on ART. [19] Poor VL monitoring is still a major hindrance in resource limited areas. The consequence of this is late detection of VF, delayed switching to 2nd line regimens and poor treatment outcomes. Paediatric studies have demonstrated the high rates of ART failure within the first 2 years of ART and the risk of VF declining with an increasing time on ART. [2, 14, 20] This emphasizes the need of treatment support and intense adherence counselling within these critical early years of ART. Accumulation of Drug Resistance Mutations (DRM) over time

on ART increases the risk of VF, as was illustrated in a Zimbabwean study where VF was associated with duration of more than 4 years on ART. [21] More than 80% of patients in this study were on a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)-based regimen and there was a high incidence of significant mutations among those who developed VF.

Baseline clinical parameters which have been linked to VF in children include CD4 count, viral load, and WHO staging. While some studies[9-10,22-24] have demonstrated that low baseline CD4 counts of less than 5-25% and absolute CD4 counts of less than 200 are associated with an increased risk of VF, other cohorts did not show any association with both low baseline CD4 counts and WHO clinical stages.[1,25] Clinical stages 3 and 4 were associated with an increased risk of VF in some cohorts,[9,25-26] further showing that weaker immune status predispose to opportunistic infections and poor viral response to ART. The timely initiation of ART regardless of CD4 counts as per WHO guidelines will ensure that children are started on ART early and improve viral suppression rates. With regard to the association between baseline VL and VF, two African studies found that a higher baseline log VL is associated with an increased risk of VF, [27, 28] while another cohort conducted in Asia didn't show any association. [14]

Studies have found conflicting association between Tuberculosis (TB) infection and the risk of VF. Studies which found an increased risk of VF in children with TB diagnosis at ART initiation stated drug interactions, side effect profile and regimen complexity as possible reasons for the finding.[2,26] Conversely, children with and HIV co- infection in another cohort achieved better viral outcomes and this was attributed to the contribution of intense adherence counselling offered for such patients, the role of directly observed therapy (DOTS) and frequent visits and monitoring.[1] The effect of TB co- infection on viral outcomes was dependent on the type of ART regimen in a different cohort where a lopinavir based regimen produced poor viral outcomes compared to a NNRTI–based regimen.[29]

For ART to be fully effective, 95% adherence is required. [30, 31] This is particularly challenging in a paediatric population as children mostly need adult supervision, assistance and support to adhere to treatment. A qualitative systematic review on adherence to ART in a paediatric population was conducted by Simoni Jane. M et al in 2007. [32] It reviewed papers which showed adherence estimates and barriers to adherence. Papers which assessed adherence estimates in a paediatric population found varied estimates and it was difficult to obtain a conclusive result. This is mainly because of different methods which were used to estimate adherence. Estimates also varied depending on patient demographics and regimen type. The systematic review also investigated papers which reported on factors causing a barrier to adherence. These were mostly classified into patient, drugs, healthcare and caregiver related factors. They include amongst others complex regimens, drug adverse events, large poorly palatable pills, storage requirements, drug cost and availability, stigma, disclosure, type of caregiver, adolescence stage and psychological factors. Poor adherence leads to sub therapeutic drug

levels, resulting in failure of a drug to reach its therapeutic effect, increasing risk of drug resistance [33] and has been associated with increased VF rates in several reports. [9, 13, 16, 34-35] However some studies showed no association between VF and sub optimal adherence [1, 36] and this is attributed to the retrospective nature of the studies or reporting bias from indirect methods used to estimate adherence. Indirect methods like self-reports, pill count, and caregiver reports have been found to overestimate adherence compared to direct methods which measure plasma drug levels. [37,38] The implications of the over estimation are that on-adherent patients tend to be missed and sub-optimally managed. More studies are needed to address the issue of adherence in a paediatric population.

HIV disease has adversely disrupted many family structures and increased the number of orphans in SA, as was indicated in a UNICEF report in 2016 which showed that SA had more than 2 million children who had been orphaned by HIV/AIDS.[39] Caregiver involvement plays a significant role in ART success in a paediatric population. Younger children are entirely dependent on caregivers for treatment administration and follow up. Successful treatment outcomes require a caregiver who is well informed about the child's condition and treatment. A study describing the importance of caregivers in the outcome of paediatric HIV management conducted in Kenya found that having multiple caregivers accompanying the child to the clinic and being cared for by a non-immediate family member increased the risk of VF.[40] Orphan hood has been associated with an increased risk of VF[17,25,41] and the reason mostly stated is poor adherence support in orphaned children .This was also demonstrated in a Vietnamese study and a Tanzanian study which showed that having a biological parent as a caregiver is a key determinant of adherence in children and teenagers.[42,43] A time period study in Togo also investigated the relationship between VF and being an orphan but did not find any association.[44]

Disclosure of the HIV status to the child is part of paediatric HIV care and non-disclosure was a risk factor for VF in some cohorts. [13,45] Disclosure should be done in a systematic manner suited for a child's age, development and maturity. It should be individualized to suit the child and caregiver's needs and take into consideration family, cultural and religious factors.[46] A systematic review performed to investigate the impact of disclosure on adherence to ART amongst children in low resource areas reviewed 14 articles of which 5 articles did not show any association between adherence and disclosure, 5 articles showed a positive association and 4 showed a negative association.[47] Stated reasons for the positive association were that disclosure empowers children to participate in their care and knowing the reason for taking the treatment improved their willingness to take the ART and therefore improving virologic outcomes. The reasons for the negative association between disclosure and adherence were related to the fear of social stigma and status denial, leading to children hiding and refusing to take their medication. There were also increased depressive symptoms associated with full disclosure, all resulting in poor adherence and virological failure. It is important to highlight that the methods of assessing adherence were different in these studies and this could have influenced the findings.

As stated earlier, ART formulation and dosing is complex in a paediatric population and drive the risk of VF in this age group. Due to the rapid scale up of PMTCT and the proven low genetic barrier of NNRTIs to resistance, NNRTI-based first line regimens, especially in PMTCT exposed children lead to increased rates of pre-drug resistance (PDR) and VF.[16,25,27-28,34,36,48] The WHO recommends a Protease inhibitor (PI) based first line regimen for all children less than 3 years of age.[8] Unfortunately this is still a work in progress in low income countries as it is taking longer to fully implement the WHO recommendations. Poor VL monitoring and lack of genotypic drug resistance testing in LMIC worsen virologic outcomes as children with VF tend to be switched late and staying on a failing regimen leads to acquisition and accumulation of further resistance mutations. [17,49]

First line PI regimen for younger children has markedly improved virologic outcomes but it is worth to note that it does not have a complete barrier to resistance and has a significant side effect profile. A retrospective cohort study conducted in SA on children who developed VF at a median age of 21 months of a Lopinavir/ritonavir (LPV/r) containing first line regimen demonstrated that significant PI-associated mutations do occur, though infrequently. In this study half of the patients who stayed on a failing PI- regimen achieved virologic suppression, suggesting suboptimal adherence as a likely cause for the VF.[50] A cohort study conducted in Asia on HIV –infected adolescents to identify predictors of post suppressive virologic rebound demonstrated an increased risk of VF on a 2nd line PI based regimen. Poor adherence related to pill burden of PI-containing regimens and limited age-and weight - appropriate fixed-dose combinations (FDC) were the suggested contributing factors.[51] NNRTI resistance mutations have been shown to decay mostly after 36 months and the benefit of switching children who are suppressed on a PI –containing 1st line regimen back to an NNRTI based regimen after this age has been demonstrated.[52] Anti-retroviral drugs with a low side effect profile and a high genetic barrier to resistance need to be developed to adequately control VF in a paediatric population

It is evident from the presented literature that children's demographics, clinical characteristics and the type of ART regimen influence virologic outcomes. Viral suppression rates are lower in children as revealed in the literature review. The management of children with virological failure is challenging and requires a dedicated multidisciplinary team to achieve better virologic outcomes. The focus of HIV management has evolved significantly over the years and has now shifted from merely initiating children on treatment to focusing on retention in care. To further improve the move towards the third arm of the 90-90-90 treatment goals in our setting, knowledge of the characteristics of children who are experiencing VF, and the outcomes of these children is required. By providing a description of patients with VF in our setting, the proposed study can potentially assist health care providers to easily identify and manage patients with characteristics which are more likely to result in VF and hopefully in turn contribute towards reducing VF rates in a paediatric population in our setting.

2. Study aims and objectives.

2.1 Aim

To describe the characteristics and outcomes of patients referred and managed at HSCC for virological failure.

2.2 Objectives

- To describe the demographic and clinical characteristics of patients referred to HSCC for virological failure.
- To determine the factors associated with virological failure.
- To describe the virologic outcomes of patients referred to HCSS for virological failure.

3. Study setting.

The study will be conducted at Harriet Shezi Children's Clinic HSCC (HSCC). HSCC is located at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg in SA. The clinic was established in 2000 and serves the clinical and psychosocial needs of HIV-infected children and adolescents. HSCC has one of the largest cohorts of HIV-infected children and adolescents, over 8000 to date. Since 2009, HSCC has transferred out stable, virally suppressed children and adolescents remaining with those with more complex HIV disease such as those who develop opportunistic infections, side effects on ART, virological failure and resistance to ART requiring second- or third-line ART. From 2013 onwards, HSCC has focused on complicated HIV cases, serving as a referral site for surrounding clinics and hospitals. In 2014, HSCC established dedicated Virological Failure and Resistance clinics in order to offer differentiated care packages for this cohort of patients. Additionally, HSCC manages complex paediatric and adolescent patients with co-morbidities that require specialist care from fields such as cardiology, nephrology, endocrinology, pulmonology and oncology.

4. Methodology

4.1 Study design.

A retrospective descriptive study of patients referred and managed for VF in HSCC

4.2 Sample population.

The study will include HIV infected patients aged 0-14 years who were referred and managed in HSCC for VF from January 2010 to December 2019. The reason for the selected time period is that patients could be referred from

the clinics to HSCC for VF from the year 2010. A patient will be excluded from a particular analysis if they have incomplete records for that particular analysis.

4.3 Data collection

Definitions

Baseline: defined as the time at or just before ART initiation

Referral: the first time the patient is seen at HSCC

VF: patients will be considered to have VF if they have 2 viral load measurements of >1000 copies/ml taken at least 3months (+/-2) apart with the first VL taken at least 6 months after initiating ART.

Duration on ART: time period from time of ART initiation to time of referral to HSCC Duration of VF: period from the first VL of > 1000 after at least 6 months on ART to time of referral

Hospital medical records of the described population will be retrieved from the HSCC records which are routinely collected for all patients referred to the clinic. Baseline information will be entered into the provided data sheet in Appendix A and will consist of the patient's age at initiation of ART, and clinical characteristics which include baseline CD4 count, WHO disease stage, TB status at initiation, baseline log VL, PMTCT exposure status (maternal or infant), and baseline ART regimen. Parameters at referral will include patient's current age and gender, caregiver type, disclosure status, current TB status, duration on ART, duration of VF, most recent CD4 count, most recent VL (log), and current ART regimen. Interventions at HSCC which include intense adherence counselling, regimen modification or switch and disclosure facilitation and other will be entered into the data sheet. A VL at 6 +/_ 3 months and at 12 +/- 3 months following an intervention at HSCC will be used to describe the outcomes and will be entered into the data sheet.

4.4 Data analysis

The collected data will be entered into a Microsoft excel spread sheet and basic statistics analysed by Statistica. (Statssoft USA, version 12). Continuous variables will be reported as means and standard deviations if normally distributed and as medians and interquartile ranges if not normally distributed. Categorical variables will be summarized in frequencies and percentages. Where comparison is required, for continuous variables student T test and Mann Whitney U analysis will be used as appropriate and with categorical variables, comparison will be done using Chi-square analysis or fisher's exact test as appropriate.

5. Limitations

This is a retrospective study; data is likely to be incomplete and inaccurate due to missing records and poor documentation.

6. Cost

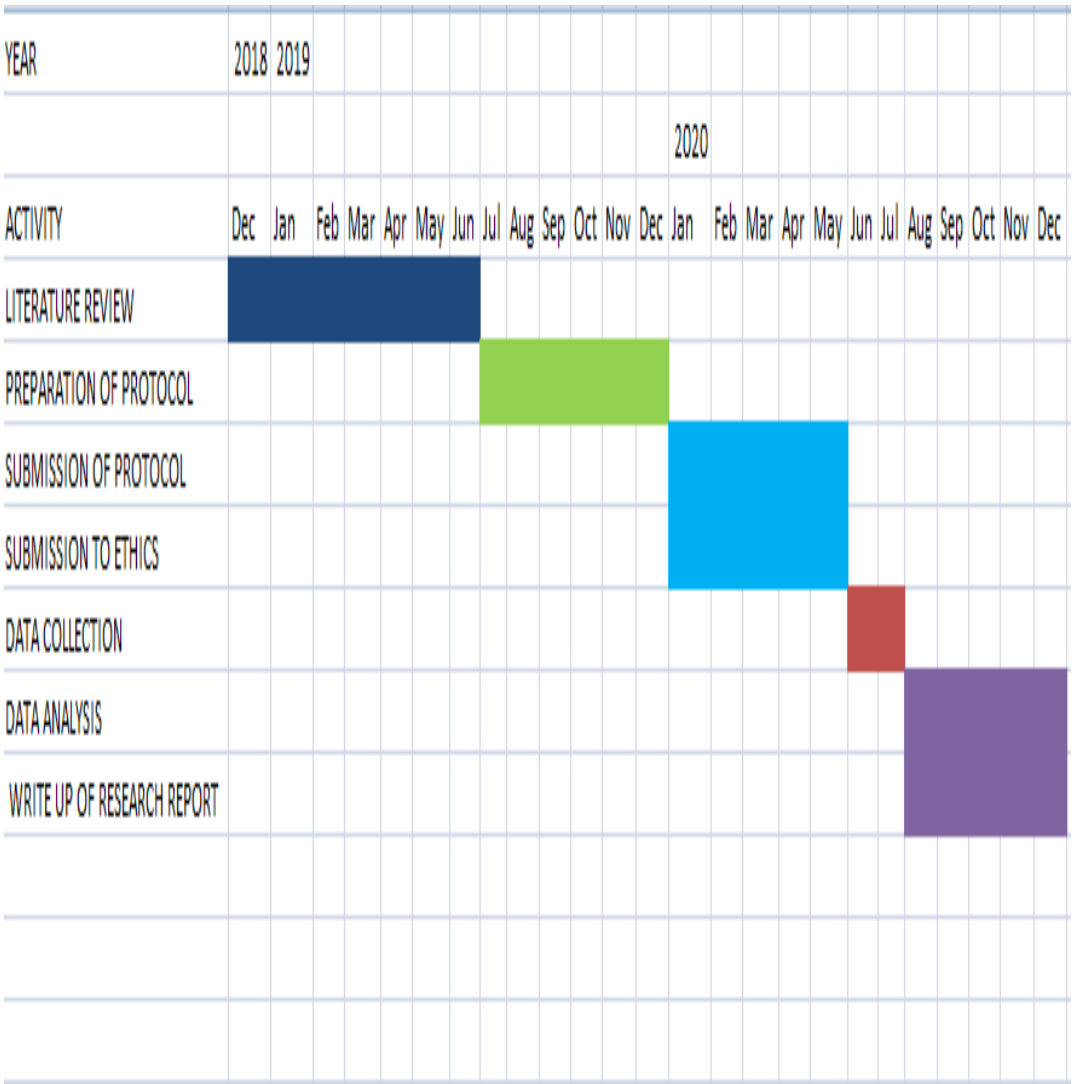
A personal laptop will be used. Stationery and printing costs will be covered by the researcher. Internal and external funding will not be required for the proposed study.

7. Ethical consideration

As this will be a retrospective study of routinely collected data, consent from study participants will not be required. Only the researcher will have access to identified data and all patient information will be handled with utmost confidentiality. Any form of patient identification by hospital numbers or name will not be included. The researcher will de-identify the data and assign a participant number to each patient.

Research protocol will be submitted to the Wits Post Graduate research committee and to the University of the Witwatersrand Human Research Ethics Committee for approval and clearance. Data collection will only commence once the above process is successfully completed.

8. Timeline


YEAR
2018 2019
2020
ACTIVITY
Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
LITERATURE REVIEW
PREPARATION OF PROTOCOL
SUBMISSION OF PROTOCOL
SUBMISSION TO ETHICS
DATA COLLECTION
DATA ANALYSIS
WRITE UP OF RESEARCH REPORT

9. References

1. Kadima J, Patterson E, Mburu M, Blat C, Nyanduko M, Bukusi EA, et al. Correction: Adoption of routine virologic testing and predictors of virologic failure among HIV-infected children on antiretroviral treatment in western Kenya. PloS one. 2019;14(1):e0210908.

2. Mu W, Bartlett AW, Bunupuradah T, Chokephaibulkit K, Kumarasamy N, Ly PS, et al. Early and Late Virologic Failure After Virologic Suppression in HIV-Infected Asian Children and Adolescents. Journal of acquired immune deficiency syndromes (1999). 2019;80(3):308-15.

3. Shiau S, Strehlau R, Technau KG, Patel F, Arpadi SM, Coovadia A, et al. Early age at start of antiretroviral therapy associated with better virologic control after initial suppression in HIV-infected infants. AIDS (London, England). 2017;31(3):355-64.

4. Tagarro A, Chan M, Zangari P, Ferns B, Foster C, De Rossi A, et al. Early and Highly Suppressive Antiretroviral Therapy Are Main Factors Associated with Low Viral Reservoir in European Perinatally HIV-Infected Children. Journal of acquired immune deficiency syndromes (1999). 2018;79(2):269-76.

5. Cotton MF, Violari A, Otwombe K, Panchia R, Dobbels E, Rabie H, et al. Early time-limited antiretroviral therapy versus deferred therapy in South African infants infected with HIV: results from the children with HIV early antiretroviral (CHER) randomised trial. Lancet (London, England). 2013;382(9904):1555-63.

6. Shiau S, Arpadi S, Strehlau R, Martens L, Patel F, Coovadia A, et al. Initiation of antiretroviral therapy before 6 months of age is associated with faster growth recovery in South African children perinatally infected with human immunodeficiency virus. The Journal of pediatrics. 2013;162(6):1138-45, 45.e1-2.

7. Crowell CS, Huo Y, Tassiopoulos K, Malee KM, Yogev R, Hazra R, et al. Early viral suppression improves neurocognitive outcomes in HIV-infected children. AIDS (London, England). 2015;29(3):295-304.

8. WHO. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. WHO Guidelines Approved by the Guidelines Review Committee. In: nd, editor. Geneva: World Health Organization,Copyright (c) 2016.

9. Yassin S, Gebretekle GB. Magnitude and predictors of antiretroviral treatment failure among HIV-infected children in Fiche and Kuyu hospitals, Oromia region, Ethiopia: a retrospective cohort study. Pharmacology research & perspectives. 2017;5(1):e00296.

10. Bacha T, Tilahun B, Worku A. Predictors of treatment failure and time to detection and switching in HIV-infected Ethiopian children receiving first line anti-retroviral therapy. BMC infectious diseases. 2012;12:197.

11. Muri L, Gamell A, Ntamatungiro AJ, Glass TR, Luwanda LB, Battegay M, et al. Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. AIDS (London, England). 2017;31(1):61-70.

12. Abrams EJ, Strasser S. 90-90-90--Charting a steady course to end the paediatric HIV epidemic. Journal of the International AIDS Society. 2015;18(Suppl 6):20296.

13. Sithole Z, Mbizvo E, Chonzi P, Mungati M, Juru TP, Shambira G, et al. Virological failure among adolescents on ART, Harare City, 2017- a case-control study. BMC infectious diseases. 2018;18(1):469.

14. Bunupuradah T, Sricharoenchai S, Hansudewechakul R, Klinbuayaem V, Teeraananchai S, Wittawatmongkol O, et al. Risk of first-line antiretroviral therapy failure in HIV-infected Thai children and adolescents. The Pediatric infectious disease journal. 2015;34(3):e58-62.

15. Duong T, Judd A, Collins IJ, Doerholt K, Lyall H, Foster C, et al. Long-term virological outcome in children on antiretroviral therapy in the UK and Ireland. AIDS (London, England). 2014;28(16):2395-405.

16. Sebunya R, Musiime V, Kitaka SB, Ndeezi G. Incidence and risk factors for first line anti retroviral treatment failure among Ugandan children attending an urban HIV clinic. AIDS research and therapy. 2013;10(1):25.

17. Njom Nlend AE, Motaze AN, Ndiang ST, Fokam J. Predictors of Virologic Failure on First-line Antiretroviral Therapy Among Children in a Referral Pediatric Center in Cameroon. The Pediatric infectious disease journal. 2017;36(11):1067-72.

18. Shiau S, Kuhn L, Strehlau R, Martens L, McIlleron H, Meredith S, et al. Sex differences in responses to antiretroviral treatment in South African HIV-infected children on ritonavir-boosted lopinavir- and nevirapine-based treatment. BMC pediatrics. 2014;14:39.

19. Ruel TD, Kamya MR, Li P, Pasutti W, Charlebois ED, Liegler T, et al. Early virologic failure and the development of antiretroviral drug resistance mutations in HIV-infected Ugandan children. Journal of acquired immune deficiency syndromes (1999). 2011;56(1):44-50.

20. Wamalwa DC, Lehman DA, Benki-Nugent S, Gasper MA, Gichohi R, Maleche-Obimbo E, et al. Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy. Journal of acquired immune deficiency syndromes (1999). 2013;62(3):267-74.

21. Makadzange AT, Higgins-Biddle M, Chimukangara B, Birri R, Gordon M, Mahlanza T, et al. Clinical, Virologic, Immunologic Outcomes and Emerging HIV Drug Resistance Patterns in Children and Adolescents in Public ART Care in Zimbabwe. PloS one. 2015;10(12):e0144057.

22. Barennes H, Virak K, Rouet F, Buisson Y, Strobel M, Vibol U. Factors associated with the failure of first and second-line antiretroviral therapies therapy, a case control study in Cambodian HIV-1 infected children. BMC research notes. 2016;9:69.

23. Teeraananchai S, Bunupuradah T, Puthanakit T, Kerr SJ, Ruxrungtham K, Chaivooth S, et al. First-Line Antiretroviral Treatment Outcomes and Durability in HIV-Infected Children Treated Through the Universal Coverage Health Program in Thailand. Journal of acquired immune deficiency syndromes (1999). 2017;75(2):219-25.

24. Mutwa PR, Boer KR, Rusine J, Muganga N, Tuyishimire D, Schuurman R, et al. Long-term effectiveness of combination antiretroviral therapy and prevalence of HIV drug resistance in HIV-1-infected children and adolescents in Rwanda. The Pediatric infectious disease journal. 2014;33(1):63-9.

25. Yihun BA, Kibret GD, Leshargie CT. Correction: Incidence and predictors of treatment failure among children on first-line antiretroviral therapy in Amhara Region Referral Hospitals, northwest Ethiopia 2018: A retrospective study. PloS one. 2019;14(6):e021790

26. Costenaro P, Penazzato M, Lundin R, Rossi G, Massavon W, Patel D, et al. Predictors of Treatment Failure in HIV-Positive Children Receiving Combination Antiretroviral Therapy: Cohort Data From Mozambique and Uganda. Journal of the Pediatric Infectious Diseases Society. 2015;4(1):39-48.

27. Davies MA, Moultrie H, Eley B, Rabie H, Van Cutsem G, Giddy J, et al. Virologic failure and second-line antiretroviral therapy in children in South Africa--the IeDEA Southern Africa collaboration. Journal of acquired immune deficiency syndromes (1999). 2011;56(3):270-8.

28. Boerma RS, Boender TS, Sigaloff KC, Rinke de Wit TF, van Hensbroek MB, Ndembi N, et al. High levels of pre-treatment HIV drug resistance and treatment failure in Nigerian children. Journal of the International AIDS Society. 2016;19(1):21140.

29. Zanoni BC, Phungula T, Zanoni HM, France H, Feeney ME. Impact of tuberculosis cotreatment on viral suppression rates among HIV-positive children initiating HAART. AIDS (London, England). 2011;25(1):49-55.

30. Nichols JS, Kyriakides TC, Antwi S, Renner L, Lartey M, Seaneke OA, et al. High prevalence of non-adherence to antiretroviral therapy among undisclosed HIV-infected children in Ghana. AIDS care. 2019;31(1):25-34.

31. Miller LG, Hays RD. Adherence to combination antiretroviral therapy: synthesis of the literature and clinical implications. The AIDS reader. 2000;10(3):177-85.

32. Simoni JM, Montgomery A, Martin E, New M, Demas PA, Rana S. Adherence to antiretroviral therapy for pediatric HIV infection: a qualitative systematic review with recommendations for research and clinical management. Pediatrics. 2007;119(6):e1371-83.

33. Buzibye A, Musaazi J, von Braun A, Nanzigu S, Sekaggya-Wiltshire C, Kambugu A, et al. Antiretroviral concentration measurements as an additional tool to manage virologic failure in resource limited settings: a case control study. AIDS research and therapy. 2019;16(1):39.

34. Kityo C, Boerma RS, Sigaloff KCE, Kaudha E, Calis JCJ, Musiime V, et al. Pretreatment HIV drug resistance results in virological failure and accumulation of additional resistance mutations in Ugandan children. The Journal of antimicrobial chemotherapy. 2017;72(9):2587-95.

35. Crowell CS, Maiga AI, Sylla M, Taiwo B, Kone N, Oron AP, et al. High Rates of Baseline Drug Resistance and Virologic Failure Among ART-naive HIV-infected Children in Mali. The Pediatric infectious disease journal. 2017;36(11):e258-e63.

36. Bunupuradah T, Puthanakit T, Kosalaraksa P, Kerr S, Boonrak P, Prasitsuebsai W, et al. Immunologic and virologic failure after first-line NNRTI-based antiretroviral therapy in Thai HIV-infected children. AIDS research and therapy. 2011;8:40.

37. Mghamba FW, Minzi OM, Massawe A, Sasi P. Adherence to antiretroviral therapy among HIV infected children measured by caretaker report, medication return, and drug level in Dar Es Salaam, Tanzania. BMC pediatrics. 2013;13:95.

38. Kouanfack C, Laurent C, Peytavin G, Ciaffi L, Ngolle M, Nkene YM, et al. Adherence to antiretroviral therapy assessed by drug level monitoring and self-report in cameroon. Journal of acquired immune deficiency syndromes (1999). 2008;48(2):216-9.

39. UNICEF. Biennial report South Africa (2014-2015) pdf. 2016.

40. Sivapalasingam S, Mendillo M, Ahmed A, Mwamzuka M, Said S, Marshed F, et al. The importance of caregivers in the outcome of pediatric HIV management, Mombasa, Kenya. AIDS care. 2014;26(4):425-33.

41. Janssens B, Raleigh B, Soeung S, Akao K, Te V, Gupta J, et al. Effectiveness of highly active antiretroviral therapy in HIV-positive children: evaluation at 12 months in a routine program in Cambodia. Pediatrics. 2007;120(5):e1134-40.

42. Nyogea D, Mtenga S, Henning L, Franzeck FC, Glass TR, Letang E, et al. Determinants of antiretroviral adherence among HIV positive children and teenagers in rural Tanzania: a mixed methods study. BMC infectious diseases. 2015;15:28.

43. Tran CT, Pham TH, Tran KT, Nguyen TKC, Larsson M. Caretakers' barriers to pediatric antiretroviral therapy adherence in Vietnam - A qualitative and quantitative study. Applied nursing research : ANR. 2017;35:1-5.

44. Salou M, Dagnra AY, Butel C, Vidal N, Serrano L, Takassi E, et al. High rates of virological failure and drug resistance in perinatally HIV-1-infected children and adolescents receiving lifelong antiretroviral therapy in routine clinics in Togo. Journal of the International AIDS Society. 2016;19(1):20683.

45. Sisay MM, Ayele TA, Gelaw YA, Tsegaye AT, Gelaye KA, Melak MF. Incidence and risk factors of first-line antiretroviral treatment failure among human immunodeficiency virus-infected children in Amhara regional state, Ethiopia: a retrospective follow-up study. BMJ open. 2018;8(4):e019181.

46. Lesch A, Swartz L, Kagee A, Moodley K, Kafaar Z, Myer L, et al. Paediatric HIV/AIDS disclosure: towards a developmental and process-oriented approach. AIDS care. 2007;19(6):811-6.

47. Nichols J, Steinmetz A, Paintsil E. Impact of HIV-Status Disclosure on Adherence to Antiretroviral Therapy Among HIV-Infected Children in Resource-Limited Settings: A Systematic Review. AIDS and behavior. 2017;21(1):59-69.

48. Vaz P, Augusto O, Bila D, Macassa E, Vubil A, Jani IV, et al. Surveillance of HIV drug resistance in children receiving antiretroviral therapy: a pilot study of the World Health Organization's generic protocol in Maputo, Mozambique. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54 Suppl 4:S369-74.

49. Boender TS, Kityo CM, Boerma RS, Hamers RL, Ondoa P, Wellington M, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. The Journal of antimicrobial chemotherapy. 2016;71(10):2918-27.

50. Meyers T, Sawry S, Wong JY, Moultrie H, Pinillos F, Fairlie L, et al. Virologic failure among children taking lopinavir/ritonavir-containing first-line antiretroviral therapy in South Africa. The Pediatric infectious disease journal. 2015;34(2):175-9.

51. Sudjaritruk T, Aurpibul L, Ly PS, Le TPK, Bunupuradah T, Hansudewechakul R, et al. Incidence of Postsuppression Virologic Rebound in Perinatally HIV-Infected Asian Adolescents on Stable Combination Antiretroviral Therapy. The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2017;61(1):91-8.

52. Coovadia A, Abrams EJ, Stehlau R, Meyers T, Martens L, Sherman G, et al. Reuse of nevirapine in exposed HIV-infected children after protease inhibitor-based viral suppression: a randomized controlled trial. Jama. 2010;304(10):1082-90.

APPENDIX

BASELINE PARAMERS

Age at initiation :< /=3 years/ > 3 years

Gender: Male/Female

Baseline CD4 counts for children < 5 years: <15%/ 15-25 %/> 25%.

Baseline CD4 counts for children > 5 years<200/200-500/ >500.

WHO staging stage I-2/stage 3-4.

Baseline VL (log):

PMTCT exposure (maternal or infant): exposed/unexposed/unknown

TB disease at initiation: yes/no

Initial ART regimen: NNRTI-based/PI- based.

REFERRAL PARAMETERS

Current age: </= 3 years/ 4-10 years/11-14 years

Gender: Male/Female

Recent VL (log):

Caregiver status: resides with one of the parents/resides with both parents/resides with non- parent caregiver.

Disclosure status: full/partial/none

Current TB disease: yes/no

Duration on ART: <2 years/>2years

Duration of VF: <1 year/> 1 year

Current ART regimen: 1st line/2nd line

INTERVENTIONS AT HSCC

Adherence counselling

Regimen switch

Regimen modification

Disclosure facilitation

Other

OUTCOMES

VL result at 6+/_3 months.

LDL

<100

100-1000

>1000

VL result at 12+/_3 months.

LDL

<100

100-1000

>1000 Appendix B

SAMJ **Author Guidelines**

Manuscript preparation

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g., 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state ‘none’.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g., 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g., '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal; therefore, for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008; 17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been

published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out.
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, and Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed.

- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc.) that may have an impact on the study results. Clearly define how participants were enrolled and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study

- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers.
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.

- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others. [3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between (1).
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.

- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references
- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by: 1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned.

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

NOTE: no. after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '... (Prof. Michael Jones, personal communication)'.

Appendix C: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Xitshembiso Confidence Magomani (Student number: 0600404G) am a student registered for the degree of Master of Medicine in Paediatrics in the academic year 2024.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 04/06/2024

Appendix D: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Xitshembiso Magomani

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M200722 MED20-07-006

NAME: (Principal Investigator)	Dr Xitshembiso Magomani
DEPARTMENT:	Paediatrics Harriet Shezi Children's Clinic(HCSS) in Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Characteristics and outcomes of HIV-infected children on antiretroviral therapy referred for virological failure
DATE CONSIDERED:	31/07/2020
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Nosisa Sipambo and Dr Fikile Mabena
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	14/08/2020

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

20/08/2020
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix E: Turnitin report

Revised research Report Xitshembiso-Sipambo-Mabena May 2024.docx_NS.docx

ORIGINALITY REPORT

19%	16%	15%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	wiredspace.wits.ac.za Internet Source	2%
2	apps.who.int Internet Source	1%
3	journals.plos.org Internet Source	1%
4	open.uct.ac.za Internet Source	1%
5	www.researchgate.net Internet Source	1%
6	bmcinfectdis.biomedcentral.com Internet Source	1%
7	aidsrestherapy.biomedcentral.com Internet Source	1%
8	pure.uva.nl Internet Source	1%
9	etd.uwc.ac.za Internet Source	<1%