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FACTORS AFFECTING VIROLOGICAL OUTCOME OF PAEDIATRIC PATIENTS ON ABACAVIR/STAVUDINE-BASED FIRST-LINE REGIMEN

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A research report submitted to the Faculty of Health Sciences,

University of the Witwatersrand, Johannesburg, in partial fulfilment of

the requirements for the Degree

of

Master in Medicine

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DECLARATION

I, Duduzile Precious Msiza, declare that this research report is my own, unaided work. It is being submitted for the Degree of asters in Medicine of South Africa at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

_____

(Signature of candidate)

_____day of_____20_____in_____

DEDICATION

To my mother Maruping Msiza, who has been my rock and always believed in me, all her sacrifices have been so that I can one day call myself a doctor.
To my husband Mmuso Mogwera, my soul mate, for always seeing the best in me and for your love and support.
To my son Motheo Kgolagano Mogwera, all I do is for you to create a better and brighter future for you.
To my lovely family: My sisters Penny Msiza and Zanele Masina and my brother Oupa Msiza for always believing in me.

To my late dad Bongane Msiza and my late sister Funi Msiza, your love will forever keep me going, no matter what challenges may come my way.
To God my creator for making it all possible.
Thank you so much.

ABSTRACT

Background

Viral load (VL) testing is recommended as the preferred monitoring approach for assessing the effectiveness of antiretroviral therapy (ART). Considering the factors which may predispose patients to treatment failure and the high rate of virological failure among paediatric patients, we investigate which characteristics have an effect on virological outcomes of young children.

Objective

To identify factors associated with an increased probability of first-line ART regimen failure and to report the rate of virological suppression in children below three years old initiated on stavudine (d4T) - or abacavir (ABC)-based first-line regimens.

Methods

This was a retrospective cohort study conducting a secondary analysis of an existing human immunodeficiency virus (HIV) treatment database of paediatric patients at the Empilweni Clinic based at the Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa, complemented by retrospective file review.

Results

From a population of 3,728 children attending the Empilweni clinic between 2008 and 2012, 296 were eligible for the study. The pre-treatment characteristics were gender, age, weight and height for age z-score, viral load, and cluster of differentiation 4 (CD4) count percentage at pre-ART. There was an upward trend in the VL suppression rate for all the variables during the study, with an average of 24% after 6-12 months of ART and 37% at 24-36 months of ART. The majority of patients were started on more than five different drugs in the first year of ART (99%), with an average adherence rate of 94%. Only a small percentage had treatment interruptions. Data on tuberculosis (TB) was available for 68% of the patients, of which 40% received HIV-TB co-treatment, mostly (90%) in the first year of ART. A total of 60% of patients had been exposed to prevention of mother-to-child transmission (PMTCT) therapy.

Patients on a d4T-based first-line regimen had superior VL suppression compared to those on an ABC-based first-line regimen ($p<0.0001$). None of the other variables had a significant association on the VL suppression rate.

Conclusion

The study found that there was a delay in the VL suppression rate, with most patients still not being suppressed at 36 months of ART (40%). In this study ART regimen was found to be the only factor associated with viral load suppression.

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ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AZT	Zidovudine
ART	Antiretroviral therapy
ARV	Antiretroviral drug
BCG	Bacille Calmette-Guérin
CD4	Cluster of differentiation 4
CPC	Clinic pill count
CYP3A	Cytochrome P450 3A
d4T	Stavudine
EAMD	Electronic adherence monitoring device
EFV	Efavirenz
EPTB	Extra-pulmonary tuberculosis
FTC	Emtricitabine
HAZ	Height for age z-score
HIV	Human immunodeficiency virus
INH	Isoniazid
IRIS	Immune reconstitution inflammatory syndrome
IQR	Interquartile range
LPV	Lopinavir
LPV/r	Lopinavir/ritonavir
MTCT	Mother-to-child transmission

NEVEREST	Nevirapine Resistance Study
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitor
PMTCT	Prevention of mother-to-child transmission
PR	Pharmacy refilling
RIF	Rifampicin
RMMCH	Rahima Moosa Mother and Child Hospital
RTV	Ritonavir
SDNVP	Single dose nevirapine
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
VL	Viral load
WAZ	Weight for age z-score
WHO	World Health Organisation

CHAPTER 1
INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV) infection in infants and young children results in much more rapid disease progression and earlier mortality than in adults. Over 2 million children globally are living with HIV and among these, about 700 die of HIV-related illnesses every day.(1) About 52% of HIV-infected children will die before two years of age if antiretroviral therapy (ART) is not provided.(1-3) Children who are older than five years, on the other hand, have slower disease progression, similar to that of young adults.(4)

South African guidelines from june 2013 state that all children and adults should be initiated on ART on diagnosis regardless of their CD4 count or World Health Organisation (WHO) HIV clinical stage.(4) However, at the time of the study which is between 2009-2012, children over five years were initiated on ART only if their CD4 count was below 350 or if they were WHO clinical stage 3 or 4 .(4)

Early detection of HIV and early ART initiation in HIV-infected infants has a dramatic effect on the course of HIV, decreasing infant mortality by 76% and disease progression by 75%.(2, 5) Although early initiation of ART in infants is important, it does not come without its challenges. ART is a lifelong commitment and this may be difficult to achieve successfully due to various factors. Infants and children are dependent on an adult caregiver to regularly administer their medication, and drugs available in a formulation appropriate for infants are often unpalatable, difficult to administer, and may need to be refrigerated. Subsequent suboptimal adherence can result in an increased risk of drug resistance developing, as can past exposure to drugs used as part of prevention of mother-to-child transmission (PMTCT), such as nevirapine (NVP).

There is still a lack of knowledge when it comes to drug efficacy, safety, and metabolism in a number of antiretroviral drugs (ARVs) used in paediatric patients, particularly when combined with other drugs used to treat tuberculosis (TB).(6, 7) Selecting the best drug combination for children therefore remains a challenge for

policy-makers.(8) The preferred first-line regimens are those based on a combination of two nucleoside reverse transcriptase inhibitors (NRTI) with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) for children older than three years, or a protease inhibitor (PI) for children younger than three years.(9) The 2010 WHO guidelines recommend the use of a lopinavir/ritonavir (LPV/r)-based first-line regimen in children less than three years because a PI-based regimen has been shown to perform better in this age group than a NNRTI-based regimen.(10)

Stavudine (d4T) is no longer part of the NRTI backbone in first-line ART in either adults or children, owing to its long-term toxic effects, such as lipoatrophy and lactic acidosis. Since 2010, South African guidelines have recommended using abacavir (ABC), instead of d4T, together with lamivudine (3TC) and either efavirenz (EFV) or LPV/r, depending on the age of the child.(4) Clinicians in South Africa have since raised concerns about the efficacy of ABC-based regimens, especially in infants and children who have high viral load (VL).(11)

One South African cohort study comparing treatment outcomes in paediatric patients in Soweto on ABC versus d4T containing first-line regimens demonstrated a non-inferior virological outcome in patients on ABC-based regimens when compared to those on d4T-based regimens.(12) A multi-cohort South African study which included data of children on ABC/ 3TC or d4T/3TC with either EFV or LPV/r from two sites in Johannesburg and four sites in Cape Town showed earlier findings of poorer performance of ABC-based regimens; ABC-based regimens performed poorly with respect to virological efficacy when compared to d4T-based first-line regimens in both the younger children on LPV/r and the older children on EFV.(13)

A study done to assess ABC plasma pharmacokinetics in the absence and presence of atazanavir (ATV)/ritonavir (RTV) or LPV/r in HIV-infected patients showed that a combination of ABC with either one of these drugs resulted in a decrease in ABC plasma concentration of about 17% to 32%.(14) Although the clinical significance of this decrease in plasma concentration is still unclear, it is difficult to ignore and may be of particular relevance to young children who receive LPV/r-based first-line regimens together with ABC. Research has also shown that ABC-containing triple

regimens have a suboptimal virological performance in patients with higher pre-treatment VL levels (>100,000 copies/ml).(15)Therefore it is concerning that infants and younger children often have a much higher pre-treatment VL than older children and adults, at >100 000 copies/ml.(9)

TB remains a major health challenge in South Africa (1% of the population acquires the disease annually), and HIV is one of the main drivers of this high incidence.(16, 17) Unfortunately, rifampicin (RIF), a common drug for treating TB, induces cytochrome P450 enzymes, specifically the cytochrome P4503A (CYP3A) isoenzymes, which metabolise LPV/r. Co-administration of RIF and LPV/r therefore results in a significant decrease in lopinavir (LPV) concentration (by 90-99%), which could account for lower HIV viral suppression rates.(18) However, previous studies have shown that adding extra RTV to LPV/r can prevent this reduction since RTV inhibits cytochrome P450.(19) On the other hand, adding extra RTV and four TB medications to ART may result in adherence difficulties due to the large number of medicines the child needs to take and the bitter taste of LPV/r and RTV syrup. This poses a serious challenge because there are effectively no other treatment options for infants and children less than three years old, and no fixed dose combinations of TB and ART drugs for children.

Adequate PMTCT is an extremely effective prevention intervention and has resulted in a significant decreased transmission of HIV from mother to infant. However, in cases where HIV is transmitted despite PMTCT, it is more likely that in utero infection (as opposed to intra-partum or post-partum infection) occurred.(20) In utero transmission of HIV is associated with faster disease progression and poorer virological suppression rates.(20) In addition, maternal exposure to drugs used as part of PMTCT can result in children who are HIV-infected being more likely to develop drug resistance.(21)

PMTCT has been more aggressive and more accessible in recent years and this has coincided with the change from the use of d4T to ABC as part of first-line treatment for children. Mothers who fail to access adequate PMTCT and transmit HIV to their infants in this setting are more likely to have social and adherence problems. This will also make it ultimately more difficult for them to give ART regularly to their HIV-infected infants.

The EARNEST study, a randomised control trial on second-line ART in HIV-infected patients (both adults and children >12 years old) living in sub-Saharan Africa who failed NNRTI-based first line ART, showed that even with extensive cross-resistance, NRTIs still make a significant contribution to VL suppression when used in second-line therapy together with a PI. (22)This suggests that, in line with the WHO treatment guidelines recommending that resistance testing is not done when switching to second-line ART in a public health approach, routine resistance testing for patients who have failed first-line ART is not beneficial.(22) This may results in missing patients who fail therapy due to drug resistance.

The factors of adherence, PMTCT exposure history, and TB co-treatment are therefore particularly important to consider when assessing virological outcomes of infants and younger children.

1.2 Rational for the study

Technau et al. conducted a research in which results showed a poor early virologic performance and durability of abacavir-based first-line regimens for HIV-infected children, in an effort to update the existing cohort ,work by adding an analysis of more recent data (including three-year outcomes). This study did a more detailed review of adherence, PMTCT exposure history, and TB co-treatment than was possible in the larger cohort study to assess virological outcomes in paediatric patients comparing different first-line regimen treatment options. Virological suppression is one of the main goals of ART, and the fact that this may not be achieved at the same rates in children on ABC as those on d4T, as suggested by the cohort study at Rahima Moosa Mother and Child Hospital (RMMCH), is cause for concern. The reasons for this need to be investigated.

The overall aim of the study was therefore to examine adherence, PMTCT exposure history, and TB co-treatment in more detail to see if there were significant differences in virological outcomes of young children (under three years old) when controlling for these factors in addition to the NRTI backbone (ABC- or d4T-based).

It is hoped that the findings of this study will improve the understanding of the causes of virological failure among HIV-infected children. This, in turn, could ultimately curb the expenditure associated with HIV care, improve patient outcomes, and provide policy-makers and facility managers with the valuable information they need to optimise patient care and improve healthcare services.

1.2.1 Aim

The study aimed to identify the effect of certain factors on the HIV virological suppression rate in the first three years of treatment in HIV-infected children who started ART (LPV/r-based therapy) under the age of three after 2008 till 2012 at RMMCH.

1.2.2 Objectives

The study had three main objectives, which were to:

1) Describe pre-treatment characteristics (gender, age at starting ART, VL, CD4 count, and weight and height for age Z-score), concurrent NRTI (ABC- or d4T-based therapy) used, and virological outcome (virological suppression defined as suppression rate to <50 copies/ml at 6, 12, 24, and 36 months).
2) Describe adherence, concurrent TB treatment, and PMTCT exposure using the following characteristics related to the first three years on ART:
    i. Adherence:
        - Lowest adherence score obtained for the first, second, and third year of treatment.
        - Presence or absence of treatment interruptions for >1 month in the first, second, and third year of treatment.
        - Maximum number of concurrent individual medicines in the first, second, and third year of treatment.
    ii. Concurrent TB treatment:
        - Presence or absence of concurrent RIF containing anti-TB drugs during the first three years of ART. If present:
            - Timing: In the first, second, or third year of ART;
            - Type: Pulmonary TB, extra-pulmonary TB, Bacille Calmette-Guérin disease;
            - Boosting of LPV/r with RTV as either double dose lopinavir/RTV or addition of extra RTV to LPV/r, or sub-optimal unboosted lopinavir/RTV.
    iii. PMTCT exposure during pregnancy for mother and baby:
        - Presence or absence of PMTCT exposure;
        - Type of PMTCT exposure, if given as a single dose NVP, AZT- based or triple ART.
3) Assess the effect of the above factors (pre-treatment characteristics, NRTI choice, TB, PMTCT, and adherence) on the virological suppression rate at 6, 12, 24 and 36 months using univariate and multivariate analysis.

CHAPTER 2
LITERATURE REVIEW

Following a search of PubMed, Cochrane library, SUM research 2, Up to date, Google Scholar, and TRIP Database, relevant studies were selected for the literature review component of this study. The information collected from this research is presented below, including the proportion of patients living with HIV, estimated number of patients on ART, treatment failure in patients on ART, and factors which may predispose patients to poor virological suppression.

2.1 HIV infection and ART coverage

According to the WHO, 34.6 million people are living with HIV globally (2016), of which 2.1 million are children below 15 years old and a massive 79% (25.6 million) are living in Africa.(23) In 2005, only 2.1 million people were on ART, but by 2017 this had increased to an estimated 20.9 million, of which 13,799,000 (54%) are in Africa. The WHO's goal is to further increase the number of patients on ART to 30 million by 2020.(23)

The WHO has recommended the initiation of ART in all HIV-infected children, regardless of CD4 count or HIV clinical staging.(23) This change in the criteria for initiation of ART in children has resulted in an increase in the number of children on ART and consequently there has been a reduction in HIV-related mortality and morbidity rates.(5, 24) Unfortunately, early ART initiation also means prolonged duration of therapy (life-long therapy from infancy), which comes with its own challenges, including with treatment monitoring strategies, adherence, development of drug resistant (poor adherence), and switching of therapy (especially with limited second-line options). It is therefore critical to be able to recognise and appropriately manage treatment failure.(23, 25)

2.2 Treatment failure

There is no consensus on the precise definition of treatment failure although some criteria have been proposed,treatment failure can be classified into three categories: clinical failure, immunological failure, and virological failure.(26, 27) Factors associated with treatment failure can be classified into: patient-related factors, such as poor adherence, malabsorption (vomiting), primary or acquired drug resistance, and other underlying diseases; and ART-related factors, such as adverse effects, suboptimal pharmacokinetics, incorrect dosing, and high pill burden or drug-to-drug interaction.(28, 29)

2.2.1 Clinical failure

Clinical failure is defined as the development of new opportunistic infections and/or evidence of severe immunodeficiency (WHO clinical stage 3 or 4) following six months of effective therapy.(24) This must be differentiated from immune reconstitution inflammatory syndrome.(30)

2.2.2 Immunological failure

Persistent CD4 levels below 200 cell/mm^3 or below 10% in children less than five years and below 100cells/mm^3 in children over five years is considered immunological failure.(31) It can take at least one year to achieve immune recovery (CD4 cell count >500cells/mm^3), although virological suppression occurs within a shorter period. Immunological failure with good virological outcome is uncommon in paediatric patients.(26) In such a scenario, it is important to exclude laboratory error in CD4 or viral load measurements. Opportunistic infection or any concomitant illness which may suppress immunological response also needs to be ruled out.(26) A systemic review has shown that both WHO clinical and immunological failure criteria have low sensitivity and predictive values for identifying patients with virological failure.(32)

2.2.3 Virological failure

Plasma VL has been shown to be the best single marker of progression to HIV-related disease and death, and VL monitoring can identify failure significantly earlier when compared to immunology testing.(33) When VL is below the lower levels of detection, using highly sensitive assays with lower limits of quantitation of 20 to 75 copies/ml, a patient is considered virally suppressed.(34) Conversely, a repeated plasma VL of above or equal to 1000 copies/ml following six months of ART is considered virological failure.(31) This can occur as an incomplete initial response during therapy or a rebound following VL suppression. Ongoing non-suppression, particularly with NNRTI-based regimens is a cause for concern because it is highly associated with increased risk of drug resistance.(35, 36) Viral blips is a transient isolated episode of low-level increase in plasma VL (>50 -1000 copies/mL), followed by a return to viral suppression.(37) It occurs commonly and is not associated with subsequent virological failure. It can be due to laboratory error, poor adherence intermittently, or a transient burst of HIV replication.(38) The South African Department of Health and Southern African HIV Clinicians Society (SAHCS) have designed a stepwise approach to VL monitoring and management, as outlined in Table 1.(39)

Table 1. South African Department of Health and Southern African HIV Clinicians Society (SAHCS) VL monitoring and management approach

Department of Health	**SAHCS**
<400: no specific actions	>50: adherence counselling and repeat viral load in two to three months
400-1000: adherence counselling and repeat viral load six monthly	>1000 on two occasions two to three months apart – switch therapy
>1000: adherence counselling and repeat viral load in two to three months • If repeat <1000, repeat viral load in six months • If repeat >1000, switch therapy	

2.3 Adherence

There is still a high rate of infant HIV infection annually in sub-Saharan, however, as a result of successful ART use, children are surviving well into adolescent and adult years.(40) These children are started on life-long therapy mostly early during infancy, and adherence soon becomes a challenge. Nevertheless, a systematic review of paediatric ART adherence studies in low- and middle-income countries reported more than 75% adherence, which was a better outcome when compared to high-income countries who showed ART adherence of less than 75%.(18) Suboptimal therapy secondary to poor adherence may result in virological failure and drug resistance.(41, 42)

Factors contributing to paediatric non-adherence can be classified into: child-related factors, due mostly to the developmental age, caregiver, and regimen (burden of medication, side effects); and society-related factors (stigma).(43) Although most of the causes of poor adherence may be similar to the causes of poor adherence in other chronic diseases, the stigma associated with HIV infection or AIDS is a unique phenomenon. A study done in Ghana on caregivers of paediatric patients who had not disclosed their child's HIV status found that issues such as level of education, health literacy, and HIV-associated stigma are challenging barriers to paediatric HIV disclosure. One way this could be overcome is through properly designed HIV education campaigns.(44)

There are various strategies that can be used to assess adherence and predict virological failure and drug resistance, such as clinic pill count (CPC), pharmacy refilling (PR), and electronic adherence monitoring device (EAMD). Self-reporting (SR) is less reliable and a poor predictor of virological failure or development of resistance. With limited available second-line options, it is vital to use more reliable measures when assessing adherence.(43)

2.4 HIV-TB co-infection

HIV-TB co-infection in Africa was about 79% in 2012, and is the leading cause of death from infectious diseases worldwide, with the highest incidence in developing countries.(45-48) HIV-TB co-infection has an accelerated disease process, which leads to more severe symptoms and complicated types of TB.(45, 49) The risk of extra-pulmonary TB (EPTB) or disseminated TB is increased in HIV-positive patients, particularly with low CD4 cell counts. This also results in further reduction of the CD4 count and causes an increase in the of HIV VL.(50)

Diagnosis of TB in children remains a huge challenge, especially with the high incidence of HIV co-infection among children in sub-Saharan Africa. The currently used TB screening tests have poor sensitivity, particularly in patients who are immunologically suppressed; the tuberculin skin test has about 20% sensitivity and gastric aspirates only about 10% sensitivity.(45, 51, 52) Children are started on treatment based on a history of close contact with an adult who has pulmonary TB (the risk of transmission is 60-80% in sputum acid fast bacilli positive source), clinical presentation, presence of constitutional symptoms, and chest radiographic changes suggestive of TB (which could also be due to other HIV-associated lung conditions, thus making over-diagnosis of TB a possibility).(49, 53)

Because HIV-positive children are 20-30 times more likely to develop active TB disease compared to HIV-negative children, having a high index of suspicion for TB infection and starting HIV positive children on TB treatment even without laboratory evidence is necessary at times.(54) In 2016, 1 million children(globally) aged between 0-14 years acquired TB, of which 25% (including children with HIV-associated TB) died from the disease.(54)

Early initiation of ART is associated with a significant drop in the incidence of TB in HIV-positive children. Before ART, the incidence was 16.4 per 100 person yearly, and after ART 6.3 per 100 person yearly.(55) The treatment of TB in HIV-infected patients is complex, and it is important to note the potential drug interactions and side-effects as well as complications related to the immune reconstitution inflammatory syndrome (IRIS). First a combination of RIF, isoniazid (INH), ethambutol, and pyrazinamide is given for two months, followed by four months continuation phase of RIF and INH. A supplement of pyridoxine is also given to reduce the risk of peripheral neuropathy associated with INH.(56) South African guidelines recommend PI-based ART with LPV/r for HIV-infected children less than three years old or less than 10kg with ABC and 3TC as part of the first-line regimen.(57) RIF is a strong inducer of cytochrome P450 enzymes, which results in sub-therapeutic plasma concentrations of ARVs, specifically PIs, however, RTV used as an add-on booster has been shown to optimise the PI plasma level.(58)

Another drug-to-drug interaction is the increased risk of drug toxicity from both ART and TB treatment, such as hepatic toxicity, peripheral neuropathy, and other gastrointestinal signs and symptoms.(57) In addition, patients tend to find it more difficult to be adherent to their treatment due to an increased number of medications, and this can subsequently lead to the development of drug resistance.(59-61)

2.4.1 Bacille Calmette-Guérin vaccine

The Bacille Calmette-Guérin (BCG) vaccine is used globally for the prevention of meningeal and military tuberculosis in children.(62) Complications associated with BCG vaccination can be classified as localised or disseminated.(63) Localised BCG reactions include regional lymphadenitis, and abscess formation on the injection site, which can occur in healthy individuals or as a result of IRIS in HIV-positive patients.(63) Disseminated BCG reactions can be characterised by two or more of the following signs and symptoms suggestive of systemic mycobacteria disease: weight loss, fever, lymphadenopathy or cutaneous abscesses, pneumonia, osteomyelitis, hepatomegaly, and splenomegaly. In patients with localised BCG reaction, expectant management is advocated and anti-TB drugs should not be used. In patients with disseminated BCG, TB drugs effective for BCG disease should be used.(64)

Because of the high exposure to TB in children in South Africa, BCG vaccination is offered to all new born babies and this is in line with the WHO recommendations for a TB endemic area.(65) The use of BCG in HIV-infected babies is associated with an increased incidence of disseminated Mycobacterium bovis disease, but early initiation of ART ameliorates this risk.(65)

2.5 ART used in PMTCT

Successful strategies for PMTCT have led to a decline in the number of infants acquiring HIV perinatal or through breast milk.(66) In fact, in 2015, a few African countries, came close to achieving the target of reducing MTCT by 90%, including South Africa (84%) and Uganda (86%).(67)

In line with the new WHO guidelines for PMTCT, all HIV-infected pregnant woman are started on lifelong ART (tenofovir [TDF] and 3TDF/emtricitabine [FTC] + EFV) regardless of CD4 count and HIV clinical stage.(68) HIV-exposed infants are started on a NVP daily dose from birth to six weeks and/or zidovudine (AZT) in 12-hourly doses. The chance of HIV passing from mother to child without treatment is about 15-45%, but effective PMTCT can decrease the risk to less than 2%.(23)

The choice of ART in children is largely influenced by PMTCT exposure history, in which case they would been exposed to NNRTIs for a prolonged duration (at least six weeks), as opposed to a single dose at birth. In the Nevirapine Resistance Study (NEVEREST), the protease inhibitor LPV/r was shown to be superior when compared to NVP in children less than three years old among HIV-positive children with and without previous PMTCT exposure. This supports the use of a LPV/r-based ART regimen in all children less than three years old.(69)

2.6 Effect of age on VL suppression rate

Disease progression in HIV-infected infants is much more rapid compared to older children or adults. Furthermore, a Ugandan study done on factors associated with virological non-suppression among HIV-positive patients on ART demonstrated a higher rate of virological failure among a group of patients aged 0-4 years (29%) and adolescents aged 15-19 years (27%), while older patients had a failure rate of only 7%.(28) In addition, a study done in the United Kingdom and Ireland showed that younger children have a poorer virological response, and thereby increased risk of resistance, but also have a better immunological response.(70) These findings support early initiation of ART in children.(71)

CHAPTER 3

MATERIALS AND METHODS

3.1 Study design

This longitudinal retrospective cohort study used historic data to analyse virological outcomes of a group of 296 children who started ART (LPV/r-based therapy) under the age of three between 2008 and 2012 at RMMCH with at least one year of post-ART initiation follow-up. This was a secondary analysis of an existing HIV treatment database of paediatric patients at RMMCH, complemented by a retrospective file review.

3.2 Study site

The study was conducted at the Empilweni Clinic, situated at RMMCH, which functions as a research unit as well as offering routine services. Empilweni is one of the largest paediatric treatment clinics in the country, with over 1,600 children in active follow-up. The clinic manages both HIV-exposed and HIV-infected infants and children and has been conducting clinical trials since 2003 with the aim of finding ways to improve the lives of women and children living with HIV. The unit conducts investigator-driven research projects in conjunction with international collaborators, carrying out work that has not only influenced South Africa's treatment guidelines for children and pregnant women, but international guidelines as well.

3.3 Study inclusion criteria

- HIV positive children aged 3 years and below started on ABC/ d4T first line based regimen with lopinavir/ritonavir and 3TC.
- Patients who initiated treatment after 2008 till 2012 at RMMCH with at least 12months of post ART initiation follow-up.

3.4 Study exclusion criteria

- Children above 36 months
- Patients on other ART regimen
- Patients with follow-up from base line of less than 12 months
- Patients who did not attend between 2008 -2012 and those transferred in after ART initiation.

3.5 Data collection

Data was gathered from two sources. The first source of data was the existing database from where the following variables were directly extracted to a Microsoft Excel spreadsheet (Appendix A):

- Pre-treatment characteristics (age at ART initiation, gender, CD4 count, viral load, and anthropometric measurement [weight and height for age z-scores]). The pre-treatment CD4, VL, weight and height for age z-score values were taken as any value before or on the date of ART initiation and if more than one value was available, the value closest to ART initiation was used.
- NRTI backbone (either ABC/3TC or d4T/3TC).
- Virological outcomes (suppressed or not suppressed to <50 copies/ml at 6, 12, 24, and 36 months).

The second source of data was a retrospective file review conducted to collect more detailed information on PMTCT exposure, concurrent TB treatment, and adherence. To facilitate this file review, the form presented in Appendix B was used and this data was added to the spreadsheet (Appendix B). Each patient's database number was noted on the form, which otherwise contained no other identifying information. In assessing adherence, the score that is routinely calculated for patients at each visit was used. It is standard practice at the clinic for the counsellor or nurse assessing the patient before their doctor's visit to complete a score sheet (Appendix C). A total score of 8-10 is considered as good adherence and a score below 8 is considered as poor adherence. The lowest score achieved in each year of treatment after ART initiation was recorded from the files.

Data was limited to after 2008 to reduce the possible calendar effect of the evolving clinic and to limit data to the time two years prior to the policy change of d4T to ABC use in 2010. Permission has been obtained from the data gatekeeper (Sr Annie Jordan) from the Empilweni Clinic as well as from the RMMCH hospital CEO.

3.6 Data analysis

All data was captured and stored in Microsoft excel. Data was then imported to a statistical package (STATA version 12, SAS version 9.4). Different methods of data analysis were used. For the first two study objectives, data was analysed by descriptive methods. Categorical data was expressed in proportions and percentages. Medians and inter-quartile ranges were used for continuous data. In this way, all the variables listed in the objectives were described. For the third objective, virological suppression as the outcome variable was assessed against each of the other factors in a univariate analysis. For example, virological outcomes were compared for each gender, then for each age group at ART initiation, then for children started on d4T vs those started on ABC, etc. Univariate analysis employed both Chi-square test (for proportions) and t-tests or Wilcoxon tests (depending on the distribution of continuous variables). Multiple analysis of variance method was used to analyse the treatment response where more than two groups were compared.

Once the univariate analysis was done, the final virological suppression as outcome was assessed by multivariate analysis (multiple logistic regression) against all the factors above in order to determine which factor had the greatest effect of achieving or not achieving virological suppression. This process was done for the virological outcome of ever being virally suppressed between 6-36 months. This enabled the comparison of study findings with those in the previous cohort study where the choice of NRTI of either d4T or ABC had the most significant effect on achieving suppression or not.

3.7 Ethical considerations

The study proposal was submitted and approved by the Human Research Ethical Committee of the University of Witwatersrand as well as the departmental research protocol committee for approval (clearance certificate number M140747 - Appendix D). Data collected was used only for the purpose of this study and all details will remain confidential. Study numbers were used for data processing and reporting in order to ensure confidentiality.

As this was a retrospective study, no individual patient consent was needed. Caregivers of children attending RMMCH HIV services are routinely invited to sign a prospective data-sharing consent form. Any cases where this was refused were excluded from the analysis.

CHAPTER 4

RESULTS

4.1 Study population selection process

Between 2008 and 2012, there were 3,728 children attending the Empilweni Clinic. Of these, 3,432 did not meet the inclusion criteria and were excluded from the study. There was therefore a starting population of 296 patients (Figure 1). For file review, only 211 patients were captured for data analysis due to missing files. The children included in the study were below 36 months at the time of ART initiation, on either ABC or 3TC for at least 12 months, and were started on ART at RMMCH after the year 2008.

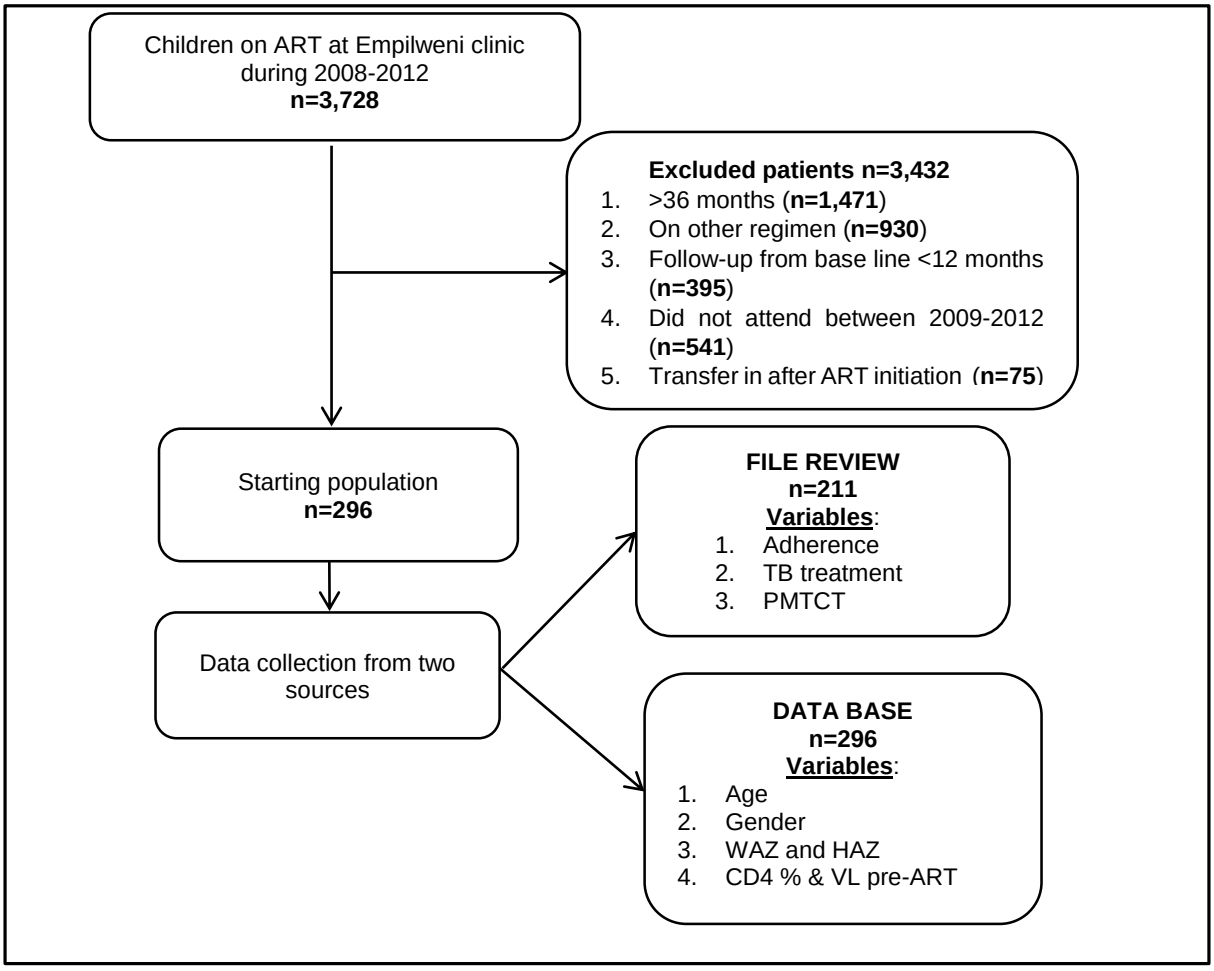

Figure 1. Flow diagram of study population

ART: antiretroviral therapy, HAZ: height for age z-score, HIV: human immunodeficiency virus, PMTCT: prevention of mother-to-child transmission, TB: tuberculosis, VL: viral load, WAZ: weight for age z-score.

4.2 Demographic characteristics of infants at ART initiation

The median age of ART initiation was eight months (interquartile range [IQR]: 4-16), but the largest group of patients were between 0-6 months old, at 41% (120), and 51% of patients were female. Out of 296 patients included in the study, 254 patients had records on pre-ART weight for age z-score (WAZ) and height for age z-score (HAZ). Almost half of the patients 49% (125) were underweight for age, and 41% (105) were stunted. The median base line CD4 count percentage was 21.1% (IQR: 14-29) among 63% of patients (189) patients with available pre-ART CD4 count records. The median pre- ART HIV viral load in 63% (190) of patients was 5.8 $\log_{10}$ copies/ml (IQR: 5-6). More patients were on a d4T-based regimen (60%) than an ABC-based one (40%). Table 2 captures these demographic characteristics.

Table 2. Demographic characteristics of infants at ART initiation

Variables	Frequency=N (%)
Total number of patients	296
Age ranges 0 – 6 months 7 – 12 months 13- 24 months 25-36 months	 120 (41) 69 (23) 80 (27) 27 (9)
Gender female	152 (51)
Pre-ART weight < -2 z-score >/= -2 z-score Missing	 125 (49) 129 (51) 42
Pre-ART height < - 2 z-score >/= - 2 z-score Missing	 105 (41) 149 (59) 42
Pre-ART CD4 % Missing	Median: 21.1 IQR (14.3-29.0) 113
Pre-ART VL copies/ml Missing	Median: 5.8 IQR (5.3-6.4) 107
ART regimen d4T ABC	 179(60) 117(40)

All statistics presented as N (%) unless otherwise indicated.

ABC: abacavir, ART: antiretroviral therapy, d4T: stavudine, IQR: interquartile range, VL: viral load.

Z-scores calculated according to WHO 2007 igrowup SAS macro package.

4.3 Proportion of children virologically suppressed at 6, 12, 24, and 36 months

There were a higher number of available VL records earlier during therapy, with 84% (249) for children at six months of ART. This number declined over time, to 79% (234) for children at 12 months of ART, 72% (215) for children at 24 months of ART, and only 55% (164) for children at 36 months of ART (Figure 2). Using the VL threshold of 50 copies/ml, the VL suppression rate was lowest at six months (19%), but there was a progressive upward trend in the virological suppression rate later during therapy, at 28% (12 months), 33% (24 months), and 40% (36 months).

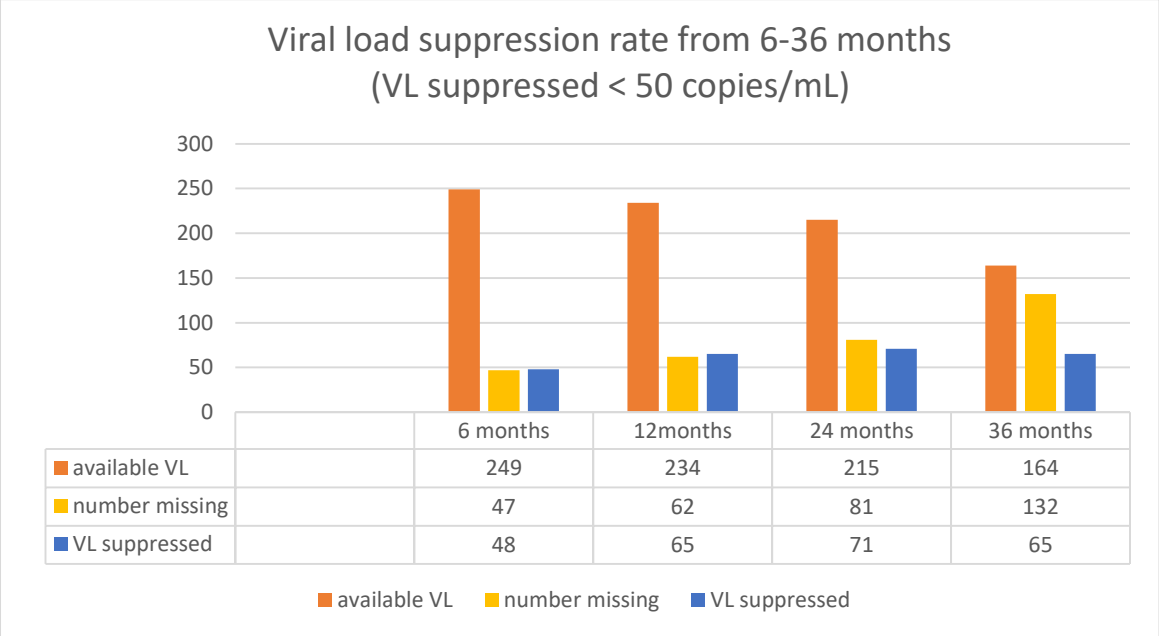

Figure 2. Virological outcomes at 6, 12, 24, and 36 months of ART

4.4 Description of adherence score, treatment interruptions, and number of medications at first, second, and third year of ART

During the first year of ART, there was available data on adherence for 199 out of 296 patients. The median adherence score was 8, and only 12% of patients had an adherence score below 8 (poor adherence). The median adherence score for both the second and third year of ART was 9. In the second year of ART, data on adherence was available for 193 patients, and only 9% had a poor adherence score. In the third year of ART , data on adherence was available for only 153 patients and 10% of these patients had a poor adherence score.

The majority of patients were started on more than four different drugs in the first year of ART (99%) (co-trimoxazole, multivitamins, ART and TB treatment with booster RTV), but this number dropped to 63% in the second year, and only 39% by the third year. As with adherence, only a small percentage of patients had treatment interruptions during ART. In the first year of ART, there was available data on treatment interruption for 203 patients, and only 8% of patients had treatment interruption. In the second year of ART, the total number of patients with available data on treatment interruption was 198 and the rate of treatment interruption was slightly higher, at 12%. In the third year of ART, there were again fewer number of patients with available data (162), but the rate of treatment interruption was only 6%.

Table 3. Adherence score, number of medications, and treatment interruption during the first, second, and third year of treatment

Treatment variables	First year	Second year	Third year
Lowest adherence score			
Data available	199	193	153
Median lowest score	8	9	9
% below 8	12	9	10
Maximum meds			
Data available	204	198	160
Median number of medications	5	5	4
% above 4	99	63	39
Interruptions			
Data available	203	198	162
% of interruptions	8	12	6

Adherence assessed using the adherence score sheet, completed by a counsellor or nurse assessing the patient on each visit. A good adherence score was a score between 8 -10 and a score below 8 was classified as a poor adherence score. Treatment interruptions were present if a patient had treatment interruption lasting >1 month.

4.5 HIV-TB co-treatment

Data on TB was available for 68% (202) of patients, of which 40% (80) were treated for TB during ART. Of these, 75% (60) had pulmonary TB, 12% (10) EPTB, and 4% (3) BCG adenitis. A total of 90% (72) were started on TB treatment in the first year of ART. Patients on HIV-TB co-treatment, 71% (57) were boosted with RTV, 26% received double dose kaletra as a booster, and 3% also did not receive any booster at. Some patient in the study where bostered correctly initially but due to stock outs had to be given double kaletra.

Table 4. HIV-TB co-treatment

Study population	**296**
Available data on HIV-TB cotreatment	**202 (68%)**
Children with HIV-TB cotreatment-total available	**80 (40%)**
Timing	**N (%)**
TB before ART start	72 (90%)
TB after ART start	8 (10%)
Site of TB	**N (%)**
Pulmonary TB	60 (75%)
Extra-pulmonary TB	10 (12%)
Bacillus Calmette-Guerin (BCG adenitis)	3 (4%)
Unknown	7 (9%)
PI dosing	**N (%)**
Double dose kaletra	21 (26%)
RTV boosted	57 (71%)
Unboosted	2 (3%)

ART: antiretroviral therapy, HIV: human immunodeficiency virus, TB: tuberculosis.

4.6 PMTCT exposure for mother and child

There were 156 patients (53%) who had records on PMTCT, of which 90 (58%) had received some form of PMTCT. Out of 90 mothers with records on PMTCT, 21 (23%) received a single dose of NVP, 43 (48%) received AZT-based PMTCT, 13 (14%) received triple ART, and 13 (14%) received no PMTCT. Of the 89 children with records on PMTCT, 21 (24%) received a single dose of NVP, 43 (48%) received AZT- and NVP-based PMTCT, 35 (39%) received daily NVP, and 8 (9%) received no PMTCT.

Table 5. PMTCT exposure for mother and baby

PMTCT exposure	Frequency	Percentage %
Total patients with PMTCT records	156	53
Received any PMTCT	90	58
Mother – N=90		
PMTCT- SD NVP	21	23
AZT-based PMTCT	43	48
Triple ART PMTCT	13	14
None	13	14
Baby – N=89		
PMTCT- SD NVD	21	24
AZT+NVP-based PMTCT	35	39
Daily NVP	26	29
None	8	9

ART: antiretroviral therapy, AZT: zidovudine, PMTCT: prevention of mother-to-child transmission, SD NVP: single dose nevirapine.

4.7 Virological suppression rate for pre-ART characteristics

4.7.1 Age categories

The Chi-square statistics for the association between VL suppression rates and the four age categories at 6, 12, 24, and 36 months of ART did not show any statistically significant differences.

Table 6. Virological suppression rate for different age groups at 6,12,24 and 36months of ART

Age group in months	Number of available VL at 6,12,24 and 36months of ART			
	6months	12months	24months	36months
0-6	103	94	88	64
7-12	55	52	55	38
13-24	69	64	53	48
25-36	22	23	19	14
Total number VL available	**249**	**233**	**215**	**164**
Age group in months	**VL suppression rate (VL<50 copies/ml) at 6,12,24, and 36months of ART**			
	6months N (%)	**12months N (%)**	**24months N (%)**	**36months N (%)**
0-6	21(20)	23(25)	34(39)	29(45)
7-12	10(18)	16(31)	16(29)	14(37)
13-24	11(16)	18(28)	15(28)	17(35)
25-36	6(27)	8(35)	6(32)	5(36)
P value	**0.670**	**0.720**	**0.530**	**0.690**

Number VL available: number of available viral load for this variable (age group)

ART: antiretroviral therapy, VL: viral load.

4.7.2 Gender

The chi-square statistics for the association showed no statistically significant difference between gender and VL suppression rate at 6, 12, 24, and 36 months of ART. It was poorer at six months for both females (20%) and males (19%), but improved dramatically at 36 months, at 42% in females and 38% in males.

Table 7. HIV virological suppression rate for gender at 6, 12, 24, and 36 months of ART

	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART			
GENDER	**6 months N (%)**	**12 months N (%)**	**24 months N (%)**	**36 months N (%)**
Number VL available	**122**	**117**	**104**	**77**
Female	25 (20)	37 (32)	35 (34)	32 (42)
Number VL available	**127**	**116**	**111**	**87**
Male	23 (19)	28 (24)	36 (32)	33 (38)
P value	0.630	0.200	0.840	0.630

Number VL available: is the number of available viral load for this variable (gender)

ART: antiretroviral therapy, HIV: human immunodeficiency virus, VL: viral load.

4.7.3 Weight for age and height for age z- scores

In terms of the Chi-square for the association between weight for age and height for age at pre-ART and at 6, 12, 24, and 36 months of ART, there was no significant difference between patients who had normal weight or height at ART initiation and patients who were underweight or stunted at ART initiation. At 6 months, the VL suppression rate was 20% and 21% for patients with normal weight and underweight respectively, and at 36 months these went up to 31% and 44% respectively. The VL suppression rate at six months was 18% and 23% for patients with normal height for age and those who were stunted at pre-ART respectively. Again, this rate increased to 40% and 35% respectively at 36 months.

Table 8. Viral load suppression rate for pre-ART weight and height for age z-score

Z-SCORE	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART			
	6 months N (%)	12 months N (%)	24 months N (%)	36 months N (%)
Weight for age z-score (WAZ)				
Number VL available	**107**	**101**	**98**	**75**
Below -2 z-score	22 (21)	28 (28)	29 (30)	33 (44)
Number VL available	**113**	**104**	**91**	68
Above/equal -2 z-score	22 (19)	31 (30)	32 (35)	21 (31)
P value	0.830	0.740	0.410	0.100
Height for age z-score (HAZ)				
Number VL available	**93**	**83**	**80**	**62**
Below -2 z-score	21(23)	25(30)	27(34)	25(40)
Number VL available	**127**	**122**	**109**	**81**
Above/equal -2 score	23(18)	34(28)	34(31)	29((36)
P value	0.410	0.720	0.710	0.580

Number VL available: number of viral load available for this variable (weight/height) ART: antiretroviral therapy, HIV: human immunodeficiency virus.

Weight for age < - 2 z score: underweight, weight for age > /= - 2 z score: normal weight for age, height for age z score of < - 2: stunted, height for age z score of >/= - 2: normal height for age.

4.7.4 Viral load at ART initiation

The subgroup analysis of the baseline VL over 100,000 copies/ml showed no difference in the proportion of children virologically suppressed when compared to those with the baseline VL below 100,000 at six months (p = 0.911) and 36 months of ART (p=0.647). Further analysis with the multivariate logistic regression model gave the following results: OR 1.61, 95% CI: 0.5 to 5.2 p=0.423. In the logistic regression calculation, the outcome was categorised into patients with VL >100,000 versus patients with VL <100,000. There was a poorer VL suppression rate, particularly earlier during therapy, with a majority of patients only being suppressed at 36 weeks of ART.

Table 9. Viral load suppression rate for pre-treatment VL of <100,000 and VL of >100,000 at 6, 12, 24, and 36 months

Pre-ART Viral load (copies/mL)	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART			
	6 months N (%)	12 months N (%)	24 months N (%)	36 months N (%)
Number VL available	**29**	**27**	**18**	**13**
VL <100000	5 (17)	8 (30)	7 (39)	4 (31)
Number VL available	**138**	**125**	**123**	**102**
VL >100000	25 (18)	32 (26)	37 (30)	38 (37)
P value	0.911	0.666	0.451	0.647

Number VL available: number of available viral load for this variable (pre-ART)

ART: antiretroviral therapy, VL: viral load.

4.7.5 CD4 count at ART initiation

With regard to the Chi-square for the association between baseline CD4 count and the VL suppression rate at 6, 12, 24, and 36 months of ART, the subgroup analysis of the baseline CD4 count above 20% showed no statistically significant difference in the proportion of children virologically suppressed when compared to those with the baseline CD4 count below 20% at six months (p = 0.830) and at 36 months of ART (p =0.880).

Table 10. Comparison of VL suppression at 6, 12, 24, and 36 months between patients with a pre-ART CD4 count of <20% and >20%

Pre-ART CD4 Count (%)	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART			
	6 months N (%)	12 months N (%)	24 months N (%)	36 months N (%)
Number VL available	**73**	**75**	**67**	**54**
CD4 <20	13 (18)	16 (21)	16 (24)	20 (37)
Number VL available	**89**	**77**	**74**	**56**
CD4 >20	17 (19)	25 (32)	25 (34)	20 (36)
P value	0.830	0.120	0.190	0.880

Number VL available: number of available viral load for this variable (CD4 count)

ART: antiretroviral therapy, VL: viral load.

4.8 Comparison of VL suppression rate between ABC- and D4T-based first-line regimen

Comparison between the VL suppression rate for patients on ABC-based vs. d4T-based first-line regimen showed that patients on d4T had a superior VL suppression rate. The proportion suppressed (VL <50copies/ml) were significantly lower in the ABC groups at six months (ABC 9% vs.d4T 34%, p=<0.000), 12 months (ABC 18% vs.d4t 43% p= < 0.000), 24 months (ABC 28% vs.d4T 40% p=0.064), 36 months (ABC 31% vs.d4T 50% p=0.010). At six months of therapy, the ABC group had a higher virological failure rate, which did improve over time, remaining substantially higher when compared to the d4T group.

Table 11. Comparison of HIV suppression rate between ABC- and D4T-based first-line ART over a period of 6, 12, 24, and 36 months

NRTI START	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART			
	6 months N (%)	12 months N (%)	24 months N (%)	36 months N (%)
Number VL available	**149**	**140**	**128**	**88**
Abacavir	14 (9)	25 (18)	36 (28)	27 (30)
Number VL available	**100**	**93**	**87**	**76**
Stavudine	34 (34)	40 (43)	35 (40)	38 (50)
P value	< 0.0001	< 0.0001	0.060	0.010

Number VL available: number of available viral load for this variable (ART)

ART: antiretroviral therapy, NRTI: nucleotide reverse transcriptase inhibitors, VL: viral load.

Figure 3 shows the overall VL suppression proportion for the ABC and d4T groups over a period of 6-36 months. Patients on d4T had a significantly higher viral load suppression rate (70%) when compared to those on ABC (38%) with the value of <0.0001.

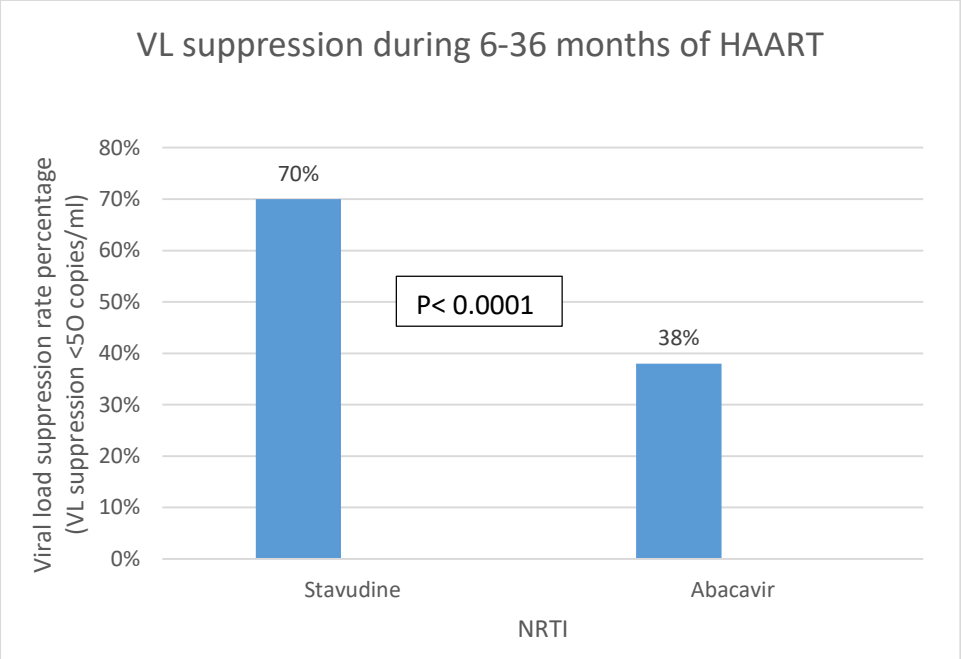

Figure 3. Comparison of VL suppression rates between ABC- and d4T-based first-line ART ever between 6-36 months of ART

HAART:highly active antiretroviral therapy, VL: viral load, NRTI: nucleoside reverse transcriptase inhibitors.

4.9 VL suppression rate of patients who received HIV-TB co-treatment

The Chi-square statistics for the association between HIV-TB co-treatment and the viral load suppression rate for patients ever between 6-36 months of ART showed that patients with concomitant HIV-TB treatment had a significantly higher VL failure rate (56%), when compared to the group never treated for TB (40%).

Table 12. VL suppression rate ever between 6-36 months of patients treated for TB

HIV-TB CO-TREATMENT	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART	
	Viral load suppressed N (%)	No Viral load suppression N (%)
TB	35 (44)	45 (56)
No TB	73 (60)	49 (40)
P value	0.025	

ART: antiretroviral therapy, HIV: human immunodeficiency virus, TB: tuberculosis, VL: viral load.

4.10 Effect of the number of drugs taken by the patient on the VL suppression rate

The Chi-square statistics for the association between patients on a higher number of drugs (more than five) and patients on a lower number of drugs (less than five) in the VL suppression rate for patients ever between 6-36 months of ART showed no statistically significant difference between the two groups (p=0.43).

Table 13. VL suppression rate ever between 6-36 months of ART in patients who received more or less than five drugs

NUMBER OF MEDICATION	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART	
	Viral load suppressed N (%)	Not virologically suppressed N (%)
More than 5	40 (50)	40 (50)
Less than 5	69 (56)	55 (44)
P value	0.430	

ART: antiretroviral therapy, VL: viral load.

4.11 Virological suppression rate for patients with previous PMTCT exposure

Previous PMTCT exposure did not have any statistically significant effect on the VL suppression rate in patients ever between 6-36 months of ART. Patients with a previous history of PMTCT exposure had a VL suppression rate of 53% and those with no previous PMTCT exposure had a VL suppression rate of 51%.

Table 14. VL suppression rate ever between 6-36 months for patients on ART with previous PMTCT exposure

PMTCT	VL suppression rate (VL<50 copies/mL) at 6, 12, 24, and 36 months of ART	
	Virologically suppressed N (%)	Not virologically suppressed N (%)
PMTCT exposure	47 (53)	42 (47)
No PMTCT exposure	34 (51)	33 (49)
P value	0.79	

ART: antiretroviral therapy, HIV: human immunodeficiency virus, PMTCT: prevention of mother-to-child transmission, VL: viral load.

4.12 Multiple logistic regression

Due to the missing data in some patients, the final multiple logistic regression model only contains 98 patients with all data available. The outcome was chosen as ever recording a VL <50 copies/ml between 6-36 months on ART. When assessing the variables of pre-ART VL (more or less than 100,000 copies/ml), WAZ (more or less than -2 z-Scores), ART regimen (containing d4T vs. ABC), number of drugs used (more or fewer than five), TB treatment (present vs. absent), and PMTCT exposure, only the regimen variable yielded a significant result (OR 3.41,95% CI:1.2 to 9.7 p=0.021).

Table 15. Assessing the likelihood of reaching suppression among the treatment characteristics using multivariate analysis

Treatment characteristics	N/%	Unadjusted Odds ratio (95%CI)	P	Adjusted Odds ratio (95%CI)	P
VL					
>100,000	78 (50)	1.54 (0.5-4.6)	0.434	1.61 (0.5-5.2)	0.423
<100,000	16 (48)	Ref		Ref	
WAZ					
<-2z-score	26 (52)	0.95 (0.4-2.1)	0.900	1.01 (0.4-2.3)	0.989
.-2z-score	25 (50)	Ref		Ref	
ART regimen					
ABC	67 (38)	3.14 (1.2-8.0)	0.017	3.41 (1.2-9.7)	0.021
D4t	79 (70)	Ref		Ref	
PMTCT					
PMTCT-present	47 (53)	0.78 (0.35-1.76)	0.549	0.74 (0.31-1.8)	0.501
PMTCT-absent	34 (51)	Ref		Ref	
TB treatment					
TB-present	35(44)	1.59(0.69-3.70)	0.279	1.75(0.70-4.36)	0.228
TB-absent	73(60)	Ref		Ref	
Number of drugs					
More than 5	40(56)	0.73(0.31-1.75)	0.480	1.14(0.42-0.09)	0.794
Less than 5	69(56)	Ref		Ref	

ABC: abacavir, ART: antiretroviral therapy, d4T: stavudine, PMTCT: prevention of mother-to-child transmission, TB: tuberculosis. VL: viral load, WAZ: weight for age z-score. Ref: reference group

CHAPTER 5
DISCUSSION

This chapter contains a discussion of key findings of the results in the preceding chapter in light of the literature review.

5.1 Proportion of children virologically suppressed on ABC- vs. d4T-based first-line regimen

The study showed a higher rate of non-suppression among HIV positive patients under three years started on ABC-based first-line regimen when compared to those started on d4T-based first-line regimen (failure to suppress the VL below 50 copies/mL). Patients on ABC-based first-line regimen had a significantly lower VL suppression rate mostly earlier during ART and there was a delay in the VL suppression rate, with most patients still not being virologically suppressed at 36 months of ART.

5.2 VL suppression rate for pre-ART variables

Non-modifiable factors such as the different age groups, gender, and weight and height categories did not have any statistically significant effect on the viral load suppression rate. Patients in this study where all below three years old at ART initiation, with 41% in the 0-6 months age category, and had a mean pre-treatment viral load of 5.8 log10 copies/ml, which could also be a contributing factor to poor VL suppression rate in patients placed on ABC-based regimen. A study done on adult patients with a high pre-treatment VL showed ABC to be less effective in those with a high pre-ART VL.(15)A South African multi-cohort analysis which compared virological responses in children treated with ABC- vs. d4T-based ART also showed a poorer virological outcome in the ABC-based regimen group, which showed a reduced VL suppression rate at both 6 and 12 months of therapy.(13) Another double-blinded study on patients on ABC/3TC or tenofovir (TDF)/FTC versus patients on TDF/FTC/EFV or ATV/RTV showed a consistent VL failure in the ABC/3TC arm associated with higher VL (> 100000 copies/mL).(72)

While one pharmacokinetics study done showed a reduction of ABC level by 32% when used in combination with LPV/r, another study comparing virological responses to initial ART regimens containing ABC or TDF showed no significant difference in the VL suppression rate between the two drugs.(73) A possible explanation for having such a difference in results might be related to different age groups among the study populations in most studies, as well as the different combination of drugs used.

In this study, patients' weight and height pre- ART did not seem to have any significant effect in the virological suppression rate. However, another South African study did show a poorer virological suppression rate among severely underweight children initiated on ART when compared with normal or moderately underweight children.(74) The reason for these different results may be because the patients in this study who were underweight were not further classified as either severely or moderately under-weight.

5.3 Number of drugs and adherence influence on VL suppression rate

While patients mostly had a higher number of drugs earlier during ART, this did not appear to have any influence on the adherence score. Patients who were treated for TB had an overall higher number of total medications.

Adherence is important in order to achieve and sustain virological suppression. Pill burden (number of medication, frequency of dosing), palatability of ART, and development of adverse drug reactions may result in poor adherence to ART.(43) In this study, there was no statistically significant difference in the VL suppression rate between patients who had a low adherence score when compared to patients who did not. However, the assessment scoring system used in this study was subjective in that it relied on the information provided by the caregiver at the time of the interview and there was no objective way used to confirm if the information given was accurate. From the various methods that can be used to assess adherence, self-report is the most frequently used method but has been shown to overestimates adherence.(74)

There is a direct relationship between virological suppression, development of drug resistance, and adherence. A study assessing the incidence of risk factors for first-line ART failure among Ugandan children showed that one in three children are likely to develop VL treatment failure after the first 24 months of therapy mainly secondary to poor adherence to ART or prior exposure to SDNVP.(69) Conversely, this study found that patients had a poorer VL suppression mostly earlier during therapy (before 36 months) even among patients with good adherence, however, this may be because a high number of patients had a positive history of PMTCT exposure, and therefore the development of drug resistance in the study population should also be considered as a possible cause of a poor VL suppression.

5.4 HIV-TB co-infection rate and virological suppression outcomes

In this study, 40% of patients were treated for TB, which correlates with the overall rate of HIV-TB co-infection of 35-52% in childhood TB cases in South Africa. This is a high rate when compared to TB infection in HIV-negative children, which is only 10%.(75)

The majority of patients were diagnosed and received TB therapy earlier during or just before ART was initiated (first year of ART). The diagnosis of TB was mainly based on presence of constitutional symptoms and signs, chest x-ray features suggestive of TB, and in patients with a positive tuberculin skin test. Diagnosing TB in children is challenging, especially if they are HIV-positive. TB and HIV have overlapping clinical manifestations which could lead to high probability of false diagnoses of TB.(57, 58) Considering the fact that microbiological confirmed diagnosis of TB in children is only achieved in a minority (10%), the number of children treated for TB may overestimate the proportion of TB infection.(53, 55)

Most patients in the study had pulmonary TB and only a small percentage had EPTB, although HIV-positive patients have a higher risk of developing EPTB.(52) The rationale to start patients on TB treatment even without positive microbiological results cannot be argued; TB is the second leading cause of death from infectious disease worldwide, and HIV-infected children with TB have a 23% mortality rate, versus 4% in HIV-negative children.(76)

A four-drug combination TB treatment is recommended for high-risk patients, those who are immunosuppressed, and those with severe acute malnutrition.(61) RIF is a potent CYP3A4 inducer when given with a PI, for example LPV/r (LPV is metabolised through a cytochrome P450 enzyme) it causes a decrease in the plasma concentration of LPV.(77) RTV inhibits the cytochrome P450 (CYP3A4) enzyme, super boosting LPV/r with RTV for a 1:1 ratio is considered effective, based on pharmacokinetic studies.(18, 77) In this study, most of the patients where correctly boosted with RTV when on TB treatment. It is concerning that there were some cases where boosting was not done correctly and this should be addressed with further training, as inadequate boosting may lead to HIV drug resistance.

This study showed that TB was associated with a statistically significantly lower virological suppression rate compared to patients who never received TB therapy during ART. However, this finding was not maintained in the multivariate regression. The concern is that most patients had TB early during therapy in this study, and there was a higher rate of virological failure earlier during therapy for most patients as well. Nevertheless, contrary to these findings, other studies have shown that patients with HIV-TB co-infection have a high rate of virological failure, secondary to drug-drug interaction, poor adherence, and subsequent development of drug resistance.(64, 66)

5.5 PMTCT coverage and virological suppression

Increase in PMTCT coverage and ART for adults could also lead to an increased proportion of HIV-positive children with primary ARV drug resistance, and transmission of the HIV drug mutation M184V may reduce ABC activity.(78) This study did not show any statistically significant difference in the virological suppression rate between patients with previous PMTCT exposure and those without. Since no data was collected for resistance testing in this study, mutation/drug resistance could not be excluded as a possible contributing factor to poor virological suppression rates.

5.6 Limitations of the study

The study had several limitations, including patients lost to follow-up and significantly fewer children who had available VL results at 24 and 36 months. In addition, the final clinical outcome of patients with poor VL suppression rate could not be assessed, which is an important factor in the classification of treatment failure. When looking at the VL suppression rate between children on d4T compared to children on ABC-based first-line regimen, there may have been a calendar effect, however the study period was limited to the two years before and after the change of d4T to ABC to avoid this. There was also a limitation with regard to gaining adherence, TB, and PMTCT information from the files. The adherence measures used in the clinic may also have overestimated adherence and this may therefore not have shown up as a factor in the analysis. A further limitation of the study was the small sample size and lack of availability of all variables for all patients, which in turn limited the quality of the multivariate regression significantly. Imputation could have been considered but was deemed too complex for the purpose of this report; however, it should certainly be considered for further analyses.

As the study was limited to the patients at RMMCH, a large central academic unit, results may not be generalisable to all settings in South Africa, such as rural areas or non-academic sites. Data was collected retrospectively; therefore some important information may be missing from the files. Missing data is acknowledged.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study showed that the main contributory factor to VL suppression rates was the type of ART taken by the patient. Patients below three years started on an ABC-based first-line regimen had a significantly poorer VL suppression rate when compared to patients started on a d4T-based first-line regimen. There's a possibility that children on d4T could be a different population compared to the children on ABC-based first-line regimen. Taking into consideration other factors at baseline, TB drug exposure, PMTCT history, and adherence, no other factor could be identified on multivariate regression, but TB did emerge as a potential contributor to poorer viral outcomes.

6.2 Study recommendations

There is a need for more prospective studies in sub-Saharan countries that include a range of age groups with a longer follow-up plan and that assess the clinical outcome of patients with poor VL suppression. Other factors such as drug resistance in paediatric patients also need special attention, especially with the high rate of virological failure in the current first-line regimen.

HIV-TB co-infection is still a serious matter, and more attention needs to be given to improving the accessibility to faster and more sensitive diagnostic TB screening tests. There is a need to further evaluate the paediatric first-line regimen, with the aim of improving the treatment outcome of all patients.

7. REFERENCES

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8. APPENDICES

Appendix A: Spreadsheet for collected data

PID	Gender	Age at ART initiation	Baseline CD4 - absolute	Baseline CD4 - percentage	Baseline Weight for age Z-score	Baseline Height for age Z-score	NRTI backbone	Viral load at six months	Viral load at twelve months	Viral load at twenty four months	Viral load at thirty six months

This table will be extracted directly from the pre-existing database. Following the file review using Appendix B, the data collected there will be appended as follows.

Appendix B: Adherence, PMTCT exposure history, and HIV-TB co-treatment

STUDY NUMBER:

Notes: to verify on database or from file				
Timeframe		1st Year	2nd Year	3rd Year
ADHERENCE*	**Lowest adherence score ….. /10**	……/10	……/10	……/10
	Maximum number of concurrent medicine	……Meds	……Meds	……Meds
	Treatment interruptions for >1 months	YES/NO/ND	YES/NO/ND	YES/NO/ND
TUBERCULOSIS*	**Any**	YES/NO/UNK		
	Timing	**SITE OF DISEASE**	**Kaletra Boosting**	
	first year / second / third year	pulmonary / extrapulmonary TB / BCG	double dose kaletra/ RTV boosted kaletra / unboosted kaletra	
	first year / second / third year	pulmonary / extrapulmonary TB / BCG	double dose kaletra/ RTV boosted kaletra / unboosted kaletra	
PMTCT	**Any**	**Mother**	**Baby**	
	YES / NO / UNK	single dose NVP / AZT+ sdNVP / triple ART/ none	single dose NVP/ AZT+sdNVp / daily dose NVP/ none	

*Note that all data on adherence and Tuberculosis treatment applies to those factors experienced during the first three years of ART only. If no data available, complete "ND"

Appendix C: Adherence questionnaire RMMCH

ADHERENCE QUESIONNAIRE RMMCH

(To be completed for each child on ART at each visit)

Question	Options	score
1) How many days have you missed this month?	Less than 3 between 3 and 7 more than 7 Score: 2 1 0	
2) How many times were you more than for 4 hours late with the dose?	Less than 3 between 3 and 7 more than7 Score: 2 1 0	
3) Check the return date on the script- did the care giver come/child come?	On right date earlier than date late than 5 days Score: 2 1 0	
4) Let the caregiver demonstrate the dose/ measurements. Are they doing it correctly?	Exactly right need small correction total wrong Score: 2 1 0	
5) Is the child vomiting doses straight after taking them	Never up to every third dose most doses Score: 2 1 0	
	The final adherence score is:	**/10**

Appendix D: Ethics approval

M140747

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140747

NAME: (Principal Investigator)	Dr Dudusile P Msiza
DEPARTMENT:	Department of Paediatrics CM Johannesurg Academic Hospital
PROJECT TITLE:	Factors Affecting Virological Outcome of Under Three Year Old Paediatric Patients on ABC/d4T Based First Line Regimens
DATE CONSIDERED:	25/07/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Karl G Technau
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/07/2014

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

DECLARATION OF INVESTIGATORS

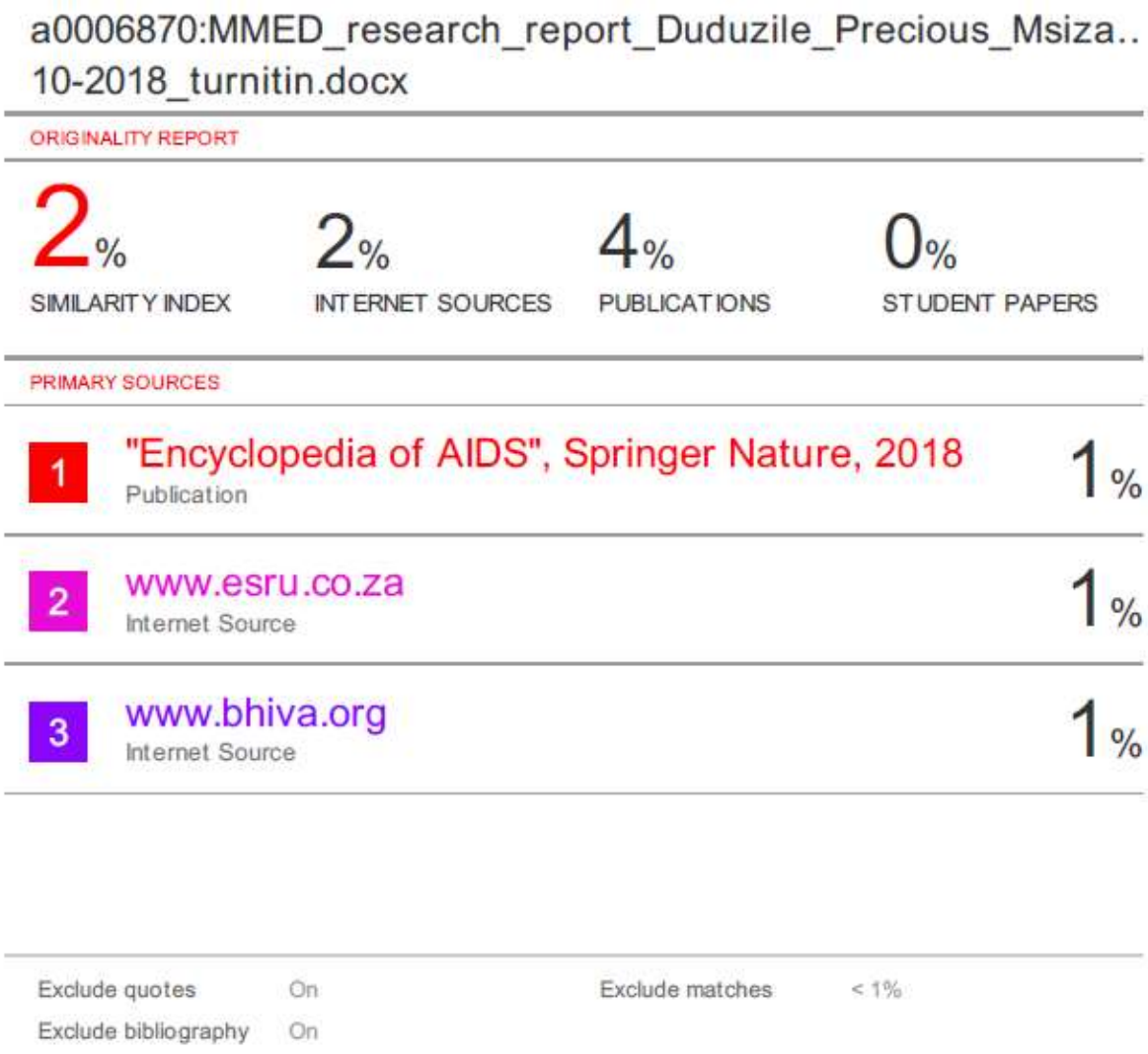
To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

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.**

Principal Investigator Signature — M140747Date 21/08/14

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix E: Turn- it-up



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