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COMPARISON OF VIROLOGIC OUTCOMES IN HIV–INFECTED ADOLESCENTS AND PRE-ADOLESCENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN SOWETO, SOUTH AFRICA

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376101



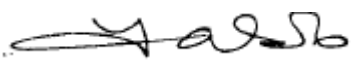
A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg; in partial fulfilment for the degree of

Master of Science in Medicine in Epidemiology and Biostatistics

Johannesburg, 2010

DECLARATION

I Tonderai Mabuto declare that this Research Report is my own work. It is being submitted for the degree of Master of Science in Medicine in Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. No prior submissions of this material have been made for any degree or examination at this or any other university.



Tonderai Mabuto

29 October 2010

DEDICATION

****

In memory of my beloved brother

Canaan Nhamoinesu Shambare

1969 -2009

****

ABSTRACT

Objectives: To evaluate differences in virologic outcomes between adolescents and pre-adolescents initiated on HAART and to determine the patient baseline variables associated with virologic suppression.

Design: Retrospective cohort study using routinely collected clinic and outcome data.

Setting: Public sector HIV paediatric facility at Harriet Shezi Children's Clinic (Chris Hani Baragwanath Hospital) Soweto, South Africa.

Patients: HIV infected pre-adolescents (5 to < 11 years) and adolescents (11 to <18 years) initiating HAART between 1 April 2004 and 31 December 2008.

Main outcomes and measures: Primary: virologic suppression (HIV viral load ≤ 400 copies/ml) and viral rebound (single HIV viral load ≥ 400 copies/ml after initial suppression) at 24, 48, 72 and 96 week follow up intervals. Secondary: determination of baseline variables associated with virologic suppression. Survival analysis was performed using the Kaplan Meier method and modelling was based on Cox proportional hazards.

Results: Both groups exhibited similar incidence rates of virologic suppression by the 24th week from HAART initiation. Adolescents had a slightly lower incidence rate of early virologic suppression in comparison to pre-adolescents (197/100 person years vs. 203/100 person years). However, the observed difference was not statistically significant at 5% significance level (IRR: 0.97, 95%CI: 0.81 - 1.15). In a sub-group of children who had not virologically suppressed by the 24th week (168 days) of follow up, adolescents were 42% less likely to achieve virologic suppression after this time point than pre-adolescents ([IRR: 0.58, 95%CI: 0.35, 0.93). In the sub-group of all female participants, lower hazards of virologic suppression by the 24th week (aHR 0.76, 95%CI 0.59-0.99) and 96th week (aHR 0.70, 0.55-0.90) of follow up were observed among female adolescents when compared with female pre-adolescents. Additionally, clinically advanced disease was observed as a risk factor for non-virologic suppression by the 96th week of follow up among participants of all ages (aHR 0.75, 95%CI 0.64 -0.87). After 60 weeks from the initial virologic suppression, adolescents were twice more likely to experience rebound after this point than pre-adolescents (IRR: 2.33, 95%CI: 1.00 - 5.13).

Conclusion: Given the potential for resistant strains of the HIV virus and the public health threat this presents, health care teams face complicated dilemmas regarding initiation of HAART to adolescents, particularly female adolescent patients who are likely to be non-adherent. Findings from the study advocate for intensified adherence and treatment support for all adolescents initiated on HAART to achieve virologic suppression within the first 6 months of treatment, a time after which they have been shown to exhibit inferior virologic suppression rates. Once virologic suppression has been attained, adolescents require prolonged treatment support to maintain long term virologic suppression at levels observed among pre-adolescents. We recommend further research into the comparison of virologic outcomes between pre-adolescents and adolescents on HAART, through prospective study designs. Qualitative study designs are also important to bridge the knowledge gaps on the barriers to HAART encountered by female adolescents.

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Special thanks to my family, especially my parents, Diana, Sharon, Richard and Tsungai for believing in me. To Wadzanai, thank you for being patient with me throughout this period. I also acknowledge my colleagues and the incredible staff in the School of Public Health for providing academic, emotional and administrative support throughout the life cycle of this project.

Finally, all glory and honour be unto God.

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NOMENCLATURE

aHR	Adjusted hazard ratio
AIDS	Acquired Immunodeficiency Syndrome
ASSA	Actuarial Society of South Africa
CI	Confidence Interval
HAART	Highly Active Antiretroviral Therapy
HAZ	Height for age z-score
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
HSCC	Harriet Shezi children's Clinic
IRR	Incidence rate ratio
Log	Logarithm
NHLS	National Health Laboratory Service
NNRTI	Non-nucleoside reverse transciptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
P	Probability
PACTG	Paediatric Action Trial Group
PMTCT	Prevention of Mother to Child Transmission
REACH	Reaching for Excellence in Adolescent Care and Health
RNA	Ribonucleic acid
SD	Standard deviation
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
uHR	Unadjusted hazard ratio
UNICEF	United Nations Children's Emergency Fund
WAZ	Weight for age z-score
WHO	World Health Organisation

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CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Epidemiology of Paediatric HIV/AIDS

The World Health Organisation (WHO) estimates that globally 2.1 million [1.2 million-2.9 million] children under the age of 15 were living with the Human Immuno-deficiency Virus (HIV) in 2008. In addition, children within this age group accounted for 6 % of all HIV infections, 16% of new infections and 14% of all HIV related mortality. Sub-Saharan Africa remains the region most affected by the HIV epidemic among children. In 2008, the region accounted for 91% of new HIV infections among children less than 15 years of age worldwide. The HIV epidemiologic estimates for children older than 15 years of age are subsumed into adult categories, therefore not well described [1]

South Africa is home to the world's largest population of people living with HIV (5.7 million). Moreover, the country has a young population; with approximately 40% of the total population being children under 20 years of age [2]. Of the children living with HIV, 61% are pre-adolescents (<10 years old) and 39% are adolescents (10 -19 years old) [2]. The Actuarial Society of South Africa AIDS and Demographic Model (ASSA 2003), estimates that by mid-2010; children (<20 years) will contribute 9% of people living with HIV in South Africa [3].

1.1.2 Role of Highly Active Antiretroviral Therapy

Highly active antiretroviral therapy (HAART) has been harnessed as one of the key interventions in mitigating the HIV epidemic. The primary goal of HAART is to decrease HIV-related morbidity and mortality [4]. Whilst on HAART it is expected that; the patient should experience fewer HIV-related illnesses, their CD4 count should rise and remain above the baseline count and the viral load should become undetectable (<400copies/ml) and remain

undetectable. The South African Government and its partners expanded the availability of HAART in the first quarter of 2004, through the implementation of The South African Comprehensive HIV and AIDS Care, Management and Treatment Plan [5]. By the end of 2007, 32,000 children in South Africa were receiving HAART [6].

One of the sites that has benefited from the HAART roll out programme in South Africa since 2004 is the Harriet Shezi Children's Clinic (HSCC), a public sector paediatric HIV clinic at Chris Hani Baragwanath Hospital, in Soweto, South Africa. By June 2009, the clinic had initiated more than 3000 children on HAART making it one of the largest single site paediatric HAART cohorts known. [7].

1.1.3 Monitoring of HAART treatment outcomes

Monitoring of the children's HAART treatment outcomes is essential in evaluating the effectiveness of HAART. Laboratory monitoring of plasma HIV-RNA (viral load) has been developed as a reliable and reproducible marker of HIV related disease progression that facilitates the evaluation of HAART effectiveness [4]. Research has shown that patients may respond with a rapid reduction in plasma viral load to undetectable levels within a median of 4 months [8]. However, the viral load may start to increase in a significant proportion of patients, due to the emergence of drug resistant viruses, complexities in maintaining long-term adherence and a limited number of available drugs [9-11]. Therefore, assessment of virologic response in patients initiated on HAART is essential in supporting efforts to avoid the development of drug resistance and to enable preservation of treatment options [4].

1.1.4 Adherence Issues

Notwithstanding the importance of virologic markers, adherence to HAART medication remains the greatest patient-enabled predictor of treatment success, drug resistance, progression of disease and mortality for children initiated on treatment [12, 13]. However,

efforts to develop a clinically useful measure of antiretroviral medication adherence have been unsuccessful and still no gold standard exists [14, 15]. Traditional adherence measures are unreliable [14, 16] and prone to reporting bias and manipulation by patients. Electronic monitoring methods to counter these limitations have been implemented, but they underestimate adherence and do not provide evidence of actual ingestion of the drug [17, 18].

Given the expansion of HAART coverage in South Africa within the context of unreliable adherence measures; the monitoring of immunological and virologic markers is essential for all age groups to address group specific needs that enable implementation of interventions to improve treatment outcomes.

1.2 Problem Statement

HIV-infected adolescents (11 to <18 years) are faced with unique and complex psychosocial adherence issues which include daily lifestyle burden and non-disclosure of HIV status. In addition, parent-child relationships are transformed as adolescents learn to make decisions independently and autonomously. Consequently, adolescents are often given independence in taking medications despite not fully understanding regimens [19-21]. As a result, HAART may fail to reach its intended goal of sustained suppression of viral replication in the adolescent population. Interruptions in medication adherence allow the virus to recommence typical rapid replication (up to 10^{10} viral particles per day) [22, 23].

Despite the demonstration of existent barriers to HAART adherence in adolescents and the absence of methods that accurately measure adherence, limited information exists on the patterns and correlates of viral replication among adolescents on HAART in South Africa. This underscores the problems faced by health teams in managing and directing efforts to adolescents who would require more intensive adherence support to achieve long term treatment success. This knowledge gap threatens the success of HAART programs in South Africa, as the number of adolescents enrolled and eligible for HAART continues to rise [3].

1.3 Justification of the study

Virologic outcomes at large paediatric HIV/AIDS care clinics in resource limited settings are poorly described beyond the first year of HAART. The cost implications of the South African National Strategic Programme constrain the feasibility of extending the ARV Program to sufficiently cater for complexities in first line treatment failure [5]. Furthermore, the failure to match national strategies to evidence based research and documented experience is perilous to the success of initiatives by the government and its partners. Therefore, research into the effectiveness of HAART treatment in all sub-populations is important to inform and strengthen existing treatment programs.

Despite having a large HIV-infected adolescent population and well published barriers to HAART adherence, relatively few studies have been conducted describing the virologic outcomes of HAART among adolescents in South Africa. Instead, significant research attention in this field has focused on survival outcomes of children initiated on HAART [25-28]. As most of these studies have demonstrated prolonged survival in children, attention in HIV paediatric research requires a focus shift to the evaluation of achievement and maintenance of long-term virologic suppression, as the younger children progress into adolescence.

This study was carried out in an endeavour to contribute to the existing body of knowledge on HAART treatment outcomes from a paediatric clinic population which has a wide spectrum of patients initiating HAART. A similar study conducted in South Africa by Nachega et al (2009), evaluated virologic outcomes in 154 adolescents (aged 11-19 years) initiated on HAART [30]. While the sample size was adequate, it is uncertain whether the results are generalisable to public sector HAART facilities due to the strict inclusion of adolescent dependents of adult employees in private medical insurance schemes participating in the program. Previous studies of adolescents initiated on HAART, have faced the

limitation of small sample sizes, high rates of loss to follow up and death leading to smaller numbers for analysis [29-31].

The study at Harriet Shezi Children's Clinic embraced the fact that in South Africa most HIV infected children gain access to HAART through tertiary public health facilities [24]. Therefore, with a study sample coming from one of the largest single site paediatric cohorts known; the rationale was to improve the external validity of study results in comparison to other adolescent studies that have been conducted thus far in South Africa.

Adolescent and pre-adolescent HAART outcomes are frequently clustered together in studies, but dissimilarity in developmental issues between the groups, creates a distinctive environment which frames differences in their interactions with health care systems. Comparisons in treatment outcomes have been done between adolescents (aged 11-19 years) and adults (aged ≥ 20 years) initiated on HAART [30,31], but a paucity of data and documented experience exists in the comparison of treatment outcomes between adolescent and pre-adolescent children. The above mentioned concerns further motivated for the study to be conducted.

1.4 Literature Review

The achievement and maintenance of viral suppression is directly related to the long term efficacy of HAART [31]. Although, clinical efficacy and feasibility of HAART in adult HIV patients in Africa is well documented [32-35], few studies specifically address the treatment response in HIV-infected adolescents in the same population [30]. This has led to heavy dependence upon a few studies in the developed countries to inform African HAART programs and clinicians on the treatment outcomes in adolescent populations [28, 29]. However, the older age and greater disease severity at the time of HAART initiation in sub-Saharan Africa hinder direct comparisons with adolescents in developed countries who are immunologically robust [36, 37].

Studies of treatment outcomes in adolescents initiated on HAART have recently been executed in an African setting. Nachega et al (2009) have demonstrated lower rates of virologic suppression in South African adolescents compared to adults at all time points after initiation of HAART. The proportion of adolescents achieving viral suppression at 6,12 and 24 months was 63%, 45.7% and 45.3%, compared to 69.3%, 62.1% and 62.3% in adults [30]. This study compellingly evaluates adolescent treatment outcomes and contextualises findings to resource limited settings. However, the eligibility for enrolment into the study was restricted to adolescent dependents of adult employees in private medical insurance schemes. Gaps still exist to assess the generalisability of these study results to the public sector where most adolescents gain access to HAART.

Treatment outcome data of adolescents initiated on HAART has only been compared with data from adults [28-31]. Non-formal, indirect comparisons with younger paediatric cohorts suggest better virologic response in pre-adolescents compared to adolescents initiated on HAART. In a younger South African paediatric cohort (83% of children <10years) , Reddi et al (2007) reported 84% and 80.3% of children with virologic suppression at 6 and 12 months, compared to 63% and 45.7% in an older adolescent cohort at the same time points [13]. Furthermore, data from child studies suggest that as the age range of enrolled children increases to encompass more adolescents, the proportion of participants achieving viral suppression at 12 months falls from 80% to below 50% [38-40]. Due to small study samples of children initiated on HAART meeting inclusion criteria and the underrepresentation of adolescents, most studies have evaluated preadolescent and adolescent children as homogeneous groups despite greater complexities in treatment adherence in the latter group [38-41]. Research that focuses on direct comparisons of these two sub-populations bears the potential of identifying risk factors for viral suppression in adolescents who would require appropriate interventions to enhance treatment outcomes.

Once viral suppression has been achieved, maintenance thereof has been found to be problematic. Observational studies in adult populations have reported rates of virologic rebound of between 20-40% at 6 and 12 months after initial viral suppression [42, 43]. Higher rates have been reported among adolescents due to poor adherence to treatment. In the REACH study (United States), 35 adolescents were virologically suppressed by the third month and had viral load information for at least 4 consecutive visits (at 3 month intervals). Out of this sub-group, 48.6% experienced virologic rebound after 12 months from the initial time of suppression [28]. PACTG 381 studies (United States), report that only 24% of the adolescent participants maintained virologic suppression (< 400copies/ml) for 3 years [29]. In South Africa, Nachega et al (2009) report proportions of adolescents with virologic rebound at 6, 12 and 24 months of 31.1%, 42.4% and 38.9% compared to 16.6%, 20.2% and 24.2% in adults [30]. Short observation periods have resulted in limited knowledge on the time to virologic rebound in both pre-adolescents and adolescents. There is need for long term observational studies involving larger samples to obtain further information of prolonged use of HAART in African children.

Literature reports several patient baseline variables associated with virologic suppression during HAART. Higher baseline viral loads (>100,000 copies/ml) and lower $CD4^+$ % counts are associated with a shorter duration of viral suppression [44, 45]. Closely related to these are higher WHO stages that have been reported to be independently associated with suboptimal virologic outcomes at 12 months [45]. Nutritional status as defined by height for age Z-scores and weight for age Z-scores have been described as predictors of HIV progression among children [46]. Although, studies have shown that nutritional status improves after HAART, the effect of malnutrition on virological success has not been fully explored. Different findings on the association between virologic success and age or sex have been described in published literature [32, 47]. The lack of standardised

monitoring and assessment systems makes data synthesis and summary difficult to compare the progress of children initiated on HAART.

The association between the various variables and virologic suppression discussed above is context specific and may vary in different resource settings or populations when patients present with different stages of disease before HAART initiation or due to differences in ascertaining of virologic responses in participants across studies. Therefore, collecting information and adjusting observed relationships for these variables is critical in maintaining internal validity of studies that evaluate virologic outcomes of patients initiated on HAART.

1.5 Research Question

Do HIV-infected adolescents (11 to <18 years) have lower virologic suppression rates and higher rates of virologic rebound compared to HIV-infected pre-adolescents (aged 5 to <11 years), in a clinic cohort of HIV-infected children initiated on HAART between 1 April 2004 and 31 December 2008 at Harriet Shezi Children's Clinic, Soweto, South Africa?

1.6 General objective

To determine and evaluate differences in virologic suppression rates and time to virologic rebound between adolescents (11 to <18 years) and pre-adolescents (aged 5 to <11 years) after initiation of HAART, in a clinic cohort of HIV-infected children initiated on HAART between 1 April 2004 and 31 December 2008 at Harriet Shezi Children's Clinic, Soweto, South Africa.

1.7 Specific Objectives

- To describe baseline demographic and clinical characteristics of participants at the time of initiation of HAART.

- To evaluate differences in virologic suppression rates between pre-adolescents and adolescents after initiation of HAART.
- To determine patient baseline variables associated with viral load suppression in pre-adolescents and adolescents after initiation of HAART.
- To evaluate differences in the time from virologic suppression to virologic rebound between pre-adolescents and adolescents on HAART.

CHAPTER 2

METHODS

2.1 Introduction

This chapter presents the key methods implemented in this study to achieve both internal and external validity of research findings. The description of the study approach and key method elements is adopted from the collaborative initiative of strengthening the reporting of observational studies in epidemiology (STROBE Statement) [48]. This comprehensively incorporates the description of method components that include; study design, participant selection, follow-up methods, definition of outcomes, potential confounders, effect modifiers, potential sources of bias and statistical considerations.

2.2 Study design

A retrospective cohort study was performed. Clinic records of all study participants were retrospectively reviewed for virologic outcomes and other study variables of interest. The study cohort was stratified into pre-adolescents (5 to < 11 years) and adolescents (11 to <18 years) to generate two comparison groups.

2.3 Study setting

The study was performed at Harriet Shezi Children's Clinic. This is a public sector HIV paediatric clinic at Chris Hani Baragwanath Hospital, reputedly the world's largest hospital (by bed count) located in Soweto, South Africa [49]. It is situated in the south west of Johannesburg, on the southern border of Soweto. It is the only public hospital serving approximately 3.5 million people in Soweto and this population has historically been overwhelmingly black. The clinic is one of the sites that has benefited from the roll out of HAART programmes in South Africa since 2004. The support of international donors and research organisations to the clinic facilitates the provision of paediatric HAART services at

no cost to the patient. By June 2009, the clinic had initiated more than 3000 children on HAART making it one of the largest single site paediatric HAART cohorts known in Africa. Most of the children are referred to the clinic after being admitted for HIV related illness at the Chris Hani Baragwanath Hospital.

These children are managed by a health team comprising of paediatricians, medical officers, primary health care sisters, professional nurses, lay adherence counsellors, a dietician, psychologist and pharmacists. The average age of children is higher than in other HIV clinics because the clinic is not the referral site for HIV- infected children identified through the prevention of mother to child transmission programme, and because prior to April 2004 the clinic only managed children over two years of age. [7].

2.4 Sample size

The study cohort was selected based on non-random convenience sampling. To evaluate the adequacy of the study sample; the power of the study was computed after assuming a minimal 10% difference in virologic suppression and virologic rebound between the 1002 pre-adolescents and 232 adolescent participants. After further assumptions of an alpha of 0.05 with a two-sided level of significance, this study had over 80% power to detect a minimal 10% difference and any other difference larger than this. This computation of study power was performed in STATA 10 (STATA Corporation, College Station, TX) using the two-sample comparison of proportions method.

2.5 Study cohort

At the commencement of data extraction (October 2009), 3167 children had been initiated on HAART at Harriet Shezi Children's Clinic. However, patients had to meet the following inclusion criteria for study participation:

i. Aged 5 to < 18 years at time of HAART initiation.

ii. At least 6 months of follow up data available or one known viral load measurement after initiation of HAART.

iii. A baseline (pre-HAART) HIV viral load >400 copies /ml.

iv. Initiated on an efavirenz based therapeutic regimen.

v. No known prior exposure to HAART except exposure through Prevention of Mother to Child Transmission (PMTCT).

Children < 5 years of age were excluded from the study because they have different treatment regimens compared to older age groups [5]. Integrating these children into the study would have amplified the heterogeneity within the pre-adolescent group, resulting in an amplification of residual confounding. In this study, the upper boundary of pre-adolescence was fixed at 11 years. Research has shown adolescence to begin between 11 and 13 years of age, with individuals undergoing physical, psychological and personality changes [50, 51]. In addition, care workers at Harriet Shezi Children's clinic have observed the emergence of unique psychosocial issues among children on HAART, commencing at 11 years of age (Personal communication. Dr Harry Moultrie. 22 May 2009). Hence, the demarcation was made on the basis of context specific rationale.

2.6 Data Source

The clinic utilises an Access™ based hybrid paper-electronic chronic care management system which contains data from clinic visits. At each visit, clinical information and laboratory results are recorded on forms, which are then entered into the database. Information is captured by data clerks under the supervision of a data manager, who performs data cleaning and monitors data quality. Despite inherent data quality checks, the database was not designed as a research instrument. Poor data quality in relation to missing information presented study limitations owing to the retrospective nature of the study. For

purposes of this study, data was imported from Access™ to STATA 10 for coding, cleaning, merging and statistical analyses.

2.7 Study variables

Age, gender and ethnicity were the three demographic variables measured in this study cohort. The patient's age (in years) at HAART initiation was calculated as the difference in years between the date of HAART initiation and date of birth. This was further stratified into the age groups of pre-adolescents (5 to <11 years) and adolescents (11 to < 18 years). All study participants were analysed according to their age group at initiation. This was particularly important for pre-adolescents who traversed into adolescence over the course of the study period. As a result of minor non-black ethnic groups, the ethnicity was categorised into black and non-black ethnic groups. In Soweto, where the clinic is situated and sources its catchment, the population is predominantly of black ethnic origin. Since almost all of the study participants were of the black ethnic group, the ethnicity variable was dropped in model building.

Plasma HIV-RNA was measured using the Roche Amplicor 1.5 Assay (detection 401-750,000 copies/mL) up until mid-2004, after which the Nuclisens EasyQ assay (detection 25 -3,000,000 copies/mL) was used. Patient samples were analysed at the South African National Health Laboratory Services (NHLS). Based on the South African Antiretroviral Guidelines, viral load measurements were scheduled for assessment before HAART initiation and every 24 weeks thereafter. Viral loads were quantified and reported as copies/mL. For the purposes of this study, the viral load measurements were also log transformed ($\log_{10}$ viral load) and further categorised into high burden ($\log_{10}$ viral load ≥ 5) or low burden ($\log_{10}$ viral load < 5).

CD4 assays were performed using dual platform flow cytometry until late 2004 when PanLeucogated CD4 method was implemented at the NHLS. CD4 measurements were

scheduled for assessment before HAART initiation and every 24 weeks thereafter. CD4 cells were quantified in counts (cells/mL) and as percentages (%). CD4 measurements were further categorised into severely immunocompromised (CD4 count <200 cells/mL, CD4 percentage < 15%) or non-severely immunocompromised (CD4 count ≥200 cells/mL, CD4 percentage ≥15%).

Weight measurements (in kgs) were performed using electronic scales. These measurements were scheduled for assessment at each clinic visit. Sex and age standardised z-scores for weight (WAZ scores) were calculated for all children in EpiInfo (version 3.5.1) using WHO (1978) growth reference standards [52]. The computations were based on the WHO (1978) reference standards because they include a reference population up to the end of adolescence (18 years), contrary to the limit of the recently revised growth curves [53]. WAZ scores were categorised according to United Nations Children's Emergency Fund (UNICEF) definitions; using the standard deviations (SD) from the median WAZ of the reference population. Using these definitions, patients with WAZ scores < -3 SD, -3SD to -2SD or ≥ -2 SD were considered severely malnourished, moderately malnourished or well nourished, respectively [54].

Height measurements (in cm) were performed at each clinic visit using stadiometres. Sex and age standardised z-scores for height (HAZ scores) were calculated for all children in EpiInfo (version 3.5.1) using WHO (1978) growth reference standards [52]. The same rationale to using the WHO (1978) reference standards for WAZ scores was extended to the calculation of HAZ scores. HAZ scores were also categorised according to UNICEF definitions; using the standard deviations from the median HAZ of the reference population. Using these definitions, patients with HAZ scores < -3 SD, -3SD to -2SD or ≥ -2 SD were considered severely stunted, moderately stunted or not stunted, respectively [54].

The clinic changed from the WHO 3-stage to WHO 4-stage classification of disease severity in 2006 [55]. For the purposes of this study, participants with WHO stage III or IV disease under either system were categorised as having clinically advanced disease. The reference group was comprised of the rest of the study participants with WHO stage I or II disease and considered as having non-clinically advanced disease.

Inclusion into the study was restricted to patients initiated on an efavirenz based regimen. Considering that most patients were initiated on an efavirenz based regimen, the rationale of this exclusion was based on eliminating the confounding effect of initial HAART regimen on the relationship between age group and virologic outcomes. Analyses were based on "intent to continue initial therapy principle", in that eligible study participants were analysed according to initial regimen regardless of whether they eventually discontinued or modified the HAART regimen.

2.8. Follow up

Follow up was commenced from the date of HAART initiation or date of initial virologic suppression for virologic suppression and virologic rebound, respectively. Patients were declared lost to follow-up if they did not return to the clinic at six months from their last clinic visit date. Additionally, patients that remained in care but did not experience virologic suppression and/or virologic rebound were censored after two years of follow up (for both outcomes) or at study end on 31 June 2009 (6 months after last eligibility date).

Follow up time was further categorised into 24 week intervals for both virologic suppression and rebound using the following criterion:

i. Follow up > -90 days and ≤ 0 days = baseline
ii. Follow up > 84 days and ≤ 252 days = 24 weeks
iii. Follow up >252 days and ≤ 420 days = 48 weeks
iv. Follow up >420 days and ≤ 588 days = 72 weeks

v. Follow up >588 days and ≤ 756 days = 96 weeks

Virologic outcomes that were observed closest to the mid-point of each 24 week follow up interval were deemed valid for that time interval. In situations where two observations were equidistant from the midpoint, the latter of the two observations was selected for the follow up period.

2.9 Primary outcomes

2.9.1 Virologic suppression

Virologic suppression was defined as a viral load ≤ 400 copies/mL. The cut-off value was chosen to incorporate values based on the detection limit of the non-ultra sensitive laboratory test that was used until mid-2004.

Two analytic approaches were used to compare virologic suppression between the two age groups. In the first approach, the proportion of participants ever-suppressed (cumulative incidence) were compared at 24 weeks intervals after HAART initiation, between the two age groups. This analysis assumed an intention to treat approach, in which participants were analysed according to their age group at initiation, regardless of being lost from care. Analysis was based upon comparison of the proportion of patients in the two age groups that had experienced virologic suppression (at the end of each defined 24 week interval) out of the total number of participants that were in each study arm.

In the second method, differences in the time to first virologic suppression were evaluated using survival analysis. This approach facilitated comparison of incidence rates of ever being virologically suppressed between the two age groups, while accounting for loss to follow-up. Additionally, the two age groups were further stratified by gender to evaluate differences in time to virologic suppression based on this criterion. In contrast to the evaluation of the cumulative incidence, this analysis used follow-up time on a continuous scale (days from HAART initiation).

2.9.2 Virologic rebound

Virologic rebound was defined as, the first viral load >400 copies/mL after achieving initial virologic suppression (<400 copies/mL). Additionally, sensitivity analysis was performed using a more strict definition in which virologic rebound was defined as the first of two consecutive viral loads >400 copies/ml after initial virologic suppression. However, the less strict definition was favoured over the second one due to the frequency of missing viral load measurements. These analyses were restricted to the sub-population that had ever achieved virologic suppression and had at least one viral load measurement after virologic suppression.

Similar to virologic suppression, two analytical approaches were used to compare virologic rebound between the two age groups. In the first approach, the proportion of participants with virologic rebound (cumulative incidence) at 24 weeks intervals after initial virologic suppression was compared between the two age groups. This analysis also assumed an intention to treat approach, in which participants were analysed according to their age group, regardless of being lost from care after initial virologic suppression.

In the second approach, survival analysis was used to evaluate the differences in time to virologic rebound between the two age groups. Additionally, the two age groups were further stratified by gender to evaluate differences in time to virologic suppression based on this criterion.

2.10 Statistical analyses

2.10.1 Descriptive statistics

For purposes of this study, baseline parameters were the nearest measurement within 3 months before HAART initiation or at the date of initiation. The baseline demographic and clinical characteristics were described according to the defined age strata.

Mean (standard deviation) or median (inter-quartile range) were used to summarise continuous variables if data were normally or non-normally distributed respectively.

Additionally, categorical and discrete random variables were reported as frequencies (n) and percentages (%). Two-way comparisons for the frequency and distribution of baseline categorical variables were performed using the Pearson's Chi-square test. Alternatively, the Fisher's exact test was applied when the expected values in any of the cells were below 5. In addition, baseline continuous variables were compared using the Student's t-test if data were normally distributed; otherwise the Wilcoxon rank-sum test was applied.

2.10.2 Inferential statistics

As part of the primary analysis, Kaplan Meier survival techniques were used to evaluate differences in the time to virologic suppression and virologic rebound stratified by age group and additionally by gender. As a result of sparse patient data after 756 days (96 weeks), assessment of individual patient data was discontinued at this point or at the cohort specific close of the data base; if either of these occurred sooner. The Log rank test for equality of survival functions was used to determine the existence of a statistically significant difference at 5% significance level, in the rate of virologic suppression and rebound, between the two age groups.

The second part of the analysis employed Cox proportional hazards modelling to determine the patient baseline variables associated with viral suppression at 24 (early virologic suppression) and 96 weeks after HAART initiation. Univariate and multivariate analyses were performed. Multivariate (adjusted) models included all patient baseline demographic and clinical characteristics. For purposes of these analyses, variables were categorised and missing values were assigned a category for each variable. Hazard ratios from the missing category did not possess any meaningful interpretation, but the category facilitated in maintaining a constant denominator that allowed comparison of different models in multivariate analysis.

The final multivariate models were built using backward elimination, based on the Likelihood ratio test (Lrtest). In this method, the initial step involved fitting a model containing all the study covariates and cofactors. Study variables were sequentially dropped from the model starting in descending order of the magnitude of the Wald p-values. Retention of study variables was based upon an Lrtest Chi-squared p-value of 0.05 or less. Evaluation for effect modification was performed by introducing interaction terms in the model. The assumption of proportional hazards was evaluated by the model based test for time by log (t) interaction.

2.10.3 Study bias and missing data

This study was based on an urban heterogeneous clinic population; hence the ideal situation of well measured study variables at specific time points could not be met. Furthermore, the database from which patient records were retrospectively evaluated was not originally implemented as a research tool; hence, potentially compromised data quality.

To assess selection bias, baseline comparisons between patients who did not have 6 months of follow up data and who met eligibility criteria were performed using the Pearson's Chi-square test. The same test was also performed to evaluate differential losses to follow up at 24 and 96 weeks between the two age groups stratified by baseline variables.

Missing data was a consequence of evaluations not being performed or the non-attendance by patients to the clinic for monitoring. The first approach to handle missing data was to consider measurements for each variable closest to the midpoints of each 24 week period. This was necessitated by differences in the frequency of clinic visits within each 24 week period, owing to different states of health among the patients. The second approach involved setting the missing data as a unique category for each study variable. The rationale of this approach was to maintain the number of patients in analysis constant during model building, thereby allowing accurate comparison of models in backward elimination. Finally, a

missing data analysis was performed using the Pearson’s Chi-squared test to evaluate whether data was missing at random or whether informed censoring had occurred.

CHAPTER 3

RESULTS

3.1 Cohort profile

During the period of 1 April 2004 to 31 December 2008, a total of 3167 children were initiated on HAART at HSCC (Figure 3.1). However, 1931 (61%) children did not meet the inclusion criteria for study participation. Of those excluded; 1647 (85%) did not meet the age inclusion criteria, 137 (7%) were not initiated on HAART in the defined period, 4 (0.2%) were not initiated on an efavirenz based regimen, 21(1%) were initiated on HAART with a viral load ≤ 400 copies/mL and 126 (6.8%) did not have any viral load follow-up data. Ultimately, the final dataset comprised of 1234 children, 1002 (81%) pre-adolescents and 232 (19%) adolescents. These children contributed 29 358 person weeks of follow up over the study period. Ninety-two (9.2%) of the pre-adolescent participants were lost to follow up by 96 weeks, compared to 24(10%) of all adolescent participants at the same time point. There was no statistically significant difference in loss to follow up rates over the study period between the two age groups (Incidence rate ratio [IRR]: 1.2, 95% CI: 0.78 – 2.04). At the 96th week of follow up, < 50% of the remaining study participants were assessed for viral loads. However, these proportions were comparable between the two age strata.

3.2 Baseline demographic characteristics

Pre-adolescents presented for HAART initiation at a mean age of 7.6 years (SD; 1.6 years), while adolescents presented at a mean age of 12.9 years (SD; 1.5 years). Males and females were evenly distributed in the two age strata and this was representative of the distribution in the combined study sample. As anticipated, the majority of patients (99%) in all age categories were of black ethnic origin. Demographic characteristics of the study cohort at HAART initiation are described in Table 3.1.

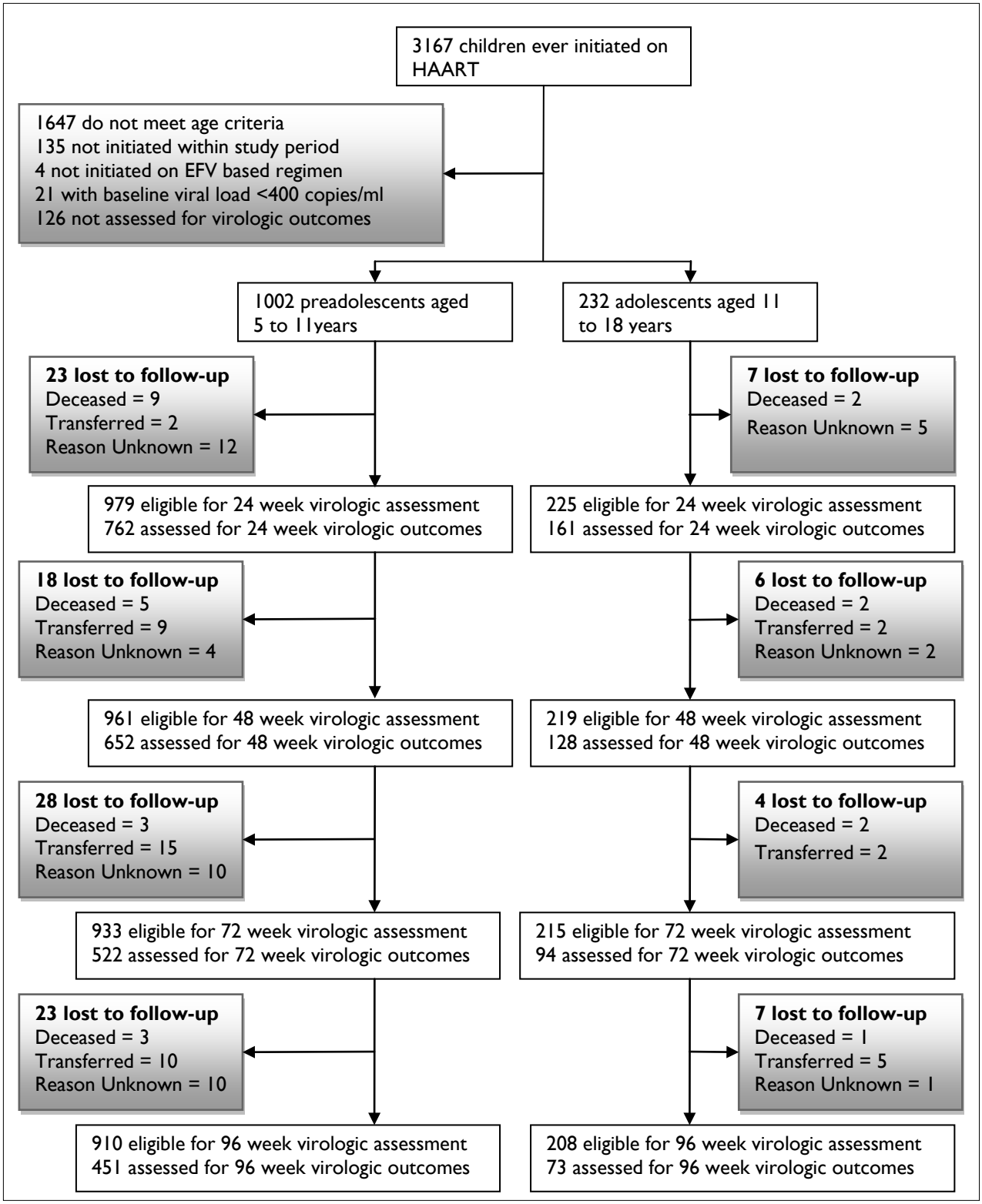

Figure 3.1: Profile of age stratified study cohort at Harriet Shezi Children's Clinic at 24 week follow up intervals

Table 3.1: Baseline demographic characteristics of the combined and age-stratified study cohort at initiation of HAART

Characteristic	All (N=1234)	Pre-adolescents (N=1002)	Adolescents (N=232)
Age			
Years, mean (SD)†	8.6(2.6)	7.6(1.6)	12.9(1.5)
Gender			
Male, n (%)	631(51.1)	522(52.1)	109(46.9)
Female, n (%)	603(48.9)	480(47.9)	123(53.1)

† Data are mean and SD (standard deviation); else data are n (number) and % (percentage).

3.3 Baseline clinical characteristics and anthropometric indicators

Adolescent status was significantly associated with an advanced stage of disease at HAART initiation (68.8% vs. 56.9%, P = 0.003). Additionally, adolescents were less immunologically competent than pre-adolescents, exhibiting a lower mean CD4 count (199.9 cells/μL [SD: 19.5] vs. 313.3 cells/μL [SD: 269.4]). Contrary findings were obtained when immuno-suppression was determined using CD4 percentages. In this analyses, there was strong evidence of no significant differences in advanced immuno-suppression (CD4 < 15%) observed between the two age groups (P= 0.92). Additionally, there was no evidence of any significant difference in the mean baseline viral load between the two age strata (P= 0.25), refer to Table 3.2.

Severe malnutrition and wasting was also a noteworthy baseline characteristic among the study participants at HAART initiation. The mean WAZ scores for the complete and age stratified study cohort were lower than -2 standard deviations (Table 3.2). Adolescents were more likely to present with severe malnutrition (WAZ < -3SD) at HAART initiation than preadolescents (35.3% vs. 26.1%, P = 0.011). At baseline, both age groups presented with poor cumulative linear growth as indicated by observed mean HAZ scores of less than -2 standard deviations. Additionally, both age groups had similar proportions of participants with severe stunting (HAZ < -3SD) at HAART initiation (31.3% vs. 28.8%, P= 0.054).

Table 3.2: Anthropometric and disease-related indices of study participants at initiation of HAART stratified by age group

Characteristic	All‡ (N=1234)	Pre-adolescents (N=1002)	Adolescents (N=232)	P value
Clinical stage††, n	977	794	183	
Less clinically advanced	399(40.8)	342(43.1)	57(31.2)	0.003*
Clinically advanced	578(59.2)	452(56.9)	126(68.8)	
WAZ-score, n	1170	952	218	
Mean, (SD)†	-1.7(1.1)	-1.6(1.1)	-1.9(1.0)	0.011*
≥-2	730(62.4)	613(64.4)	117(53.7)	
≥-3 to <-2	114(9.7)	90(9.5)	24(11.0)	
<-3	326(27.9)	249(26.1)	77(35.3)	
HAZ-score, n	1150	938	212	
Mean, (SD)	-2.2(1.2)	-2.1(1.2)	-2.4(1.2)	0.054
≥-2	532(46.3)	443(47.2)	89(42.0)	
≥-3 to <-2	264(23.0)	202(21.5)	62(29.2)	
<-3	354(30.7)	293(31.3)	61(28.8)	
CD4 count (cells/µl),n	898	726	172	
Mean, (SD)	291.5(260.0)	313.3(269.4)	199.9(190.5)	
≥200	518(57.7)	450(62.0)	68(39.5)	<0.001*
<200	380(42.3)	276(38.0)	104(60.5)	
CD4 percentage (%),n	896	727	169	
Mean, (SD)	10.6(7.4)	10.8(7.3)	9.7(7.4)	
≥15	220(24.6)	178(24.5)	42(24.9)	0.920
<15	676(75.4)	549(75.5)	127(75.1)	
Log Viral Load, n	917	747	170	
Mean, (SD)	4.8 (0.76)	4.8(0.75)	4.7(0.79)	
≥5	398(43.4)	331(44.3)	67(39.4)	0.245
<5	519(56.6)	416(55.7)	103(60.6)	

*P is significant at alpha level of 5%.
† Data are mean and SD (standard deviation) else data are n (number) and % (percentage).
‡ The total number of study participants (N) for different study variable may vary because of missing data.
†† Less clinically advanced (WHO clinical stage I and II) and clinically advanced (WHO Clinical Stage III and IV).

3.4 Virologic Suppression

3.4.1 Time to virologic suppression

Over the entire follow-up period, a total of 1025 study participants experienced their first virological suppression (Figure 3.2). The median time to the first virologic suppression for

the whole study cohort was 22 weeks (Interquartile range [IQR]: 13 – 28 weeks). Similar median times to virologic suppression were also observed in the two age groups (pre-adolescents; [22 weeks, IQR: 13-28] vs. adolescents; [22 weeks, IQR: 14-30]). Both groups exhibited similar incidence rates of virologic suppression by the 24th week from HAART initiation. Adolescents had a slightly lower incidence rate of early virologic suppression in comparison to pre-adolescents (197/100 person years vs. 203/100 person years). However the observed difference was not statistically significant at 5% significance level (IRR: 0.97, CI: 0.81 - 1.15). The incidence rate ratio (IRR) for virologic suppression over the entire 96 weeks of follow up suggested that adolescents were 23% less likely than pre-adolescents to achieve virologic suppression, but the difference was also not statistically significant (IRR: 0.87, 95% CI: 0.74 - 1.02). This finding was corroborated by a non-significant Log-rank p-value for equality of time to virologic suppression between the two age groups (P = 0.11) for the overall study period (Figure 3.2). However, further analysis showed that; within the sub-group of participants that failed to virologically suppress by 24 weeks, adolescents exhibited lower incidence rates of virologic suppression than pre-adolescents (70/100 person years vs. 119/100 person years) from this time point up to the 96th week of follow up. Adolescents that had failed to achieve early virologic suppression were 42% less likely to virologically suppress than pre-adolescents between the 24th and 96th week of HAART (IRR: 0.58, CI: 0.35 – 0.93).

When analysis was further stratified by gender, no significant differences were observed in the time to virologic suppression between pre-adolescent males (N= 522) and adolescent males (N=109) over 96 weeks of HAART (Log rank p-value = 0.281). Conversely, adolescent females (N=123) were 28% less likely to achieve virologic suppression than pre-adolescent females (N=480) over 96 weeks of HAART (IRR: 0.72, CI: 0.57-0.91). Over the general 96 week period, female adolescents experienced a consistently longer time to

virologic suppression than pre-adolescents females (Log rank p-value = 0.004, Figure 3.3). In corroboration with the two findings on females in the two age groups; pre-adolescent females had a shorter median time to virologic suppression than adolescent females (23 weeks, IQR: 13-28 vs. 24 weeks, IQR: 15 -48).

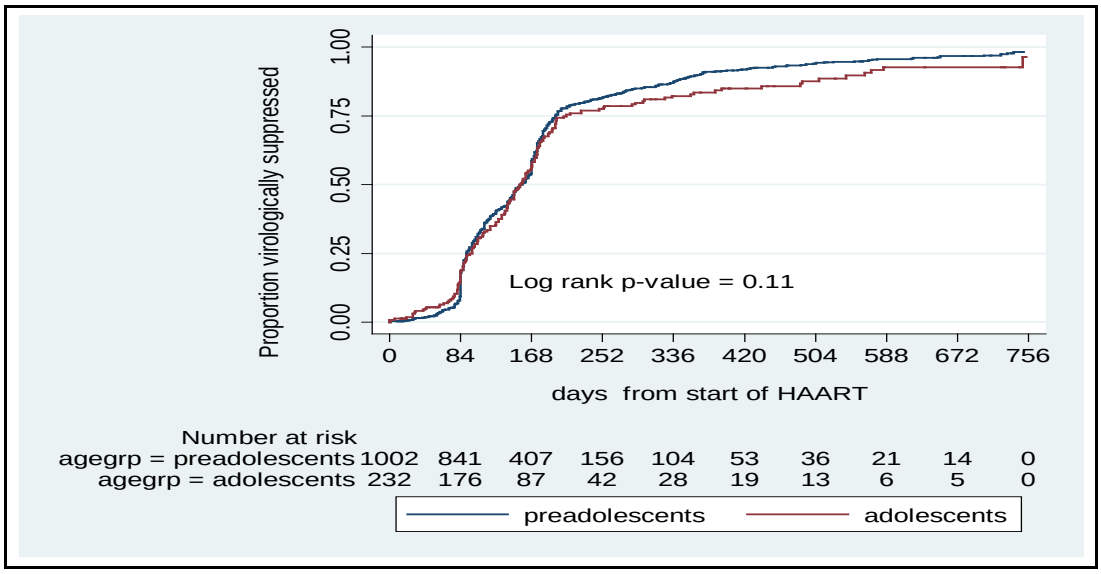

Figure 3.2: Comparison of time to virologic suppression after initiation of HAART comparing pre-adolescents (5 to < 11 years) and adolescents (11 to < 18 years)

**The log rank p-value is for the test of equality of time to virologic suppression between pre-adolescents and adolescents over the 96 week follow-up period.*

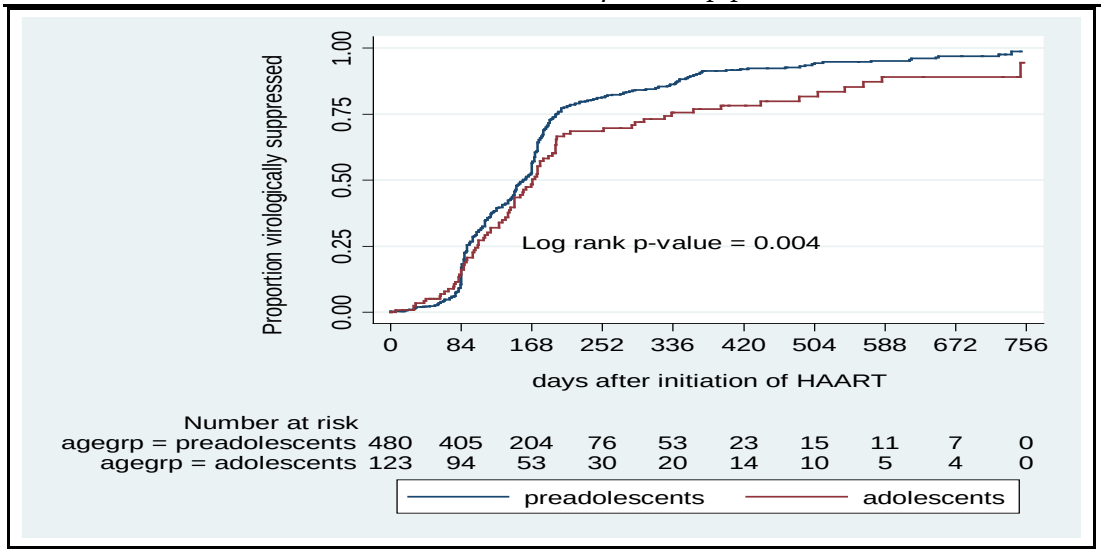

Figure 3.3: Comparison of time to virologic suppression after initiation of HAART between pre-adolescent (5 to < 11 years) and adolescent (11 to < 18 years) females.

**The log rank p-value is for the test of equality of time to virologic suppression between pre-adolescents and adolescents over the 96 week follow-up period.*

3.5 Virologic Rebound

3.5.1 Time to virologic rebound

This analysis was restricted to the 849 pre-adolescents and 176 adolescents who had ever achieved virologic suppression and had at least one viral load measurement after their initial virologic suppression. The sub-population of 1025 patients who achieved virologic suppression contributed 55 887 person weeks of follow up in the ascertainment of time to virologic rebound (Figure 3.4). Follow-up in this analysis refers to the time after initial virologic suppression. Low incidence rates of virologic rates of virologic rebound were observed in both age groups and there was no significant difference in the incidence rates of virologic rebound between the two age groups by the 24th week from initial virologic suppression (IRR: 1.34, 95% CI: 0.67 – 2.51). The incidence rate ratio (IRR) for virologic rebound over 96 weeks of follow up suggested that adolescents had a 40% increased risk than pre-adolescents of experiencing virologic rebound by 96 weeks, but the difference was not statistically significant (IRR: 1.4, CI: 0.9 -2.12). This finding was corroborated by a non-significant log-rank p-value for the equality of time to virologic rebound between the two age groups (P = 0.076) over the entirety of the study period. However, from 60 weeks (420 days) of follow-up onwards, adolescents had significantly higher incidence rates of virologic rebound and were twice more likely to experience rebound after this point than pre-adolescents ([IRR: 2.33, CI: 1.00, 5.13], [Log rank p-value = 0.018]). For adolescents, the incidence rate of virologic rebound increased from 17/100 person years (≤ 420 days from initial suppression) to 25/100 person years (> 420 days from initial suppression).

When analysis was further stratified by gender, there were no significant differences observed in the incidence rates of virologic rebound between male pre-adolescents (N= 437) and male adolescents (N=89), ([IRR: 1.33, CI: 0.72-2.3, Log rank p-value = 0.299]). Similarly, adolescent females (N=123) did not experience different rates of virologic rebound

in relation to pre-adolescent females (N=89), two years after their initial virologic suppression ([IRR: 1.46, CI: 0.72-2.7], [Log rank p-value = 0.195]).

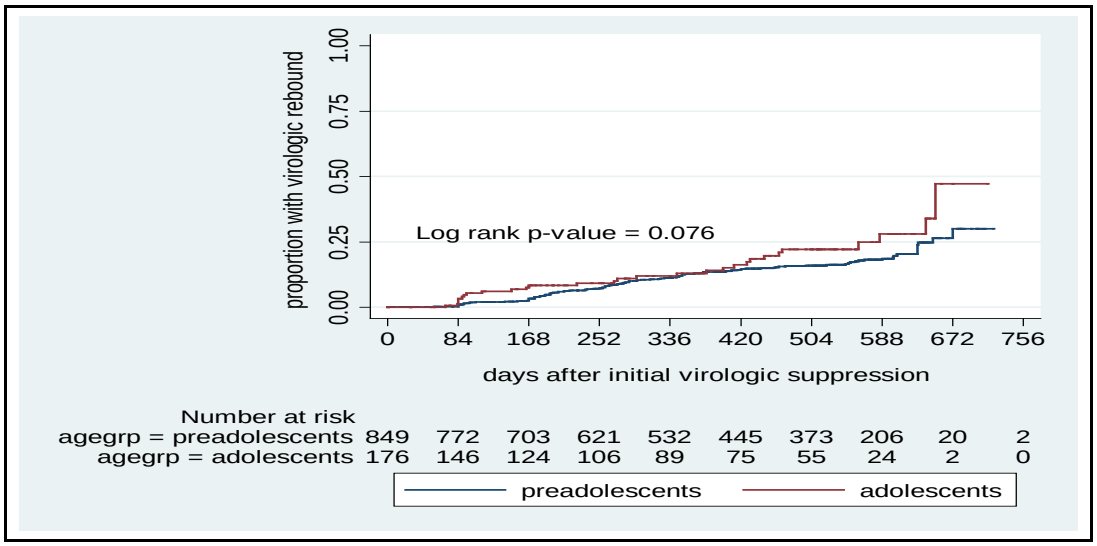

Figure 3.4: Comparison of time to virologic rebound between pre-adolescents (5 to < 11 years) and adolescents (11 to < 18 years) after initial virologic suppression.

**The log rank p-value is for the test of equality of time to virologic suppression between pre-adolescents and adolescents over the 96 week follow-up period.*

3.6 Baseline variables associated with virologic suppression

This first analysis was restricted to 762 pre-adolescents and 161 adolescents who had virologic outcomes ascertained at the 24th week of follow up. In this sub-cohort, none of the baseline characteristics were associated with early virologic suppression at both univariate and multivariate level (Table 3.3). After adjusting for baseline characteristics, the hazard ratio implied that adolescents had a 3% reduced likelihood of early virologic suppression than pre-adolescents. However, this observation was not statistically significant (aHR: 0.97, 95% CI: 0.79-1.19). When the study cohort was further stratified by gender, age group was associated with early virologic suppression among females. In this finding, adolescent females were 23% and 24% less likely to achieve early virologic suppression than pre-adolescent females at univariate and multivariate levels, respectively (uHR: 0.77, CI: 0.60-1.00, aHR: 0.76, CI:

0.59-0.99). None of the baseline variables were associated with early virologic suppression when analysis was restricted to male patients.

The second part of this analysis modelled baseline variables associated with virologic suppression by the 96th week of follow-up, for all of the 1234 selected study participants (Table 3.4). There was marginal evidence that, females were less likely than males to achieve early virologic suppression (uHR: 0.89, CI: 0.79-1.01, aHR: 0.90, CI: 0.78-1.04). Advanced clinical disease was strongly associated with inferior virologic suppression by 96 weeks, at both univariate and multivariate levels. After adjusting for other baseline variables, patients with clinically advanced disease were 25% less likely to achieve virologic suppression by 96 weeks relative to patients with less clinically advanced disease (aHR: 0.75, CI: 0.64-0.87). There was marginal evidence at univariate level that adolescents were 13% less likely than pre-adolescents to achieve virologic suppression by 96 weeks (uHR: 0.87, CI: 0.75-1.03). However, stronger evidence of no association between age group and virologic suppression was observed after adjusting for other baseline variables (aHR: 0.98, CI: 0.81-1.18). After stratifying the cohort by gender and adjusting for baseline variables, adolescent females were 30% less likely to achieve virologic suppression than pre-adolescents females by 96 weeks (aHR: 0.70, CI: 0.55-0.90).

Females who had clinically advanced disease at baseline had 22% less likelihood of virologic suppression by 96 weeks in comparison to patients of the same gender who had less clinically advanced disease (aHR: 0.78, CI: 0.63-0.95). Advanced clinical disease retained its association with virologic suppression by 96 weeks, when analysis was also restricted to male participants. Male patients who had clinically advanced disease at baseline were 30% less likely than less clinically advanced males in achieving virologic suppression by 96 weeks (aHR: 0.70, CI: 0.56-0.87).

Table 3.3: Baseline variables associated with virologic suppression by 24 weeks of follow up, among 923 participants with 24 week viral loads

	Unadjusted Hazard ratio (uHR) [CI]			Adjusted Hazard Ratio aHR [CI]		
Baseline variables	**All children (N=923)**	**Males (N=468)**	**Females (N=455)**	**All children (N=923)**	**Males (N=468)**	**Females (N=455)**
Age group						
Pre-adolescents	Reference	Reference	Reference	Reference	Reference	Reference
Adolescents	0.96[0.81-1.14]	1.22[0.96-1.56]	0.77[0.60-1.00]*	0.97[0.79-1.19]	1.21[0.91-1.62]	0.76[0.59-0.99]*
Gender						
Male	Reference	Not included in the model		Reference	Not included in the model	
Female	0.92[0.81-1.06]			0.94[0.81-1.10]		
WAZ-score						
≥-2	Reference	Reference	Reference	Reference	Reference	Reference
≥-3 to <-2	1.09[0.86-1.40]	1.01[0.74-1.37]	1.16[0.76-1.76]	1.09[0.77-1.54]	0.97[0.61-1.51]	1.23[0.71-2.14]
<-3	1.00[0.86-1.16]	0.88[0.71-1.09]	1.13[0.90-1.41]	1.10[0.88-1.39]	1.03[0.75-1.43]	1.18[0.84-1.65]
HAZ-score						
≥-2	Reference	Reference	Reference	Reference	Reference	Reference
≥-3 to <-2	0.99[0.83-1.17]	0.94[0.74-1.18]	1.04[0.81-1.35]	0.95[0.72-1.25]	1.03[0.69-1.53]	0.92[0.63-1.35]
<-3	0.98[0.83-1.14]	0.89[0.71-1.11]	1.07[0.86-1.34]	1.02[0.84-1.24]	1.02[0.78-1.35]	1.01[0.76-1.32]
Log viral load						
<5	Reference	Reference	Reference	Reference	Reference	Reference
≥5	0.86[0.72-1.03]	0.98[0.77-1.24]	0.85[0.65-1.10]	0.87[0.71-1.09]	0.95[0.70-1.28]	0.81[0.60-1.11]
CD4 count						
≥200	Reference	Reference	Reference	Reference	Reference	Reference
<200	1.06[0.90-1.27]	0.98[0.77-1.24]	1.15[0.90-1.47]	1.06[0.85-1.33]	0.94[0.69-1.30]	1.13[0.82-1.57]
CD4 percentage						
≥15	Reference	Reference	Reference	Reference	Reference	Reference
<15	1.05[0.85-1.28]	0.94[0.71-1.26]	1.12[0.84-1.50]	0.94[0.36-2.51]	0.93[0.64-1.35]	1.53[0.45-5.22]
Clinical Stage†						
less clinically advanced	Reference	Reference	Reference	Reference	Reference	Reference
clinically advanced	0.88[0.76-1.04]	0.90[0.72-1.11]	0.90[0.71-1.13]	0.87[0.74-1.03]	0.86[0.68-1.09]	0.92[0.72-1.17]

Values are Hazard Ratios [95% CI]. Reference HR =1. * Variable statistically significant at alpha level of 0.05%.
† Less clinically advanced (WHO clinical stage I and II) and clinically advanced (WHO Clinical Stage III and IV)

Table 3.4: Baseline variables associated with virological suppression by 96 weeks of follow-up cohort, for all 1234 study participants.

	Unadjusted Hazard ratio (uHR)[CI]			Adjusted Hazard Ratio(aHR)[CI]		
Baseline variables	**All children (N= 1234)**	**Males (N=631)**	**Females (N=603)**	**All children (N=1234)**	**Males (N=631)**	**Females (N=603)**
Age group						
Pre-adolescents	Reference	Reference	Reference	Reference	Reference	Reference
Adolescents	0.87[0.75-1.03]	1.13[0.9-1.42]	0.71[0.57-0.90]*	0.98[0.81-1.18]	1.17[0.89-1.54]	0.70[0.55-0.90]*
Gender						
Male	Reference	Not included in the model		Reference	Not included in the model	
Female	0.89[0.79-1.01]			0.90[0.78-1.04]		
WAZ-score						
≥-2	Reference	Reference	Reference	Reference	Reference	Reference
≥-3 to <-2	0.94[0.75-1.17]	0.93[0.70-1.24]	0.91[0.63-1.31]	1.00[0.73-1.38]	0.89[0.58-1.38]	1.14[0.71-1.85]
<-3	1.02[0.88-1.18]	0.96[0.79-1.17]	1.08[0.87-1.33]	1.09[0.88-1.35]	1.08[0.81-1.46]	1.08[0.79-1.47]
HAZ-score						
≥-2	Reference	Reference	Reference	Reference	Reference	Reference
≥-3 to <-2	0.96[0.82-1.13]	0.94[0.92-1.18]	0.97[0.76-1.23]	0.99[0.77-1.27]	1.11[0.77-1.59]	0.93[0.65-1.33]
<-3	1.00[0.87-1.16]	0.92[0.75-1.14]	1.07[0.88-1.32]	1.06[0.88-1.27]	1.14[0.88-1.48]	0.94[0.76-1.28]
Log viral load						
<5	Reference	Reference	Reference	Reference	Reference	Reference
≥5	0.95[0.81-1.13]	0.99[0.78-1.26]	0.93[0.72-1.18]	0.98[0.80-1.21]	1.04[0.78-1.39]	0.96[0.74-1.24]
CD4 count						
≥200	Reference	Reference	Reference	Reference	Reference	Reference
<200	0.94[0.80-1.11]	0.93[0.75-1.29]	0.96[0.76-1.21]	1.03[0.84-1.28]	1.08[0.81-1.46]	1.01[0.78-1.32]
CD4 percentage						
≥15	Reference	Reference	Reference	Reference	Reference	Reference
<15	0.96[0.80-1.18]	0.98[0.75-1.29]	0.95[0.72-1.25]	0.88[0.70-1.13]	0.86[0.61-1.2]	0.89[0.65-1.21]
Clinical Stage†						
less clinically advanced	Reference	Reference	Reference	Reference	Reference	Reference
clinically advanced	0.74[0.64-0.86]*	0.72[0.58-0.88]*	0.77[0.62-0.95]*	0.75[0.64-0.87]*	0.70[0.56-0.87]*	0.78[0.63-0.95]*

Values are Hazard ratios [95% CI]. Reference HR =1. * Variable statistically significant at 5% significance level.
† Less clinically advanced (WHO clinical stage I and II) and clinically disease (WHO Clinical Stage III and IV).

CHAPTER 4

DISCUSSION AND LIMITATIONS

4.1 Introduction

This section states the study's major findings while offering explanations of the meaning and importance of study results with relation to findings from similar studies. Moreover, this chapter addresses the clinical relevance of study findings while acknowledging the study's limitations and making suggestions for further research.

4.2 Discussion

The outcomes in the study demonstrate good virologic responses to treatment among an urban South African HIV-infected paediatric cohort of 1234 children initiated on HAART at Harriet Shezi Children's Clinic. The study provides an assessment of virologic outcomes in children beyond those previously reported in other paediatric studies in which pre-adolescents and adolescents were merged into one homogeneous group [38-41].

The proportion of patients who achieved virologic suppression in our study cohort compares favourably to other published cohorts in which virologic outcomes of adolescents are subsumed into those of pre-adolescent children to form a single group. In the current study, 72.3% (95% CI: 69%-75%) of all study participants achieved early virologic suppression 24 weeks after initiation of HAART, which is comparable to the 74% figure described in a Ugandan paediatric cohort [56]. In a meta-analysis of 9 studies, representing 1097 children (0-15 years) in resource limited settings, Ciarenallo et al (2009) [57] reported a 12 month pooled estimate of virologic suppression of 72% (95% CI: 69%-75%). These findings are analogous to results from this study in which 80% (95% CI: 77%-80 %) of the study participants achieved virologic suppression by 12 months. Differences between the Harriet Shezi cohort and other paediatric studies (such as age distribution, viral subtypes,

HAART regimens, adherence rates, etc) render conclusions drawn from these direct comparisons to be guarded. More research is necessary to investigate the sources of these discrepancies. Nonetheless, within the context of studies described above, outcomes of this study highlight early virologic suppression as an important feature of sub-Saharan paediatric HAART. Worthy of note, is the finding that early virologic suppression in this study cohort falls within the range of treatment responses observed in Europe and North America that vary between 47% - 79% [37]. Direct comparisons between resource limited and advantaged settings may be hindered by differences in age and disease severity at the time of HAART initiation. However, this study's findings highlight that comparable HAART outcomes in children are observed in resource limited and developed settings.

While it has been shown that the overall cohort exhibited good virologic suppression rates, results from further analyses suggest that, adolescents on HAART have distinct needs that need to be addressed in order to achieve virologic suppression rates analogous to those demonstrated by pre-adolescents after 24 weeks of HAART. While both groups demonstrated similar early virologic suppression rates, adolescents failing to suppress by the 24th week showed inferior virologic outcomes between this time point and the 96th week of follow up. Similar to our study findings, a study by Nachega et al (2009, South Africa) comparing adolescents to adults; showed that the proportion of adolescents achieving virologic suppression was inferior to that of adults, although no significant differences were observed at the 24th week of follow up. In their cohort, adolescents only exhibited significantly lower rate of virologic suppression after the 24th week from HAART initiation. This may be attributed to higher rates of incomplete suppression of viral replication among adolescents once they initiate HAART. This could occur due to reduced drug levels secondary to differing pharmacokinetics or poor adherence to HAART. Once drug levels are reduced, this may ultimately result in greater longevity of the long-lived HIV infected cells, redistribution

to the plasma of a greater amount of HIV previously adherent to follicular dendritic cells or acquired viral resistance. Hence, the observed lower rates of virologic suppression [60]. These study findings underscore the importance of exploring ways of ensuring that higher proportions of adolescents initiated on HAART attain virologic suppression within 6 months of treatment in order to obtain the benefits of therapy.

In a study by Walker et al (2004, United Kingdom), adolescents (13-17 years) also demonstrated inferior virologic suppression rates compared to pre-adolescents (6-12 years) [47]. In their study, Walker et al (2004) showed that while pre-adolescents were 13% less likely than adults (30-39 years) to be virologically suppressed at 24 weeks, adolescents had an additional 9% reduced likelihood of viral suppression compared to pre-adolescents. This is in agreement with findings by Kekitiinwa et al (2008) in which a non-linear association was observed between age and virologic suppression [56]. Using odds ratios they showed that the likelihood of virologic suppression increased from infancy into pre-adolescence and then decreased rapidly in adolescence.

In corroboration with adolescent outcomes observed in this current study, previous studies have established that adolescents also have less successful virologic response to HAART compared to adults [28-31]. In a study by Nachega et al (2009, South Africa) that compared adolescents to adults; the proportion of adolescents achieving virologic suppression was inferior to that of adults, although differences were significant at 48, 72 and 96 weeks after HAART initiation. The proportion of adolescents achieving virologic suppression by the 24th week in our study cohort compared favourably to that described by Nachega et al (2009) (66% vs. 63%) [30]. These study findings are similar, despite the differences in setting. Wherein this study was conducted in a public sector clinic, the adolescents described by Nachega et al (2009) received treatment within a private sector setting. Comparably poor virologic outcomes have also been reported among adolescents in a North American study

cohort. For example, in the PACTG 381 cohort of 120 adolescents; 59% (CI: 49% - 68%) achieved virologic suppression by the 24th week of HAART [29].

Another major study finding was that pre-adolescent females demonstrated a shorter time to virologic suppression than their adolescent counterparts by the 24th and 96th week of HAART. Contrary to the observation among adolescent females, no differences were observed in time to virologic suppression between adolescent and pre-adolescent males. Inferior virologic outcomes observed among adolescents in general, may be attributed to key changes at the biological, psychological and social levels that differentiate them from other stages of development [28]. Based on our study findings, these changes appear to negatively impact the virologic outcomes of female adolescents, more than male adolescents. At this stage, peer groups become increasingly important as adolescents seek intimacy and acceptance outside the family unit; a phenomenon that may explain better treatment outcomes in pre-adolescents who are yet to encounter such concerns. Furthermore, Steinberg (1990) suggests parent-child relationships are transformed, as adolescents learn to make decisions independently and function autonomously [58]. This may explain the superior virologic outcomes in pre-adolescents who unlike adolescents may still rely on care-givers for medication adherence and are more likely to adhere to treatment. Research has shown family centred models of care to improve medication adherence and treatment outcomes among children enrolled on HAART [13]. According to findings by Belzer et al (1999), adolescents should be encouraged to disclose their HIV status and therapy regimens to their peers or school personnel, to prevent unilateral authority in terms of handling dosages and treatment schedules without a support structure [69].

Apart from cognitive and social changes, adolescents experience adjustment to changing bodies, re-defining self image and learning to manage emerging sexuality. However, psychosocial changes that interfere with HAART appear to be more pronounced

among adolescent females than adolescent males. This is based on the observation that adolescent females had a longer time to their first virological suppression than pre-adolescent females. Since no differences were observed between pre-adolescent and adolescent males, study findings suggest targeting more concerted efforts towards adolescent females on HAART.

Wherein this study; HIV-plasma RNA was used as a surrogate marker for treatment adherence, Nachega et al (2009) report low levels of adherence among adolescents using pharmacy refill data. Additionally they showed that 91% of adolescents who were perfect adherers achieved virologic suppression by 12 months in comparison to 45% of non-adherers [30]. Although it is highly suggestive that adolescent transition has a negative effect on treatment adherence and virologic outcomes, barriers to adolescent adherence extend beyond biological, psychological and social levels. Over and above the issues unique to them, adolescents also share poverty as a barrier to HAART adherence with other age groups, as described by Bikaako-Kajural et al (2006) in a Ugandan cohort [59]. Directly observed therapy (DOT) may be considered as a strategy to mitigate non-adherence concerns among adolescents. Roberts et al (2004) instituted a step-wise approach to manage non-adherence. This involved (in chronological order) visits by a home health nurse, DOT during a hospitalisation and, subsequently, a medical neglect report [70]. Although the study demonstrated the potential ability of DOT to diagnose non-adherence; similar to findings by Glikman et al (2007) and Purdy et al (2008), the decrease in viral load was not sustained in the majority of patients when evaluated many weeks after discharge [19,21]. Research gaps still exist in assessing the generalisability of these findings and potential use of the DOT approach in paediatric clinics within resource limited settings.

It is also important to note that adolescent outcomes described thus far in comparison to pre-adolescents may be in keeping with the fundamental role of the thymus in repopulating

the immune system after HAART and the fact that it is particularly active in younger children. Conversely, pre-adolescents have a less mature immune system than adolescents that allows less containment of HIV with resultant high viral replication, balancing out their superior thymic activity [60]. Rudy et al (2006) have also shown adolescents to exhibit persistent thymic activity and greater potential for immune recovery and possible viral control [62]. Owing to lack of information and its retrospective nature, the current study did not explore possible differences of immunologic reconstitution between the two age groups and its influence, if any, on the observed differences in virologic suppression between the two age groups. Additional research is essential in this regard as the rollout of paediatric HAART intensifies, with reference to resource limited settings.

While adolescents had lower proportions with virologic suppression than pre-adolescents at all time points, it was encouraging to observe that the median time to virologic suppression was 22 weeks in both age groups. Bangsberg (2006) showed that NNRTI regimens lead to virologic suppression at moderate levels of adherence (54-100%) [61], while Nachega et al (2009) have also shown that efavirenz based regimens lead to a rapid time to virologic suppression [30]. Therefore, the favourable median time to virologic suppression observed in this cohort that includes potentially non-adherent adolescents may be due to the restriction of this study sample to patients initiated on an efavirenz based regimen. To a lesser extent, this may be indicative of older age at HAART initiation being a proxy for slower disease progression and good virologic control.

Regardless of similar times to early virologic suppression between the two age groups; their differences in virologic suppression after the 24th week of follow up and virologic rebound after 420 days from initial suppression warrants raises concerns of acquired HAART resistance among adolescents. Plasma viraemia inevitably returns to individual pre-therapy set point levels in almost all HIV-infected patients who encounter interruption of

antiretroviral therapy [62, 63]. Despite concerns of treatment interruption among potentially non-adherent adolescents, patients who experienced initial virologic suppression were likely to remain suppressed, two years after their initial virologic suppression. Using a more relaxed definition of one viral load >400 copies/ml after initial suppression, this study showed that: 2% and 9% of all study participants who were initially suppressed, experienced viral rebound by the 24th and 48th week of therapy respectively. These results are slightly lower than 16 - 44% virologic rebound at 6 months reported from other observational cohorts [43-45, 64]. Although both groups in this study had similar proportions of patients experiencing virologic rebound; after 60 weeks, adolescents had faster rates of virologic rebound than pre-adolescents. This finding strongly suggests closer monitoring of adolescents in order to sustain the long term effects of HAART.

Notwithstanding that poor adherence leads to sub-inhibitory drug levels allowing partial virologic suppression and often the emergence of resistant virus; literature has shown that the level of adherence required to prevent treatment failure (including virologic rebound), varies depending on the regimen used. Lower levels of adherence are required when using NNRTIs, possibly due to higher potency and longer plasma half life [62]. This could help explain the low incidence of virologic rebound in a study sample that only had participants initiated on efavirenz. Despite concerns of sub-optimal adherence and low virologic suppression rates among adolescents, there were no differences in their time to virologic rebound compared with pre-adolescents. These data do not alter the goal to achieve the premier level of adherence among adolescents; instead, it presents partial evidence that patients on NNRTIs with moderate levels of adherence may preserve virologic suppression. The study did not measure the levels of adherence. However, since one of the inclusion criteria for virologic rebound was an initial response to HAART, these patients appear to have been adherent to therapy to a certain extent for the initial few months of HAART.

This study also set out to describe baseline characteristics of study participants. Study participants had baseline clinical characteristics and anthropometric indicators similar to those reported in other resource limited settings that also provide paediatric HAART through the government or donor organisations [65]. In this study, children presented with advanced immuno-deficiency, advanced clinical disease stages and with a high prevalence of stunting and wasting. These study findings are consistent with those of a systematic review by Sutcliffe et al (2008) describing HAART programmes in sub-Saharan Africa [37]. Similar to our finding of 57%, Sutcliffe et al (2008) report that 56% to 96% of sub-Saharan children present with less than 15% CD4 T cells at HAART initiation. However, the mean baseline viral load of our study cohort ($\log_{10}$ 4.8 copies per mL) was slightly lower compared to that reported by Sutcliffe et al (2008) (5.0 to 6.1 $\log_{10}$ copies per mL) but comparable to findings by Davies et al (2009) from 8 South African paediatric cohorts (4.7 to 5.8 $\log_{10}$ copies per mL). Additionally, similar to findings by Davies et al (2009), over half the study cohort had clinically advanced disease at HAART initiation [65]. In South Africa, this can be explained by the existence of marked inequities to HAART access with HIV-infected children constituting <15% of all patients on HAART. Additionally, limited human resources and inadequate paediatric skills at lower levels of care limit HAART delivery to tertiary institutions. Ultimately, this debilitates immediate access to treatment and depreciation of HIV-infected paediatrics awaiting therapy [24].

Although both age groups presented with comparably inferior baseline clinical characteristics, adolescent status was associated with clinically advanced disease stage, severe stunting and advanced immunodeficiency. In the South African study by Nachega et al (2009), adolescents also exhibited inferior immuno-competency at baseline, when compared to young adults aged 20-30 years (median CD4 count (IQR): 144 cells/µl (27-246) vs. 175 cells/µl (75- 278), P = 0.003) [30]. Observations among adolescents can be attributed to

difficulties in identification of older HIV-infected patients. This is consequent to non-specific clinical features of HIV infection in children, which makes it difficult for health care workers to reliably diagnose HIV infection on clinical grounds alone. This is further exacerbated by limited interaction of this age group with the health system and missed opportunities when such interactions occur for voluntary counselling and testing (VCT) [24].

In the context of other paediatric studies, baseline findings from this study, underscore the threat to paediatric HAART success in resource limited settings, since some of the inferior baseline clinical and anthropometric characteristics have been reported as risk factors for poor virologic suppression. The current study demonstrated marginal evidence suggesting that participants with clinically advanced disease at baseline were less likely to experience early virologic suppression than patients with less advanced disease. There was a stronger association between virologic suppression and advanced clinical disease by 96 weeks. At this time point, patients with clinically advanced disease at baseline were less likely to virologically suppress than the reference group. The same effect was observed when the study cohort was further stratified by gender. Noteworthy, is the fact that patients categorised as having clinically advanced disease were those staged into WHO stages 3 and 4. These findings might well characterise unidentified and unmeasured confounders, such as underlying chronic infections that are associated with advanced WHO clinical stages. More importantly, they highlight the implications of delayed commencement of HAART on treatment outcomes.

4.3 Limitations

To our knowledge, the current study is one of the first research initiatives to evaluate differences in virologic outcomes between pre-adolescents and adolescents, from one of the largest HIV paediatric cohorts. However, the reported study findings must be interpreted with regard to study limitations.

Firstly, analysis of virologic response to HAART may have been limited by differences in the frequency and timing of viral load testing, between and within the two age strata. Contrary to study cohorts in clinical trials where virologic outcomes are evaluated according to study protocols [28, 29], irregular viral load testing in this study is truly reflective of clinical practice in resource limited settings. The availability of regular viral load information is unusual in resource restricted settings. Other sub-Sahara African studies have failed to describe virologic outcomes owing to the high cost involved in viral load testing [67]. Hence the results obtained from this study are a reflection of the existent, rather than ideal virologic outcomes among pre-adolescent and adolescent children on HAART. For the purposes of this study, four follow-up intervals were established to counter the irregularity of viral load assessment and measurements closest to the interval mid-point were selected. Use of the interval mid-point assisted in averting information bias where differential misclassification would have occurred if the selection of viral measurements to determine virological outcomes was associated with the age group of study participants.

To assess the impact of missing values on the observed results, sensitivity analyses were performed. It was observed that, interpretation and conclusions drawn from reported study findings were not altered when missing viral loads were considered to represent non-virologic suppression. Due to sporadic and infrequent viral load testing, virologic rebound was defined using a less strict definition based on one viral load > 400 copies per mL after initial suppression. The definition was susceptible to artificial blips resulting from concurrent infection or measurement error, independent of the effect of HAART or age group specific issues that influence HAART response. However, due to its leniency; the employed definition presented the worst case scenario in terms of virologic rebound rates within the study participants. When sensitivity analysis was performed using a definition that required two consecutive viral loads >400 copies per mL; the incidence of virologic rebound decreased as

expected, but still no differences were observed between the two age groups. Since sensitivity analyses yielded consistent findings to primary analyses, both definitions of virologic suppression and rebound were maintained as described in the methods section.

The evaluation of virologic outcomes in perinatally infected children inherently exposes the study to potential confounding by indication. Enhanced outcomes for older children represent a survivor effect, with older age at HAART initiation being a proxy for slower disease progression. This is supported by studies showing that children who are well enough to defer treatment commencement until later ages may have improved underlying virological control or less fit virus that can respond better virologically to HAART [47]. The current study did not evaluate any variable that explained reasons for the late commencement of therapy, because the current South African Treatment Guidelines used by the clinic do not instruct the collection of such information. Since the majority of patients were perinatally infected, the generalisability of these study findings to horizontally infected adolescents is limited.

Additionally, generalisability is also limited to patients initiated on an efavirenz based regimens since this was an inclusion criterion for selection into the study. The results add to the growing body of evidence and are highly applicable to South Africa, where 53.4% of children from 11 HAART sites and at different levels of care, where initiated on efavirenz based regimens [66]. Due to a selection bias toward HAART naive patients at initiation, we may have overestimated virologic suppression and underestimated rebound rates. Treatment experienced patients have been shown to exhibit poorer virologic outcomes in comparison to treatment naive patients [68]. The exclusion of treatment experienced patients from this study is reflective of the current HAART era and how the majority of patients enter the HAART programme at the clinic, with very few treatment experienced patients coming from elsewhere.

The database used for this study was not initially set up for comprehensive research; instead it was designed as a patient management tool, hence the limited data on potential confounders, modifiers and time-updated clinical characteristics. The clinic did not capture information on opportunistic infections, treatment adherence, changes in HAART and doctor to patient ratios among other potential confounders that could explain the observed study findings. As HAART rollout expands in sub-Saharan Africa, further studies on virologic outcomes in African children are required to establish whether the results from this study are fully generalisable and to describe associations that could not be evaluated with the limited data in the current database.

The selection of the study sample from a public sector clinic may have resulted in a selection bias towards patients with lesser financial resources. Patients with greater financial resources may seek semi-private subsidised or complete private care. Although this possibility exists, the majority of paediatric HAART in resource limited settings is administered through the governments in collaboration with public benefit organisations. Additional research is essential to understand the financial constraints of this population, but limited validity of this data due to financial inequity cannot be robustly assumed.

While this study adds to the existent body of knowledge, there still exists disproportionate representation of paediatric HAART sites with a bias to tertiary care centres (e.g. Harriet Shezi Clinic) in reporting patient treatment outcomes. The comprehensive monitoring and higher number of patients at tertiary institutions probably represent best-practice examples in well-resourced clinics [24]. Hence, it is unclear whether the observed results are generalisable to lower levels of care.

CHAPTER 5

CONCLUSION and RECOMMENDATIONS

5.1 Conclusion

The current study of a large cohort of HIV-infected children demonstrates dramatic clinical benefits for those accessing HAART in a resource limited setting. These encouraging findings as a proxy of treatment success indicate improved virologic outcomes of HIV-infected children, but challenges to achieve effective and parallel treatment outcomes among all age groups still need to be addressed. In particular, adolescents showed evidence of poorer virologic responses than pre-adolescents after the 24th week of HAART; which may put this group at increased risk of HIV disease advancement, other clinical events and resistance mutations. Among all study participants, adolescent females were more susceptible to inferior virologic suppression rates. However, once virologic suppression was attained, children in both age groups were generally able to sustain the durability up to a year, after which adolescents had higher rates of virologic rebound.

The findings underscore the unique and complex adherence issues experienced by adolescents and threat to the success of HAART programs in South Africa, as the number of adolescents enrolled and eligible for HAART continues to rise. While virologic suppression is an important goal of treatment to avoid the emergence of viral resistance, the definitive aim of HAART is to prevent clinical progression and death. Despite the importance of delaying treatment to ameliorate barriers to adherence among adolescents, prolonged deferral in enrolling them onto HAART may inevitably lead to disease progression and an elevated potential for increased morbidity and mortality.

Given the potential for resistant strains of the virus that are no longer responsive to available HAART and the public health threat this presents, health care teams face

complicated dilemmas regarding initiation of HAART in adolescent patients who may have higher rates of non-adherence.

5.2 Recommendations

As has been highlighted already, adolescents are often unable to cope with side effects and complexities of HAART that meddle with their active social lives, they focus on immediate consequences and not long term disadvantages of non-adherence. Therefore a multidisciplinary approach is necessary to help determine some of the barriers to adherence and address them at patient-level, provider- level and through the health care system.

Although no gold standard exists for adherence measurement, more intensive methods such as micro-chip caps that record opening of medication bottles or drug level monitoring may be used for more accurate information. Such methods are financially demanding and they may not be sustainable, but their benefits may far outweigh the costs of initiating patients on second line therapy [17, 18, 28]. Without clear understanding and recording of adherence levels, regimen changes can lead to additional resistance, ultimately limiting treatment options. For example, in the current setting (Harriet Shezi Children's Clinic) there were no adherence data that could be used for the study. This is despite, the counselling of almost every patient/caregiver at every clinic visit and adherence forming a large part of these sessions.

To support these provider-level interventions, family-monitored medication administration should be encouraged to enhance adherence support and psychosocial needs of adolescents on treatment. Family models of care need to be developed and evaluated in South Africa to evaluate if this will lead to improved care of HAART patients. Research efforts should also be encouraged in exploring possibilities of implementing institution-based HAART directly observed treatment to improve adherence and virologic responses.

The lack in capacity of the health system to provide treatment to patients eligible for HAART, indirectly contributes to patients presenting for treatment at advanced disease stages. This study has shown advanced clinical disease to be a strong risk factor for lower virological suppression rates. Inequities in HAART access still need to be addressed. Despite the South African National Department of Health advocating for HIV-infected children to constitute 15% of all patients on HAART, most provinces have not yet achieved this target [24]. To achieve set targets, solutions should be tailored to local conditions due to the differences in infrastructure, disease burden and resources between different settings. This should be augmented by the setting up of adolescent friendly clinic services. Adolescent focused training materials should be applied and constantly updated to accommodate novel research in this age group.

Finally, we recommend further research into the comparison of virologic outcomes between pre-adolescents and adolescents on HAART, through prospective study designs. Qualitative study designs are also important to bridge the knowledge gaps on the barriers to HAART encountered by female adolescents.

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Appendix 1: Approval of title by University of the Witwatersrand, Faculty of Health Sciences

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011) 717-2745

Reference: Ms Tania Van Leeve
E-mail: tania.vanleeve@wits.ac.za
22 September 2009
Person No: 376101
PAG

Mr T Mabuto
Flat Number One Lowe
Wycombe Falts
Lot 1452
Thembelihle
Swaziland

Dear Mr Mabuto

Master of Science in Medicine (Epidemiology & Biostatistics): Approval of Title

We have pleasure in advising that your proposal entitled "*Comparison of virologic outcomes in HIV-infected adolescents and pre-adolescents on Highly Active Antiretroviral Therapy in Soweto, South Africa*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 2: Ethics clearance certificate from the University of Witwatersrand Research Ethics Committee

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Tonderai Mabuto

CLEARANCE CERTIFICATE	**M090946**
PROJECT	Comparison of Virologic Outcomes in HIV-Infected Adolescents and Pre-Adolescents on Highly active Antiretroviral Therapy in Soweto, South Africa
INVESTIGATORS	Tonderai Mabuto.
DEPARTMENT	School of Public Health
DATE CONSIDERED	2009/10/02
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 2009/10/02 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Ms S Sawry

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...