VIROLOGICAL RESPONSE TO FIRST-LINE ANTIRETROVIRAL THERAPY AMONG HIV-INFECTED CHILDREN ATTENDING SELECTED HIGH VOLUME CLINICS IN WESTERN KENYA

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SM/PGCHP/03/14

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF MEDICINE IN CHILD HEALTH AND PEDIATRICS, SCHOOL OF MEDICINE, MOI UNIVERSITY

DECLARATION

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This thesis is my original work and has not been presented for a degree in any other university. No part may be reproduced without prior permission of the author.

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ACKNOWLEDGEMENT

I would like to recognize and appreciate the efforts and dedication of the following people who contributed in one way or another to the development of this thesis:

1. My supervisors, Prof. S.O Ayaya and Prof. W.M Nyandiko for their critique, advice and guidance.
2. Consultant pediatricians and my colleagues for providing constructive criticisms.
3. Pediatric ART clinic staff at MTRH, Busia County hospital and Trans Nzoia County hospital for their cooperation which made this thesis successful.
4. My biostatistician Dr Ann Mwangi for her guidance in the development of this thesis.

DEFINITIONS

Virological suppression: Viral load less than 1000 copies per milliliter after six months of effective antiretroviral therapy.

Clinical failure: The development of new of recurrent WHO clinical stage 3 or 4 event after 6 months of effective ART

Large care programme: AMPATH clinics in Western Kenya

Child: Aged six months to 15 years

Adherence to ART: The extent to which a patient complies with intake of antiretroviral medication as described by the healthcare provider. Good adherence - ≥95%; fair adherence – 85% to 94%; poor adherence - ≤85%.

Overweight: Expected weight for age greater than 110%, without edema

Marasmus: Expected weight for age less than 60%, without edema.

Underweight: Expected weight for age between 60% and <80%, without edema

ABBREVIATIONS AND ACRONYMS

ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
AMPATH	Academic Model Providing Access to Health
ART	antiretroviral therapy
ARVS	Antiretroviral drugs
AZT	Zidovudine
EFV	Efavirenz
HIV	Human Immunodeficiency Virus
KAIS	Kenya AIDS indicator survey
LPV/r	Boosted lopinavir
NASCOP	National AIDS and STI Control Program
NNRTIs	non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
PCP	Pneumocystis pneumonia
PMTCT	Prevention of mother-to-child transmission of HIV

R	Retonavir
RAM	Resistance associated mutations
RLS	Resource limited settings
SAM	Severe Acute Malnutrition
STI	Sexually transmitted infections
TAM	Thymidine analogue mutations
TAT	Turn-around time
TB	Tuberculosis
TDF	Tenofovir
UNAIDS	Joint United Nations Program on HIV/AIDS
WHO	World Health Organization

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ABSTRACT

Background :Approximately 36.7 million people are living with Human Immunodeficiency Virus (HIV) with 1.8 million being children. More than 90% of these are living in Sub-Saharan Africa. The World Health Organization's (WHO) plan to achieve 90% antiretroviral therapy (ART) coverage with 90% virological suppression by 2020 can only be achieved by proper monitoring of treatment response which will help in the early detection of failing regimen and prevention of drug resistance development. WHO now recommends virological monitoring since clinical and immunological criteria used previously had low sensitivity. This study sought to find out the proportion of children who achieved virological suppression and the determinant factors.

Objectives: To describe virological response to ART among children attending Academic Model Providing Access to Health (AMPATH) clinics in Western Kenya.

Methods: This was a retrospective chart review of routinely collected data conducted between June 2014 and July 2016 at three AMPATH clinics. Consecutive sampling was used to recruit HIV-infected children according to selection criteria. Socio-demographic and clinical data was collected using a pretested data collection tool. Data was entered in Microsoft Access and exported to STATA version 13 for analysis. Descriptive statistics such as frequency listings were used for categorical variables, while Chi square test and Fischer's exact test were used to assess associations. Multiple logistic regression was used to determine independent variables. All analysis was done at 95% level of significance with *p*-value less than 0.05 being statistically significant.

Results: Of the 371 study participants, 194(52.50%) were males. The median age at ART initiation was 84 months (IQR 36-123) with only 47(12.70%) having used nevirapine for prevention of mother to child transmission (PMTCT) of HIV. Most of the children (98.9%) had good adherence (≥95%). Majority of the children (96%) did not develop opportunistic infections. Opportunistic infections reported included tuberculosis (0.35%) and herpes (0.14%). Majority of the children had WHO stage 1(49.30%) and WHO stage 2 (24.50%) disease with most of them having used ART for six to twelve months (73.29%). Most of the children had normal nutritional status (62.90%) and only (27.10%) and (10.00%) had under nutrition and overweight, respectively. After at least six months of ART, 78.44% (95%CI: 73.90,82.51) of children achieved virological suppression, at a median time to virological suppression of 10.84 months (95% CI: 9.72,11.93). Of the socio-demographic and clinical factors affecting virological suppression, only age at ART initiation was statistically significant (p=0.037).

Conclusions: Slightly more than three-quarters of children achieved virological suppression following at least six months of first-line ART, with majority of the children reported to have good adherence. Younger children at ART initiation were more likely to achieve virological suppression compared with older children.

Recommendations: Efforts to be made at all levels of care provision to enhance earlier initiation of ART in all HIV-infected children.

CHAPTER ONE

INTRODUCTION

1.1 Background

Acquired Immunodeficiency Syndrome pandemic is caused by the Human Immunodeficiency Virus (HIV) and it is a global public health problem. The total number of people living with HIV/AIDS is approximately 36.7 million, with global incidence of 2.1 million people. Of these, 1.8 million are children, with more than 90% living in Africa. The global incidence among children as per UNAIDS 2014 report was 260000(UNAIDS, 2017). According to the Kenya AIDS Indicator Survey 2012, the prevalence of HIV was about 5.6% among people aged 15-64 years, which translates to approximately 1.2 million people. Children aged 18 months to 14 years have a prevalence of 0.9%, which translates to 104,000 children (KAIS, 2013).

Children are mainly infected by vertical transmission from their mothers. Without any intervention, the rate of infection is usually as high as 40%. However, appropriate prevention of mother to child transmission of HIV reduces the incidence of infection to less than 2%. Most infants who acquire HIV in-utero or around delivery usually have rapid disease progression in the first few months of life, with approximately one third of them dying before their first birthday and 50% of them dying by the end of the second year(NASCOP, 2011).

Antiretroviral therapy (ART) is the mainstay of managing HIV/AIDS in both children and adults. Consequently, World Health Organization (WHO) and the Government of Kenya have scaled up ART use among all age groups. However, proper monitoring of treatment response to ART is important to achieve long term benefits. Until 2012, monitoring of treatment response in resource-limited settings (RLS) was based on clinical and

immunological criteria, with viral load monitoring reserved for suspected treatment failure and research (WHO, 2010). This was due to the financial and infrastructural constraints in these resource- limited settings (Chohan, B.H., Tapia, K., Merkel, M., Kariuki, A.C., Khasimwa, B., Olago, A., …& Wamalwa, D.C., 2013; Jobanputra, K., Parker, L.A., Azih, C., Okello, V., Maphalala, G., Jouquet, G., …& Reid, T., 2014). The current recommendation is the routine use of viral load monitoring to assess treatment response to antiretroviral therapy (WHO, 2013).

Various studies have shown that monitoring of treatment response to ART using the WHO clinical and immunological parameters has low sensitivity. Studies in the adult population have found sensitivities of immunological and clinical monitoring in the range of 10% - 17%(Chaiwarith, R., Wachirakaphan, C., Kotarathititum, W., Praparatanaphan, J., Sirisanthana, T., & Supparatpinyo, K., 2007; Gupta, R.K., Hill, A., Sawyer, A., Cozzi-Lepri, A., von Wyl, V., Yerly, S., … & Pillay, D., 2009). In a recent study among children in Tanzania, the sensitivity of clinical and or immunological criteria for detection of treatment failure was 12.9% - 25.8%, with virological failure as high as 57.1% (Mgelea, E.M., Kisenge, R., & Aboud, S., 2014).

Several studies have also shown that there is misclassification of treatment failure using clinical and immunological criteria leading to unnecessary switch of ART from first-line to second-line regimens (Kantor, R., Diero, L., DeLong, A., Kamle, L., Muyonga, S., Mambo, F.,…& Buziba, N., 2009; Westley, B.P., DeLong, A.K., Tray, C.S., Sophearin, D., Dufort, E.M., Nerrienet, E., …& Kantor, R., 2012). It is estimated that five to twenty per cent of patients initiated on first-line ART will experience treatment failure within the first four years of treatment irrespective of good treatment adherence (Kantor, R. 2006). In Africa,

virological failure has been shown to range from 13.6% to 57% (Hassan, A.S., Nabwera, H., Mwaringa, S.M., Obonyo, C.A., Sanders, E., Rinke, de Wit, T., …& Berkey., 2014). Of those patients who fail, between 35% and 70% will have developed drug resistance to ART at the time of treatment failure (Costenaro, P., Lundin, R., Petrara, M.R., Penazzzato, M., Massavon, W., Kizito, S., …& De Rossi, A., 2014). This therefore underscores the significance of consistent virological monitoring that would enable timely diagnosis of treatment failure and inform the initiation of appropriate measures to deter development of drug resistance.

The WHO now recommends the routine use of viral load testing to monitor treatment response to ART, particularly in RLS, where development of resistance to first-line regimen greatly limits the available options for second-line and third-line regimens due to cross-resistance among the various classes of ART (WHO, 2013; Arnedo, M., Alonso, E., Eisenberg, N., Ibanez, L., Ferrreyra, C., Jaen, A., …& Dalmau, D., 2012; Sigaloff, K.C., Hamers, R., Wallis, C., Kityo, C., Siwale, M., Ive, P., …& de Wit T.F., 2011). Studies among HIV-infected children in Western Kenya to evaluate virological response to ART and factors associated with success or failure of ART are lacking. This study sought to establish the magnitude of virological response to ART and the determinants of the same.

1.2 Problem Statement

Antiretroviral therapy has been in use for about two decades. Studies have been done in other countries to show the proportion of children who respond to ART, using virological criteria. However, in resource limited regions, clinical and immunological criteria have been used to assess response to ART because viral load monitoring was not affordable. Previous use of

clinical and immunological criteria to monitor treatment response has been shown to have a lower sensitivity as well as positive and negative predictive values for treatment failure (Coetzer, M., Westley, B., De Long, A., Tray, C., Sophearin, D., Nerrienet, E., ..& Kantor, R., 2013; Mulu, A., Liebert, U., & Maier, M., 2014). Usually, virological failure predates immunological failure, and studies have demonstrated that by the time treatment failure is detected by immunological criteria, 67% of the children will have developed resistance to more than one drug (Barry, O., Powell, J., Renner, L., Bonney, E., Prin, M., Ampofo, W., …& Paintsil, E., 2013).

Studies done in other countries and regions have demonstrated the magnitude of virological failure among HIV-infected children on ART of 13%-57%. In Western Kenya however, we do not have this information and the factors associated with this response. With the inception of routine viral load monitoring in Kenya, there is need to establish the virological response among children on first-line ART in Western Kenya due to the availability of resources (NASCOP, 2014). This study was carried out to provide necessary information on virological response to ART.

1.3 Study Justification

The HIV/AIDS treatment monitoring is one of the major UNAIDS goals of increasing ART coverage, reducing morbidity and mortality, early detection of treatment failure and prevention of emergence of resistance to ART (UNAIDS, 2017). In resource limited settings, previous treatment monitoring has been based on clinical and immunological criteria, including six-monthly CD4 assessment and WHO clinical staging. However, the utility of clinical and immunological criteria has been curtailed by the lower sensitivity and positive and negative predictive values compared with virological testing in the detection of treatment

failure. In some studies, the sensitivity of clinical and immunological criteria has been shown to be as low as10% (Chaiwarith, R., et al. 2007).

Studies in resource-rich countries have shown that virologic failure ranges from 8% to over 20% (Shiau, S., Kuhn, L., Strehlau, R., Martens, L., Mclleron, H., Meredith, S., …& Arpadi, S., 2014; UK Collaborative Group on HIV Drug Resistance, 2005). In resource-limited regions, a range of 13% - 57% has been documented in various studies (Chohan, B.H. et al. 2013; Mgelea, E.M., Kisenge, R., & Aboud, S., 2014 ; UK Collaborative Group on HIV Drug Resistance, 2005). The higher rate of failure in RLS has been attributed to the delayed diagnosis of sub-optimal response to ART due to the use of clinical and immunological criteria in the monitoring of treatment response. This has been shown to delay timely interventions such as enhancement of adherence counseling and appropriate switching of ART regimen (Salazar-Vizcaya, L., Keiser, O., Technau, K., Davies, M., Haas, A., Blaser, N., …& Estill, J., 2014).

To our knowledge, at the time of this study, no study had been done within Western Kenya region in children to demonstrate the degree of virological response to ART and to evaluate the determinants of virological suppression at the initiation of ART. This study was meant to inform the policy makers about the magnitude of virological suppression among HIV-infected children on first-line ART in the region and therefore the significance of routine viral load monitoring. Without this study, there will persist a knowledge gap on the local factors affecting virological response and therefore possible worsening emergence of drug resistance. Up to 98% of children with extensive reverse transcriptase resistance can be missed when using clinical and immunological criteria (Westley, B.P., et al. 2012).

1.4 Research Question

What is the virological response to antiretroviral therapy among children attending selected high volume clinics in Western Kenya?

1.5 Objectives

1.5.1 Broad Objective

To describe the virological response to ART among children attending selected high volume clinics in Western Kenya

1.5.2 Specific Objectives

1. To determine the proportion of HIV-infected children on ART who achieve virological suppression
2. To determine the socio-demographic and clinical factors associated with virological suppression.

CHAPTER TWO

LITERATURE REVIEW

2.1 Prevalence of HIV

The HIV/AIDS pandemic is a major chronic illness globally. An estimated 36.7 million people are living with HIV/AIDS globally, with approximately 2.1 million new cases reported annually. Children too are infected with this scourge with about 1.8 million children living with HIV/AIDS globally. Of these, ninety percent of children are living in Africa. The global incidence of HIV/AIDS among children is estimated at 260,000 cases (UNAIDS, 2017).

In Kenya, the prevalence of HIV/AIDS is about 5.6% among individuals aged 15 – 64 years, which translate to approximately 1, 192, 000. Children aged 18 months to 14 years have a prevalence of 0.9% which corresponds to 104000 children (KAIS, 2013). The incidence among total population is estimated at 166, 000 people, while in children it ranges between 7000 and 10000 children (NASCOP, 2011; Kenya National Bureau of Statistics and ICF Macro., 2010).

Kenya is among the 22 priority centers in the world that the Global Plan intends to scale up antiretroviral therapy use. The 22 centers collectively account for more than 90% of pregnant women living with HIV (UNAIDS, 2017). This is important because HIV transmission in children is mainly by the vertical route and without any intervention, up to 40% of babies born to HIV positive mothers will contract the disease. With appropriate intervention, this rate is reduced to less than 2% (NASCOP, 2011). Antiretroviral therapy provides the mainstay treatment of HIV/AIDS in children because it reduces the progression of the disease and therefore lowers the morbidity and mortality resulting from illnesses.

2.2 Treatment Failure

2.2.1 Definition of treatment Failure

2.2.2.1 Clinical Criteria of Treatment Failure

In its 2010 guidelines, the World Health Organization recommended the use of clinical and immunological criteria in the monitoring of treatment response to ART in resource-limited settings. Viral load testing was reserved for suspected treatment failure based on clinical and immunological criteria. This was due to financial and infrastructural constraints in these resource limited regions (WHO, 2010).

The clinical criteria for treatment failure are based on the WHO staging of the severity of HIV/AIDS. Among adults and adolescents, treatment failure is defined as the appearance of a new or recurrent clinical condition that indicates severe immunosuppression such as WHO stage 4 event and certain stage 3 events such as tuberculosis and recurrent severe bacterial infections. In children, treatment failure based on clinical criteria is defined as the development of a clinical condition indicating severe immunosuppression such as the WHO stage 3 or 4 disease. The duration of treatment should be at least six months which should be effective and with good adherence (WHO, 2010; WHO, 2013; Gilks, C., & Victoria, M., 2006). These criteria have been used over a long time, particularly in resource-limited settings due to their ease of application and affordability.

2.2.2.2 Immunological Failure

Immunological failure is based on absolute or percentage CD4 count assessed after at least six months of ART. In adolescents and adults, immunological failure is defined as fall of CD4 count from baseline or a 50% reduction in CD4 count from on-treatment peak or persistently low CD4 counts below 100 cells/ml measured after at least 6 months of effective

ART. The measurement should be done at a time when the patient is free from concomitant infection, which may lower CD4 counts (WHO 2010; WHO 2013). In children younger than five years, immunological failure is defined as CD4 count less than 200 cells/ml or less than 10%. For children above five years of age, immunological failure refers to persistently low CD4 count below 100 cells/ml after six months of ART with good adherence.

Different studies have used various definitions to demonstrate immunological failure. In Nairobi, Kenya, (Lihana, R.W., Lwembe, R., Bi, X., Ochieng, W., Panikulam, A., Palakudy, T.,…& Ichimura, H., 2011) used CD4 count of less than 200 cells/ml or less than 10% in children aged between 2 and 5 years and CD4 count less than 100 cell/ml in children above 5 years after six months of effective ART. In this study CD4 count increased from 350 cell per ml to about 700 cells per ml after 12 months of ART (Sabate, E., 2003). In a study carried out in Western Kenya, (Arnedo, M., et al. 2012) defined clinical failure as CD4 count less than 100 cells/ml or fifty percent decrease from the peak levels ever achieved or any decrease from baseline CD4 count, following 12 months of effective ART. In this study, he found a clinical failure rate of 18.2%.

2.2.2.3 Virological Failure

Various studies have used different levels of viral RNA copies/ml of blood to define virological failure, ranging from forty RNA copies per ml to as high as 5000 copies/ml (Chohan, B. H., et al. 2013; Coetzer, M., et al. 2013; Mulu, A., et al 2014). The World Health Organization has been progressively reducing the levels of viral RNA copies that define virological failure. In 2006, the WHO recommended the use of 10,000 copies/ml and above as virological failure. This was lowered to 5,000 copies/ml and above in the 2010 WHO guidelines to define virological failure. However, in its 2013 guidelines, the WHO

recommends the use of 1,000 copies/ml or more as virological failure following at least six months of effective ART.

Chohan, B.H., et al. (2013) used viral load of greater than 1500 copies/ml as virological failure following at least 24 weeks of effective antiretroviral therapy and found a virological failure rate of 13.6%. Kantor, R., et al, (2009), defined virological failure as viral load greater than 400 copies/ml after six months on ART. In that study, virological failure was 24.1% was found.

In this study, virological failure was defined based on the WHO 2013 guidelines. These guidelines define virological failure as plasma viral load above 1000 copies/ml based on two consecutive viral load measurements after three months of adherence support, with the first measurement being taken at least six months after initiation of ART (WHO, 2013).

2.3 Virological suppression

World Health Organization came up with eight HIV drug resistance early warning indicators in 2012, following a study carried out in 50 countries. The early warning indicator number 8 defined virological suppression of at least 70% of children initiated on first-line antiretroviral therapy as acceptable, if viral load of less than 1000 copies per ml was obtained following ART treatment. The other early warning indicators include drug prescription practices, adherence, retention on first-line ART, on-time pill pick up, on-time clinic appointments, drug supply continuity and percentage of patients lost to follow up (Bennett, D. E., Jordan, M. R., Bertagnolio, S., Hong, S. Y., Ravasi, G., McMahon, J. H., ... & Kelley, F., 2012).

In a comparative study that looked at the virological response among children initiated on ART in the Netherlands and Sub-saharan Africa, it was found that the country of origin did

not affect the response to treatment whereby 96% and 94% of children initiated on antiretroviral therapy achieved virological suppression in Netherlands and Sub-Saharan Africa, respectively (Cohen, S., Van Bilsen, W. P., Smit, C., Fraaij, P. L., Warris, A., Kuijpers, T. W., ... & Pajkrt, D., 2015)). In a study carried out among adults and children in Ethiopia assessing both immunological and virological response to ART, (Mulu, A., et al. 2014) found that virological suppression of 82% and 87% was achieved among adults and children, respectively.

In KwaZulu-Natal, South Africa, (Reddi, A., et al. 2007) retrospectively studied clinical, immunological and virological response to ART and found a virological suppression of 84% among children following six months of effective ART. Wamalwa D.C et al (2007) in a study done in Nairobi, Kenya, among children aged between 18 months and 12 years, a virological suppression of 67% was achieved when a viral load cut off of 400 copies per ml was used.

Therefore, different studies have demonstrated varying levels of virological response to ART, with majority of the studies showing good response to ART among children.

2.4 Advantages of using Virological Monitoring

Prior to 2012, the WHO recommended the use of clinical and immunological criteria for routine monitoring of treatment response to ART, with viral load monitoring being reserved for targeted monitoring (WHO, 2010). However, the sensitivity and predictive values of both clinical and immunological criteria in the detection of treatment failure are lower compared to virological monitoring (Chaiwarith, R., 2007).

Consequently, the WHO through its consolidated guidelines in the treatment of HIV/AIDS in 2013, strongly recommended the routine use of viral load testing to monitor treatment response to ART. This was due to the high sensitivity of virological monitoring in detection of treatment failure (WHO, 2013).

Various studies have been done to demonstrate the importance of virological monitoring. In a study done in Thailand, it was found that the sensitivity of clinical and immunological criteria was 10% and 13.3% respectively, with the combined sensitivity of the two criteria being only 20%. This implies that a large number of patients with treatment failure would be missed if the clinical and immunological criteria would be relied upon (Chaiwarith, R., 2007).

In Cambodia, a study carried out to assess use of the 2010 WHO clinical and immunological criteria showed that up to 40% of children were misclassified as having treatment failure. In this study, as high as 98% of children with extensive reverse transcriptase resistance were missed by the clinical and immunological criteria (Westley, B. P., et al. 2012).

In a multicenter study in South Africa, the use of targeted viral load monitoring was shown to increase the positive predictive value of clinical and immunological criteria from 28% using the 2010 WHO guidelines to 82%. Many children who had been diagnosed to have virological failure based on immunological criteria in fact turned out as having suppressed viral load below the 2010 WHO definition of virological failure, which was greater than 5000 copies per milliliter (Davies, M.A., Boulle, A., Technau, K., Eley, B., Moultrie, H., Rabie, H., ...& Keiser, O., 2012).

In Tanzania, a study done to compare the sensitivities of clinical and immunological criteria in the detection of treatment failure found a very low sensitivity of clinical and immunological criteria of 3.5%. In this study, a very high sensitivity of viral load testing approaching 100% was demonstrated. This study clearly shows that relying on clinical and immunological criteria alone has the potential to delay the diagnosis of treatment failure which is associated with the development drug resistance (Emmet, S. D., Cunningham, C.

K., Mmbaga, B. T., Kinabo, G. D., Schimana, W., Swai, M. E., ... & Reddy, E. A., 2010). 2010).

Another study in Tanzania assessing the sensitivity and predictive values of virological monitoring and clinical and immunological criteria in detection of treatment failure showed that the sensitivities of clinical and immunological criteria were 12.9% and 22.6%, respectively. In this study, the true rate of virological failure was 57.1%, but the use of clinical criteria identified only 11.5% while immunological criteria identified 25% of the cases (Mgelea, M., Kisenge, R., & Aboud, S., 2014). In Uganda, the correlation between clinical and immunological criteria and viral load testing was studied and found that 12.5% of children had clinical and immunological failure. However, 44.2% and 26.9% had viral loads between 1000 and 5000 copies per ml and above 5000 copies per ml respectively (Costenaro, P., et al. 2014). This further shows the magnitude of children with virological failure who would have been missed if viral load testing was not done.

Viral load monitoring has also been shown to lower the rate of misclassification of treatment failure locally. In a study done in Western Kenya among adults, it was shown that up to 58% of patients who had been diagnosed as having immunological failure due to greater than 25% CD4 count decrease were misclassified. When using CD4 count drop by greater than 50%, misclassification rate stood at 45% (Kantor, R., et al. 2009). Such misclassification is associated by premature switch of ART to second-line or even third-line regimens which are more expensive and are associated with increased toxicity.

According to a multicenter study done in Africa, relying on clinical and immunological criteria alone led to unnecessary switch to second-line ART regimen in about 46.9%

(Sigaloff, K. C., et al. 2011). Delayed diagnosis of virological failure has the effect of accumulation of resistance associated mutations (RAM). Virological failure usually predates both clinical and immunological failure such that by the time treatment failure is detected using clinical and immunological criteria, patients will have already accumulated drug resistance mutations (Rawizza, H. E., Chaplin, B., Meloni, S. T., Eisen, G., Rao, T., Sankalé, J. L., ...& Kanki, P. J., 2011)). In a Cohort-based modeling study in South Africa, it was demonstrated that there was a marked reduction in the time spend on failing ART regimen from about 27 months with CD4 monitoring to about six months with viral load monitoring. This study also showed that only one percent of children being monitored using CD4 were switched to second-line ART compared with 12% of children monitored using viral load who were switched to second-line ART (Salazar-Vizcaya, L., et al. 2014).

In one study carried out in Cambodia, (Coetzer,M., et al. 2013) found that up to 98% of children had failed to be predicted as failing on ART by the clinical and immunological criteria. Of these, 98% had varied patterns of drug resistance. Mutations to nucleoside reverse transcriptase inhibitors (NRTI) included M184V in 88% of cases, while mutations to non-nucleoside reverse transcriptase (NNRTI) included Y181C in 65% of cases. Resistance mutations to both NRTI and NNRTI stood at 51%, and multidrug resistance mutations were detected in about 21% of patients.

One study done in West Africa in 2013 showed that 16.7% of Ghanaian children initiated on first-line ART developed virological failure. About 67% of these children already had various drug resistance mutations at the time of diagnosis of virological failure with the M184V and K1O3N being the predominant resistance types (Barry, O., 2013).

In Kenya, a study done in Busia County equally showed the existence of resistance associated mutations at the time of diagnosis of treatment failure. In this study, 33% of patients who were diagnosed as having treatment failure had accumulated RAM, 81% having thymidine analogue mutations (TAM). Similarly, another study done in Nairobi, Kenya, showed that up to 29.3% of children on first-line ART had virological failure. Of these, approximately 95% of children had drug resistance mutations (Davies, M. A., et al. 2012; Lihana, R. W., et al. 2011).

2.5 Determinants of virological response to antiretroviral therapy

2.5.1 Factors that Facilitate Virological Suppression

2.5.1.1 Adherence

Virological failure has been associated with several factors such as adherence, type of ART regimen, gender and age among others. Adherence refers to the extent to which a person's behavior corresponds with the agreed recommendations from healthcare provider (Sabate, E., 2003). The WHO classifies adherence into three categories. Good adherence is above 95% or when the total number of doses missed per month is three or less. Fair adherence is between 85% and 94% or when the number of doses missed per month is between four and eight, while poor adherence is less than 85% with the total number of doses missed per month being greater than or equal to nine. Good adherence to ART is fundamental for the best outcome in the treatment of HIV/AIDS.

Some of the factors that hamper good adherence include but not limited to lack of suitable pediatric formulations, poor palatability, high pill burden or liquid volume, frequent dosing requirements, dietary restrictions and side effect profile. Poor adherence diminishes immediate and long-term benefits to the patient and also a significant public health threat of

the development of multidrug-resistance (Gordillo, V., del Amo, J., Soriano, V., & Gonzalez-Lahoz, J., 1999; Erwin, J., 1998; Imrie, A., Beveridge, A., Genn, W., Vizzard, J., & Cooper, D., 1997).

Measurement of adherence to treatment is very challenging in children. Generally, both quantitative and qualitative methods are employed to assess adherence to treatment. Quantitative methods such as pill counts are widely used because they require less time to carry out and are simple for both care providers and patients. However, the responses may not reflect true adherence as both children and guardians or parents learn to report total adherence for social desirability. Qualitative methods, though complex and time consuming, have been shown to effectively measure adherence to medication by identifying impediments to taking medication and this helps the healthcare provider in generating solutions to such impediments (Gilks, C., & Victoria, M., 2006).

In a study done to assess the effect of different levels of adherence on clinical, immunological and virological outcomes, it was found that good adherence above 95% was associated with a 22% virological failure compared with poor adherence of less than 80% which was associated with virological failure as high as 80% (Paterson, D. L., Swindells, S., Mohr, J., Brester, M., Vergis, E. N., Squier, C., ...& Singh, N., 2000). In Tanzania, it was found that virological failure of 32% occurred in patients with incomplete adherence and that disclosure of one's HIV status was protective against treatment failure (Ramadhani, H. O., Thielman, N. M., Landman, K. Z., Ndosi, E. M., Gao, F., Kirchherr, J. L., ... & Crump, J. A., 2007).

A multicenter study in Brazil assessing the association between virological suppression and adherence, showed that a relatively higher proportion of children, 57%, compared with

adolescents, 49%, achieved virological suppression. In this study, the proportion of children who reported missing no doses of ART was 93% compared with adolescents at 77% (Cruz, M. L., Cardoso, C. A., Darmont, M. Q., Souza, E., Andrade, S. D., D'AlFabbro, M. M., ... &Bastos, F. I., 2014).

According to Jobanputra, K., et al (2015) in a study done in Swaziland, adherence counseling can negate the need to switch ART regimen from either first-line to second-line or second-line to third-line. In this study assessing the impact and implications of routine viral load measurement, 60% of patients with initial detectable viral load achieved virological suppression with intensification of adherence counseling and did not require switching to second-line ART regimen.

In San Francisco, the adherence-resistance relationships for NNRTIs and protease inhibitors were assessed and showed that at both good and poor adherence levels, the risk of developing resistance was higher in the NNRTIs than in PIs. At 0-48% adherence, about 69% resistance was seen in the NNRTIs group compared with 23% in the protease inhibitors group (Bangsberg, D. R., Acosta, E. P., Gupta, R., Guzman, D., Riley, E. D., Harrigan, P. R., ... &Deeks, S. G., 2006).

2.5.1.2 Type of Antiretroviral therapy

The current WHO guidelines recommend the use of two reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor or two reverse transcriptase inhibitors and one protease inhibitor, depending on certain factors such as previous exposures to certain drugs, co-infection with other diseases and age. The preferred regimen is abacavir (ABC) or zidovudine (AZT), lamivudine (3TC) and boosted lopinavir (LPV/r). The alternative regimen

recommended is AZT or ABC, 3TC and nevirapine (NVP) (WHO, 2013). The Ministry of Health in Kenya has adopted the WHO recommendations and therefore these are the regimens used in the AMPATH clinics.

Virological failure has also been touted to have association with the type of ART regimen that the child is initiated on. Several studies have been done and have shown mixed results for different regimens. One study carried out in Botswana showed that patients initiated on non-nucleoside reverse transcriptase inhibitors (NNRTI) have different responses depending on the type of drug. In this study, 13.5% of children on efavirenz-based regimen never achieved virological suppression compared with 26.4% of children initiated on nevirapine-based regimen. The average time to virological failure was shorter for nevirapine-based therapy compared with efavirenz-based regimen (Lowenthal, E. D., Ellenberg, J. H., Machine, E., Sagdeo, A., Boiditswe, S., Steenhoff, A. P., ...& Gross, R., 2013).

Similar findings were observed in a separate study done in the UK and Ireland, where Duong et al, found that the progression to virological failure was faster for nevirapine (NVP) and two NRTI compared with efavirenz (EFV) and two NNRTI. For children who received protease boosted regimens, there was a relatively lower rate of virological failure compared to the NVP-based group, although with higher side effect profile. In children who received three NRTI and one NNRTI, progression to treatment failure was lowest (Kamya, M. R., et al. 2007).

In Thailand however, one study found that there was no much difference in efficacy between NVP-based and EFV-based ART regimens. In the study, both ART groups gave similar increases in CD4 counts among children. The only difference noted from the study was that

ART-naïve children exhibited better CD4 response compared with the ART-experienced group (Lapphra, K., Vanprapar, N., Chearskul, S., Phongsamart, W., Chearskul, P., Prasitsuebsai, W., &Chokephaibulkit, K., 2008). In a separate multicenter study, Hartmann et al, similarly found no major differences in virological outcomes between NVP-containing and EFV-containing regimens. The proportions of virological suppression were 78% and 79% in the EFV-group and NVP-group, respectively (Hartmann, M., Witte, S., Brust, J., Schuster, D., Mosthaf, F., Procaccianti, M., ...& Petzoldt, D., 2005). In a separate study done in Uganda, looking at both adults and children, it was found that the risk of virological failure was higher in patients initiated on stavudine, lamivudine and nevirapine combination compared with patients initiated on zidovudine, lamivudine and efavirenz combination (Kamya, M. R., et al. 2007).

2.5.1.3 Gender

Gender has also been touted to have an influence in the rate of viral load suppression. In a study done among HIV-infected children in South Africa, Shiau, S., et al. (2014) demonstrated that girls who switched to NVP from ritonavir-boosted lopinavir showed marked improvement in CD4 count compared with boys. However, the risk of virological failure had no gender predilection. The boys and girls who achieved viral load suppression were 78.9% and 76.9% respectively. Similarly, a longitudinal observational multicenter study carried out in Italy showed that virological failure had minimal association with gender. It was also shown that females experienced lower clinical progression compared with males (Nicastri, E., Angeletti, C., Palmisano, L., Sarmati, L., Chiesi, A., Geraci, A., ...&Vella, S., 2005).

A large multicenter study in Africa involving children in South Africa and Uganda showed that girls had a significantly lower HIV viral load compared to boys, especially in older children. However, the study showed that boys had a significantly lower CD4 percentages compared with girls, that is 15% and 18% respectively (Ruel, T. D., Zanoni, B. C., Ssewanyana, I., Cao, H., Havlir, D. V., Kamya, M., ... & Feeney, M. E., 2011). In a separate study among adults, similar results were found with women having higher CD4 count and lower viral load than men in ART naïve patients. On the other hand, ART experienced women and men had similar virological responses, though women had relatively higher CD4 cell counts (Collazos, J., Asensi, V., Cartón, J. A., &GrupoEspañolpara el Estudio Multifactorial de la Adherencia (GEEMA., 2007).

These sex differences have been linked to various genetic factors and it has been postulated that hormonal influence is important in mediating these effects. It has been demonstrated that the increased inflammatory response mediated by the HIV-1 encoded Toll-like receptor (TLR) ligands in the production of interferon-α (IFN-α) is more marked in women than men, adjusted for similar viral load. A recent study has shown similar results by demonstrating expression of higher levels of interferon-stimulated genes in females than males. These may in part explain the more rapid disease progression and the potent immune activation in HIV-1-infected females compared with males for corresponding viral load levels (Meier, A., Chang, J. J., Chan, E. S., Pollard, R. B., Sidhu, H. K., Kulkarni, S., ... & Altfeld, M., 2009; Chang, J. J., Woods, M., Lindsay, R. J., Doyle, E. H., Griesbeck, M., Chan, E. S., ...&Altfeld, M., 2013).

2.5.1.4 Age at Initiation of ART

Infants usually have very high viral loads compared with older children. This has been attributed to the immature immune system in the infants which is less effective in mounting a strong immunological suppression of HIV replication. Various studies have been done to establish the most appropriate time to initiate ART in children so as to achieve optimal virological response. A multicenter study demonstrated that early initiation of ART among infants was well tolerated and was associated with good clinical outcomes. However, there was a high rate of virological failure and development of drug resistance. However, there was poorer virological response in younger children, with a higher proportion of older children achieving virological suppression at six months of ART (Walker, A. S., Doerholt, K., Sharland, M., & Gibb, D. M., 2004).

In a separate multicenter study, it was demonstrated that early initiation of ART in children is associated with better and faster virological suppression compared with deferred treatment. In this study, more than 73% of infants who were started on ART earlier had virological suppression to undetectable levels, compared with only 30.1% of infants who were initiated on ART later. This study also showed that there was better immunological response among infants with earlier initiation of ART compared with deferred treatment group, with no infant among the early treatment group having CD4 percentage less than 15% (Chiappini, E., Galli, L., Tovo, P. A., Gabiano, C., Gattinara, G. C., Guarino, A., ...& Italian Register for HIV Infection in Children., 2006).

Faye, A., et al. (2009), also found that children initiated on ART earlier had better clinical and immunological outcome compared with delayed initiation of treatment. In this study, it was shown that infants initiated on ART before six months of age never developed new or worse opportunistic infections compared with those infants initiated on ART after six

months, who developed various opportunistic infections including encephalopathy. Similarly, in a study done to assess the effect of early initiation of antiretroviral therapy on the risk of AIDS or death, it was demonstrated that the risk of developing AIDS or dying from HIV/AIDS-related complications was higher at 11.75% in children where ART initiation was delayed compared with those in whom ART initiation was started before three months of age (Goetghebuer, T., Haelterman, E., Le Chenadec, J., Dollfus, C., Gibb, D., Judd, A., ...& Levy, J., 2009).

2.5.2 Factors that hinder virological suppression

2.5.2.1 Nutritional Status

Severe acute malnutrition (SAM) is defined as weight-for-height z-score of less than -3 or mid-upper arm circumference less than 11.5 cm in children aged 6 months to 5 years (Musoke, P. M., & Fergusson, P., 2011). Malnutrition affects over one-quarter of children below 5 years in the developing world and is postulated to contribute to one-third of deaths in this age group, estimated at one to two million deaths per year. In a meta-analysis study, Fergusson P, and Tomkins, A., (2009) found that HIV prevalence was high in children with SAM in Sub-Saharan Africa, with 29.2% of children with SAM being HIV positive. It was also shown that HIV positive children with malnutrition were more likely to die than HIV negative children, 30.4% versus 8.4%.

In a study done in South Africa, it was found that weight-to-height z-scores improved markedly upon initiation of ART in the moderately and severely malnourished children compared with the normal group. However, viral loads decreased significantly in all groups (Naidoo, R., Rennert, W., Lung, A., Naidoo, K., & McKerrow, N., 2010). In a separate study in the Democratic Republic of Congo, it was demonstrated that severe malnutrition is one of the predictors of mortality in HIV-infected children (Callens, S. F., Shabani, N., Lusiama, J., Lelo, P., Kitetele, F., Colebunders, R., ...&Behets, F., 2009).

2.5.2.2 Orphanhood

Children rely on their parents or guardians for support in HIV care. Approximately 17.8 million children worldwide, 85% of whom live in sub-Saharan Africa, have lost one or both parents. Many others are living with parents who do not know their HIV status, are chronically ill with HIV/AIDS or are on lifelong treatment (UNAIDS, 2013).

Orphanhood is associated with multiple problems that adversely affect the quality of care that children receive. Children are forced to change their living arrangements. Studies have shown that about 90% of orphans live within extended families and approximately 10% live with unrelated caregivers. The closeness between the orphan and the head of the household matters very much, with better adherence to medication being seen among children who are closely related to the household head (Sherr, L., Cluver, L. D., Betancourt, T. S., Kellerman, S. E., Richter, L. M., & Desmond, C., 2014; Chuong, C., & Operario, D., 2012).

In a retrospective study in South Africa, (Cluver, L. D., Orkin, M., Boyes, M. E., Gardner, F., & Nikelo, J., 2012), showed that orphanhood predicted increased risk of depression, anxiety and post-traumatic stress. Children who are simultaneously affected by caregiver AIDS and AIDS-orphanhood had cumulative effect for poor mental health outcomes.

2.5.2.3 Opportunistic infections

Opportunistic infections (OIs) are still a major challenge in the management of HIV/AIDS especially in third world countries. Alarcon, J.O., et al. (2012) demonstrated that as much as 78% of HIV-infected children in Latin America had at least one opportunistic infection prior to enrolment into care. The prevalence of opportunistic infections has been steadily decreasing in the Western industrialized countries, with survival increasing from 29% in 1994-1996 to about 78% in 2007.

In a study done by Ekwaru, J. P., et al. (2013), it was demonstrated that patients who had suffered from opportunistic infections had a poor outcome following initiation of ART. In this study, patients who had any opportunistic infection in the prior three months had a higher risk of poor virological suppression compared with controls. Similarly, CD4 count dropped by 24.1 cells per microliter in patients who had had any opportunistic infection in the prior three months compared with an increase by 21.3 cells per microliter in patients without OIs. In a cohort study comparing clinical outcome in patients initiated on ART earlier versus later after diagnosis of tuberculosis and initiation of anti-TB treatment, it was found that earlier commencement of ART had better outcome than later initiation. It was found that more than 27% of patients belonging to the delayed group died compared with about 17% of those who belonged to the earlier group (Blanc, F. X., Sok, T., Laureillard, D., Borand, L., Rekacewicz, C., Nerrienet, E., ...& Goldfeld, A. E., 2011).

In summary, this study intents to determine the proportion of children who achieve virological suppression following ART treatment and to establish the associated determinants.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

A retrospective chart review of routinely collected data was used in this study. This study design was chosen due to limited time for the study considering that the turn-around-time (TAT) for viral results was approximately one month. Therefore, adopting a prospective approach may have led to failure of attainment of the desired sample size by the end of the study period.

3.2 Study Site

The study was carried out at the Academic Model Providing Access To Healthcare (AMPATH) clinics, whose main clinic is situated within the Moi Teaching and Referral Hospital, Eldoret. AMPATH is a partnership between the Moi University School College of Health Sciences, Moi Teaching and Referral Hospital and a consortium of United States medical schools led by Indiana University Medical School. Founded in 2001, AMPATH is among the largest and most comprehensive HIV/AIDS –care programs in Sub-Saharan Africa. It offers services in collaboration with the Ministry of Health in Kenya. It has thirty-five clinics and twenty-six satellite clinics which enhance easy accessibility to care by the clients. The twenty-six satellite clinics were designed to help in bringing services closer to the clients. Each of these satellite clinics in linked to a particular main clinic. For instance Busia Prison satellite clinic is linked to Busia County Hospital, Riat Dispensary is linked to Chulaimbo Sub-County Hospital while Moi University satellite clinic is linked to the Moi Teaching and Referral Hospital modules, among others. The catchment area includes eight

counties namely Uasin Gishu, Nandi, Bungoma, Busia, Kisumu, Baringo, Trans Nzoia and Elgeyo Marakwet.

The Moi Teaching and Referral Hospital is the second national referral hospital. It is located in Eldoret town, in Uasin Gishu County approximately 350 kilometers from Nairobi. It has a bed capacity of 800 and serves the western Kenya, Uganda, South Sudan, Rwanda and Burundi regions with a catchment population of over 13 million people.

Western Kenya is largely an agricultural region with both large and small-scale farming. Various crops cultivated in this region include maize, beans, tea, sugarcane, wheat, cassava, finger millet, sweet potatoes, bananas, tomatoes, tea and sorghum. Maize, sugarcane and wheat are generally grown in large scale and are the main cash crops. Livestock farming is equally an important source of income for the people in this region.

This study site was chosen due to its large catchment area and population which provided adequate study population and it offers comprehensive care to both HIV-positive and sero-exposed children. There are approximately 15,105 pediatric clients below 15 years of age attending AMPATH clinics in Western Kenya, as per the March 2015 report. Of these, 10,685 children were on antiretroviral therapy. The main AMPATH clinic situated within MTRH has four modules. Modules one, two and three are used for adult HIV care, while module four is used for pediatric HIV care. Adolescents are usually seen every Friday of the week while the rest of the children are seen from Monday to Thursday. The clinics are run by different clinicians including clinical officers, medical officers and consultants. In the case of satellite clinics, the clinicians attending to the clients usually refer cases that require

specialist attention such as change of ART regimen and management of certain opportunistic infections to the main clinics.

Several services are offered in these comprehensive clinics. These include counseling and testing for HIV, laboratory investigations prior to and during treatment, adherence monitoring and counseling, administration of ART, diagnosis and treatment of opportunistic infections, nutritional services and drug resistance testing, among others. HIV testing is offered free of charge, as is administration of ART. Some of the protocols used in these clinics include both WHO guidelines and NASCOP guidelines on management of HIV/AIDS, with special emphasis on the public health approach.[9,14,16,]

3.3 Study Population

3.3.1 Target population

The target population included all HIV-infected children attending AMPATH clinics in Western Kenya.

3.3.2 Study population

This included HIV-infected children on first-line antiretroviral therapy attending AMPATH clinics in Western Kenya.

3.4 Selection Criteria

3.4.1 Inclusion criteria

1. The children should have been on ART for six to twenty-four months. This period covered the duration from adoption of routine virological monitoring by the government of Kenya and end of the study in July 2016.
2. They should have been on first-line ART regimen and aged less than 15 years.

3.4.2 Exclusion criteria

1. Confirmed resistance to any of the antiretroviral drugs.

3.5 Study Period

The study was conducted over a period of twenty six months between June 2014 and July 2016.

3.6 Sample Size Determination

For this retrospective study, sample size was computed using the Fischer's formula. The proportion of virological suppression among children initiated on first-line ART ranges between 65% and 84% in various studies globally. According to a study done by Lihana R.W. et al. (2011) in Nairobi, Kenya, the proportion of children who achieved virological suppression was 70.7%. This informed the sample size calculation because the population studied has similar characteristics to the one being studied here.

Sample size was computed using the Cochran (Cochran, 1963:75) formula.

$$n = \frac{Z^2_{\alpha/2} \times P(1-P)}{d^2}$$

Where;

$n =$ Anticipated sample size to be considered for the study

$Z_{\alpha/2} = 1.96$, standard normal variate

$p =$ Estimated proportion of viral load suppression in MTRH (70.7%)

$d =$ Margin of error (0.05)

Calculating sample size yields the following:

Sample size $n = \frac{1.96^2 \times 0.707 \times 0.293}{0.05^2} = 319$

Therefore, a sample size of 319 subjects was needed for the study to give a precision of 0.05 or less for viral load suppression.

Adjusting for 10% missing data yields

$$n = \frac{319}{0.90} = 354, subjects$$

Therefore a sample size of 354 subjects was recruited for this study.

3.7 Sampling Procedure

Convenient sampling was used to select three centers from the thirty-five main clinics that serve about twenty-six satellite clinics. This was based on the large number of children attending the clinics. The three centers were module four clinic in the Moi Teaching and Referral Hospital in Eldoret, Kitale county hospital and Busia county hospital. The number of subjects selected per clinic was proportionately calculated based on the number of children at each clinic. The total number of children in the three clinics was 680, with Kitale County hospital having the highest number (276), followed by Module four at the Moi Teaching and Referral Hospital (232) and Busia County Hospital (171). Therefore, the number of children recruited at each center was Kitale County Hospital (144), Module Four at MTRH (121) and 89 children at Busia County Hospital.

The AMPATH electronic medical record system was used to identify children who had been seen in the AMPATH clinic during the period under study. The medical files of the selected patients were then manually retrieved from the records department and subjected to a further filtering process to select only those that met the inclusion criteria.

Those meeting the inclusion criteria were then consecutively selected until the appropriate sample size was attained.

3.8 Data Collection

The study was conducted by the principal investigator and one research assistant that was hired based on his experience and skills. The research assistant was a qualified clinical officer with experience in the management of HIV/AIDS patients. The principal investigator trained the research assistant on the nature of the study and the appropriate data collection methods. The study was overseen by two supervisors from the Department of Child Health and Pediatrics who were available to guide the execution of the entire study. Sensitization was done for the in-charges of the AMPATH clinics and health information officers concerning the objectives and importance of the study. The principal investigator and the research assistant collected requisite data from the module four clinic located at MTRH, Kitale and Busia clinics, using a pretested data collection tool (appendix 1). The variables of interest included viral load, nutritional status at the time of initiation of ART, age, gender, ART regimen, adherence to ART and WHO staging.

The de-identified data collected were cross-checked by the principal investigator for its completeness and accuracy. The identity of the study participants was protected using unique numbers.

3.9 Data Entry and Analysis

Data were entered in Microsoft Access and exported to STATA version 13 for analysis. The data was backed in an external hard drive to safeguard against data loss and was stored in password-protected folders to safeguard against unauthorized access.

Descriptive statistics such as frequency listings were used for categorical variables while measures of central tendency and dispersion were used to describe numerical variables Cross tabulations and chi square test were used to test associations. In cases where the expected cell counts were below 5 the Fisher's exact test was used. Logistic regression was used to test for independent associations. Kaplan Meier survival curve was used to demonstrate time to virological suppression from ART initiation. Cox proportional hazard model was used to assess factors associated with viral suppression. All analysis was carried out at 95% level of significance, with ***p*** values less than 0.05 considered statistically significant.

3.10 Ethical Considerations

Ethical approval was sought from the Moi University's Institutional Research and Ethics Committee (IREC). Approval letter was then presented to the Moi Teaching and Referral Hospital and AMPATH management to obtain permission to execute the study.

Information from the participants, including raw data was saved in a password-protected folder. Confidentiality was maintained by the use of unique numbers and no identity of the participant was mentioned anywhere during the study.

Information obtained was only shared with the relevant authorities, and suitable disposal will be done once the stipulated time duration expires.

CHAPTER FOUR

RESULTS

4.1 Population Description:

A total of 371 children participated in the study with males being 194(52.50%). Majority of the children 244 (66.70%) lived with their mothers who were responsible for administration of medication to the children. Most of the children (65.40%) had both parents alive with only 10.80% being total orphans. The median age at ART initiation was 84 months (IQR = 36-123).

Majority of the children in our study were residing in Trans Nzoia county (figure 1)

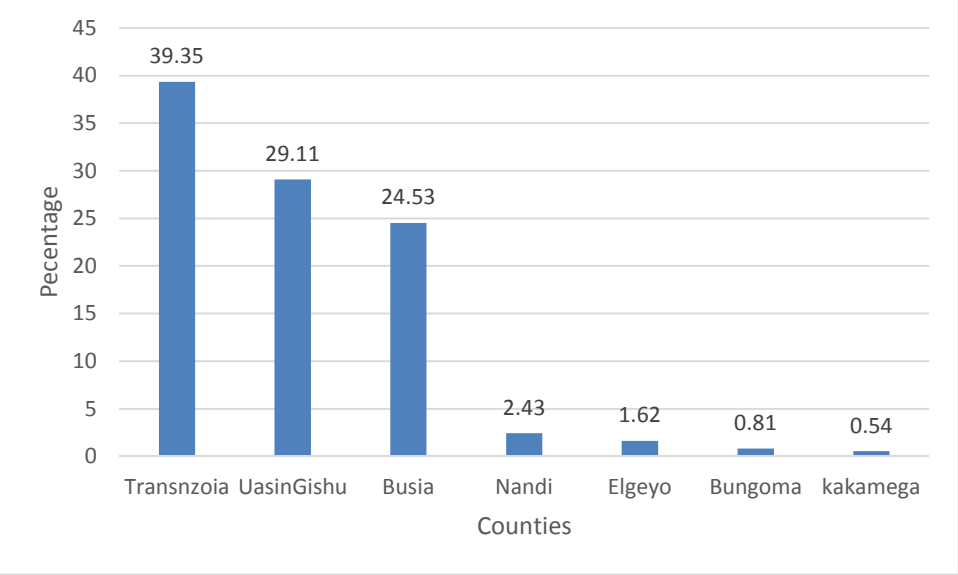

Figure 1: County of Origin

Very few children 47 (12.70%) were on nevirapine for prevention of mother to child transmission of HIV. Most of the children were reported to have had good adherence to ART at 98.9%. Majority of the children 367 (99.50%) were on cotrimoxazole for prophylaxis against opportunistic infections. Majority of the children had no opportunistic infections 354

(96%). For those who had opportunistic infections, the reported ones included Tuberculosis (5/368), upper respiratory infections (3/368) and Herpes zoster (2/368).

On nutritional status, marasmus and underweight were the common forms of malnutrition, with none of them having kwashiorkor nor marasmic-kwashiorkor(Table 1).

Table 1: Clinical Characteristics of participants

Characteristics	Frequency (%)	
Nutritional status*		
Overweight	37	10.00
Normal	232	62.90
Underweight	84	22.80
marasmus	37	4.30
WHO HIV staging		
1	183	49.30
2	91	24.50
3	87	23.50
4	10	2.70
Duration on ART		
6- <12 months	269	73.30
12- <18 months	82	22.30
18-24 months	16	4.40

*Welcome trust classification

4.2 Proportion of HIV-infected children on ART who achieved Virological Suppression

This study found that after at least six months of ART, 78.44% (95%CI: 73.90, 82.51) of the children initiated on first-line ART had achieved virological suppression.

The median time to virological suppression was 10.84 months (95%CI: 9.72, 11.93) (figure 1)

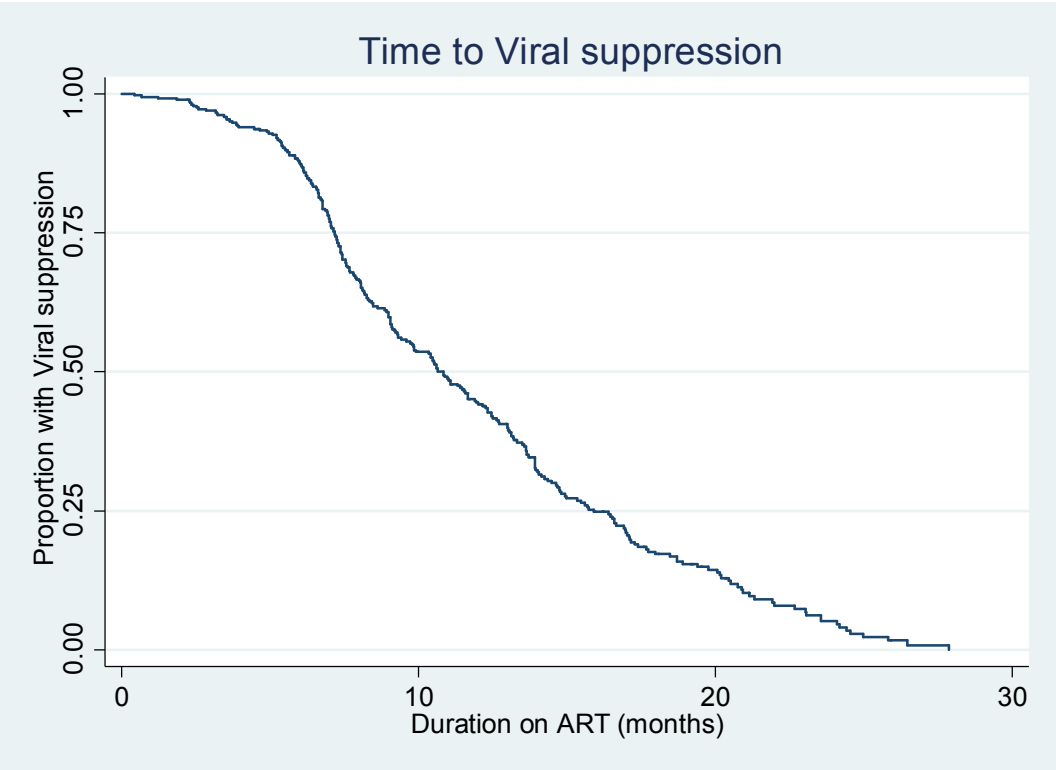

Figure 2: Time to virological suppression

4.3 Factors associated with virological suppression:

On bivariate analysis of the clinical and socio-demographic characteristics of the population studied, we found that only age at ART initiation was significantly associated with virological suppression. The younger age group below 60 months was more likely to achieve virological suppression compared with older children (Table 3).

4.3.1 The socio-demographic factors associated with Viral Load Suppression

The socio-demographic factors analysed include gender, age at ART initiation and orphan status. Of these, only age at the initiation of antiretroviral therapy was significantly associated with virological suppression. The younger age at ART initiation was associated with better virological suppression than older age.(table 2)

Table 2: Socio-demographic factors associated with virological suppression

Variable	**Viral suppression achieved**		
	No n(%)	**Yes n(%)**	***p*-value**
Gender			
Female	40 (23)	134 (77)	
Male	40 (20.9)	151 (79.1)	0.637
Orphan Status			
Non-orphan	7 (17.5)	33 (82.5)	
Double orphan	18 (20.5)	70 (79.5)	
Single orphan	55 (22.8)	186 (77.3)	0.714
Age in months at start of ART			
<60	25 (17.4)	119 (82.6)	
60 – 120	25 (19.4)	104 (80.6)	
>120	30 (30.6)	68 (69.4)	0.037

4.3.2 Clinical Factors Associated with Viral Load Suppression.

There was no clinical factor among the ones analyzed that was significantly associated with virological suppression (Table 3).

Table 3: Clinical Factors Associated with Viral load Suppression

Variable	No	Yes	*p*-value
Duration on ART			
6 to 12	53 (19.7)	216 (80.3)	0.089
12 to 18	23 (28)	59 (72)	
18 to 24	1 (6.3)	15 (93.8)	
Nutritional status			
Normal	50 (21.6)	182 (788.4)	0.77
Marasmus	5 (31.3)	11 (68.8)	
Overweight	7 (18.9)	30 (81.1)	
Underweight	17 (20.2)	67 (79.8)	
WHO HIV staging			
1	40 (21.9)	143 (78.1)	
2	20 (22)	71 (78)	
3	18 (20.7)	69 (79.3)	
4	2 (20)	8 (80)	0.995
PMTCT (Prevention of mother to child transmission) Status			
No[+]	70 (21.7)	252 (78.3)	0.686
Yes[*]	9 (19.1)	38 (80.9)	
ART regimen			
Efavirenz – based	49 (19.9)	197 (80.1)	0.532
Neverapine - based	26 (24.5)	80 (75.5)	
Others	5 (26.3)	14 (73.7)	

Key:

+ : Child not initiated on NVP prophylaxis

*: Child initiated on NVP prophylaxis

4.3.3 Regression Analysis for factors associated with Virological suppression

We subjected age in months, ART regimen, PMTCT, orphan status, nutritional status, WHO clinical status and duration on ART to cox regression analysis against time to virological suppression. Only age in months and duration on ART were statistically significant.There was a trend towards younger age group attaining virological suppression faster compared with older children (Table 4).

Table 4: Factors associated with time to Viral Suppression

	Hazard Ratio	**p-value**	**(95% Confidence Interval)**	
Age in months at ART initiation				
60-120 vs<60	0.929	0.645	0.680	1.270
>120 vs<60	0.679	0.028	0.480	0.960
ART regimen				
NVP vs EFV	0.954	0.739	0.722	1.260
Other vs EFV	0.832	0.54	0.462	1.498
PMTCT (Yes vs No)	0.863	0.487	0.569	1.308
Orphan status				
Both dead vs Both alive	1.191	0.397	0.794	1.787
One dead vs both alive	1.066	0.676	0.790	1.438
Malnutrition				
Overweight vs Marasmus	1.145	0.712	0.559	2.346
Underweight vs Marasmus	1.049	0.886	0.549	2.002
No malnutrition vs Marasmus	1.024	0.94	0.553	1.896
WHO staging				
WHO stage 2 vs 1	0.743	0.064	0.543	1.017
WHO stage 3 vs 1	0.759	0.079	0.558	1.032
WHO stage 4 vs 1	0.717	0.388	0.337	1.526
Duration on ART				
12 to 18 vs 6-12 months	0.297	0.001	0.218	0.406
18 to 24 vs 6-12 months	0.158	0.001	0.088	0.286

CHAPTER FIVE

DISCUSSION

5.1 Virological Suppression

In this study, we used the retrospective chart review of children over a period of twenty six months and found that slightly over three quarters of the children on ART achieved virological suppression. This is in line with the World Health Organization early warning indicator number 8 which set virologic suppression at ≥70% (Bennett, D. E., et al. 2012).

These findings are similar to those found in a South African study done in KwaZulu- Natal by Reddy, A., et al. (2007) where a virological suppression of 84% was attained after six months of antiretroviral therapy. This could be attributed to the retrospective design used which is similar to our study and the study population which was similar to ours. In a comparative study assessing virological suppression by country of birth, similarly high levels of virological suppression in both children born in Netherlands and Sub-Saharan Africa were found. In this study, which assessed both virological response as well as immunological response, 261 children were analyzed, majority of whom were of Sub-Saharan African Origin. This represents a similar population to ours (Cohen, S., et al. 2015). Several other studies found similar results ranging from 72% to 88% virological suppression (Van Dijk, J. H., et al. 2011; Kekitiinwa, A., et al. 2008; Vaz, P., et al. 2012).

Contrary to our study, Wamalwa D. C., et al. (2007) reported lower levels of virological suppression in a relatively similar population at Kenyatta National Hospital, Kenya. This population consisted of Kenyan children aged between 18 months and 12 years and like our study population they were ART naïve and were initiated on first-line ART. In this

prospective study, virological suppression at six months of ART was 67% and 47%, at viral load cut offs of <400 copies per ml and <100 copies per ml, respectively. This could be attributed to the lower viral load cut off used in this study. The smaller number of the children studied and the lower adherence levels in this study could also contribute to these findings. Additionally, a retrospective study conducted in the Rhode Island, USA, quite lower levels of virological suppression were achieved (Rogo, T., DeLong, A. K., Chan, P., & Kantor, R., 2015). This could be attributable, partially, to a different viral strain and to the smaller sample size studied.

5.2 Factors Associated with Virological Suppression

Several factors have been shown to influence the virological response to antiretroviral therapy. We analyzed a few factors to establish any association with virological response to first line antiretroviral therapy in our study.

5.2.1 Antiretroviral Therapy Regimen

In our study, there was a trend towards better response to efavirenz-based regimen compared with nevirapine-based regimen. However, there was no statistically significant difference. Lawrence M., et al. (2016), in a Cochrane review looking at both children and adults, similar results were noted. There was minimal difference in virological response to efavirenz-based and nevirapine-based regimens (RR 1.04 , 95% CI: 0.99-1.09). This could be attributed to the fact that like our study, the study population used was ART-naïve hence the lower risk of pre-existing resistance mutations.

Unlike our study, Lowenthal E.D., et al. (2013), found superior virological response to efavirenz-based ART than nevirapine-based ART. In this study done among children on first-

line antiretroviral therapy in Botswana aged three to sixteen years, 86.5% and 73% of children achieved virologic suppression for efavirenz-based and nevirapine-based ART, respectively, at six months of first-line antiretroviral therapy. This may be attributed to the larger sample size used in the study compared to ours which gave it the power to assess the association.

5.2.2 Age at Initiation of Antiretroviral Therapy

Our study revealed a better virological response to first-line ART among children initiated on ART at younger age compared with older age at ART initiation. In our study, younger children achieved better virological suppression, compared with older ones, which was statistically significant. Similar results were found in a study by Luzuriaga K., et al. (2016) which showed that early ART initiation especially during infancy, leads to rapid clearance of plasma HIV-1 RNA. Antiretroviral therapy has been shown to deter infection of new CD4 T cells while enhancing decline of infected mononuclear cells. This could also be due to the lower level of latent CD4+ T lymphocytes reservoirs in children initiated on ART early in life. Bitnum A., et al. (2014) also found similar results to ours whereby early ART reduced HIV-1 reservoir which favored sustained virological suppression.

Commencement of ART in children usually leads to improvement in both clinical and immunological parameters. Virological success determination is the most accurate method of defining response to ART (Costenaro, P., et al. 2014). Infants and young children usually have higher viral loads compared with older children and adults due to immature immune response. Additionally, the response to antiretroviral therapy varies with age due to the differences in the dynamics of HIV-1 replication (Luzuriaga, K., et al. 2016).

Contrary to our study, a different study found that delayed initiation of ART led to better virological suppression compared to early initiation. In their study, only 53% of children less than five years of age achieved virological suppression compared with 76% of those above nine years who achieved virological suppression (Walker, A. S., et al. 2004).This could be attributed to the prospective type of their study which allowed better monitoring of adherence to ART and response to treatment. In addition, confounding by indication may also play a role in their findings, whereby the older age group children were symptomatic before ART initiation. This therefore would make it easier for the patients and their parents or other caretakers to observe strict adherence to antiretroviral therapy.

5.2.3 Orphan Status

Our study showed that non-orphans had a slightly better response to ART, although this was statistically insignificant. Similar findings were found in a separate study which was also done in Western Kenya. Their study looked at the differences in treatment outcomes between orphans and non-orphans on ART in about 279 children with a median age at ART initiation of six years. There was no difference in immunological response to ART as well as adherence levels (Nyandiko, W. M., et al. 2006). This finding could be attributed to the retrospective type of the two studies, which made them lack the power to assess the association between orphanhood and response to antiretroviral therapy.

Orphans can either be partial or total if they have lost one or both parents, respectively. Orphanhood has been associated with several mental health problems such as depression, anxiety disorders and posttraumatic stress disorder, among others. These may affect adherence to antiretroviral therapy as well as other medications hence predisposing them to suboptimal care and subsequently treatment failure (Cluver, L. D., et al. 2012).

5.2.4 Gender

In our study, we found no association between gender and virological suppression in children on first-line antiretroviral therapy. These findings are similar to those observed in a South African study which looked at the sex differences in response to ART among 323 children. There was no significant difference in virological suppression between boys and girls both at six months during pre-randomization phase as well as post-randomization phase. Additionally, the viral load categories of <50 copies per ml, 50 – 1000 copies per ml and >1000 copies per ml during the post-randomization period were similar for both boys and girls (Shiau, S., et al. 2014). Similar to our study, adult studies including a meta-analysis looking at gender differences in response to ART, have found no differences in virological suppression between males and females (Nicastri, E., et al. 2005; Cornell, M., Schomaker, M., Garone, D. B., Giddy, J., Hoffmann, C. J., Lessells, R., ... & Egger, M., 2012).

In a study done among adults, it was found that women faired on well in many aspects such as increase in CD4 count and reduced mortality compared with men. This may be attributed to advanced disease stage at initiation of ART among men in this study. However, both men and women had similar virological response to ART (Cornell, M., et al. 2012).

5.2.5 Nutritional Status

In our study, children who had marasmus at the initiation of ART achieved lower virological suppression although overall, malnutrition did not significantly affect virological suppression. Similar findings were observed in a different study which looked at the effect of malnutrition on pharmacokinetics and virological outcomes among HIV-infected children in Uganda. In this study, malnutrition was shown to have effect on serum levels of the three drugs tested, namely lopinavir, efavirenz and nevirapine, although the virological outcome

was not statistically significant (Bartelink, I. H., Savic, R. M., Dorsey, G., Ruel, T., Gingrich, D., Scherpbier, H. J., ... & Plenty, A., 2015).

Unlike our study, Feldman M.B., et al. (2015) found out that food insufficiency had negative impact on HIV treatment outcomes, with increased risk of failure of virological suppression, (AOR 1.6, CI: 1.1 – 2.5). Similarly, other adult studies found that malnutrition was associated with low likelihood of achieving virological suppression (Wang, E. A., et al. 2011; Weiser, S. D., et al. 2009). This difference could be attributed to the differences in study populations since these studies, unlike our study, were done among adults. The difference could also be adduced to the fact that these studies concentrated more on food insufficiency than malnutrition per see. On the other hand, our study analyzed nutritional status of the children under study. Additionally, our study could not analyze malnutrition based on z-scores due to missing data.

5.2.6 Adherence to Antiretroviral therapy

Our study found good adherence among children on first-line antiretroviral therapy. However, approximately 21% of the children failed to achieve virological suppression after at least six months of ART despite good adherence. This may be attributed to the adherence assessment method used in our study. The self-report technique that was used in our study relies on recall, and therefore recall bias may be inevitable. Adherence to ART has been considered as one of the most significant factors associated with virological suppression. According to the World Health Organization, good adherence (≥95%) is associated with reduced levels of virological failure and intensified adherence counseling may negate the need for switching from first-line to second-line antiretroviral therapy (Paterson, D. L., et al. 2000; Jobunputra, K., et al. 2015).

A randomized prospective cohort study that employed various adherence assessment methods found that self-report technique had no association with virological suppression compared with medication return method (Teasdale, C. A., Abrams, E. J., Coovadia, A., Strehlau, R., Martens, L., & Kuhn, L., 2013). In this study, data was obtained from a pre-randomization phase of a clinical trial and compared virological response to ART at various adherence levels. Using medication returned method, adherence level below 85% was associated with increased odds lack of virological suppression (OR 2.5 (95% CI: 1.30-4.07). In another study comparing real-time electronic adherence monitoring with self-reporting (interactive voice response and short message service), it was clearly noted that electronic adherence monitoring gives better association outcomes between adherence level and virological response. This was true for both studies despite the self-reporting method giving higher levels of adherence (Haberer, J. E., Kiwanuka, J., Nansera, D., Muzoora, C., Hunt, P. W., So, J., ... & Bangsberg, D. R., 2013). This difference in findings between our study and these studies may be attributed to the more sensitive methods used in these studies. The micro-electronic adherence monitoring system is one of the most accurate methods of establishing adherence to ART whereby the medication caps transmit information to the researchers whenever the medication containers are opened for the patient to take the required dosages.

In a study comparing adherence among adolescents and adults with virological similar results were found, although adolescents had a higher risk of being non-adherent. Here, pharmacy refills method was employed to monitor treatment adherence and it was demonstrated that levels of adherence to ART closely determined virological suppression (Nachega, J.B., et al. 2009).

Study limitations

This was a retrospective chart review and therefore we could not ascertain the level of adherence as abstracted from the reviewed charts.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Our study demonstrated that the virological suppression occurred in slightly more than three-quarters of the children following six months of first-line antiretroviral therapy.

2. Younger children initiated on first-line ART were more likely to be virally suppressed compared to older children. No other factors were associated with virological suppression.

3. There was good adherence among HIV infected children on first line antiretroviral therapy.

6.2 Recommendations

We recommend earlier initiation of ART in all HIV-infected children to promote virological suppression. This should be as soon as diagnosis is made.

We also recommend that a larger prospective study should be carried out to further assess other possible determinants of virological suppression using a mixed methods approach.

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APPENDICES

Appendix I: Data Collection Tool

Title: Virologic Response to First-line Antiretroviral therapy among HIV-Infected children attending AMPATH clinics in Western Kenya

DATA COLLECTION TOOL

Patient ID:

A. Socio-demographic data:

1. Date of birth: / /
2. Age at initiation of ART: years……

   Months……..
3. Date of initiation of ART: …../……/….
4. Gender: Male ☐

   Female ☐
5. Tribe : Luhya ☐ Kissi ☐ Kikuyu ☐

   Kalenjin ☐ Teso ☐ Luo ☐

   Other: ☐ specify:…….
6. County:

   Nandi ☐ Uasin Gishu ☐ Bungoma ☐ Kisumu ☐

   Transnzoia ☐ Busia ☐ Elgeyo Marakwet ☐ Baringo ☐

   Other ☐

7. Orphan status:

a)Both parents alive ☐

b) Both parents dead ☐

c) Father dead ☐

d) Mother dead ☐

8. If deceased, was HIV the cause of death: Yes ☐ No ☐

No

9. Who is the child living with:

a. Mother ☐

b. Father ☐

c. Foster ☐

d. Sibling ☐

e. Children's home ☐

f. Grandparent :

1. paternal ☐
2. Maternal ☐

g. Auntie/ uncle: ☐

1. paternal
2. Maternal ☐

h. Other : ☐

Specify ……

B. **Adherence**

1.Who gives medication to the child:

1.a. Mother ☐

1.b. Father ☐

1.c. Sibling ☐

1.d. Grandparent ☐

1.e. Auntie ☐

1.f. Uncle ☐

1.g. Self ☐

1.h. Children's home ☐

1.i.Other ☐ Specify ……..

2.Any medication missed in a given month:

Yes ☐

No ☐

2. If yes, indicate drugs missed:

i. ARVS ☐

ii. Cotrimoxazole ☐

iii. Isoniazid ☐

☐

iv. Anti -TBs

v. Fluconazole ☐

vi. Other ☐ specify……..

3. If yes above, indicate reason(s): tick appropriately.

i.Travelled ☐ ii.Side effects ☐ iii. Too ill ☐ iv.Forgetting ☐

v.Ran out of drugs ☐ vi. Pill burden ☐ vii. Child refused ☐

viii.Depression ☐ ix.Other ☐ Specify…….

C. Nutritional status

1. At initiation of ART, what was the child's weight (kg)…….

2. What percentage of expected weight is this?

a. >110 ☐ b. 80 – 110 ☐ c. 60 - <80 ☐ d. <60 ☐

3. Height at initiation of ART (cm) ……

4. Presence of edema at initiation of ART: Yes ☐ No ☐

4. Indicate the type of malnutrition: tick approprietely.

a. Kwashiorkor (60-80%, + edema) ☐
b. Marasmus (<60%, no edema) ☐
c. Marasmic-kwash (<60%, + edema) ☐
d. Overweight (>110%, no edema) ☐

e. Under weight (60 – 80%, no edema) ☐

f. No malnutrition ☐

D.WHO clinical staging

1.Which WHO stage was the child at initiation of ART: Tick appropriately.

a)1 ☐ b) 2 ☐ c) 3 ☐ d) 4 ☐

2.Did the child develop any new WHO stage during ART:

a)Yes ☐

b) No ☐

3.If yes, which one: tick appropriately.

a)1 ☐ b) 2 ☐ c) 3 ☐ d) 4 ☐

4. Date of development of new WHO stage: …./…./……..

E. Antiretroviral therapy regimen

1. Which ARVS is the child on: tick appropriately.

a)ABC/3TC/LPV/r ☐

b)AZT/3TC/LPV/r ☐

c)AZT/3TC/NVP ☐

d)TDF/3TC/EFV ☐

e)AZT/3TC/EFV ☐

f) ABC/3TC/EFV ☐

g) ABC/3TC/NVP ☐

h) Other ☐ specify……

2. For how long has the child been on ART: tick appropritely.

a) 6 to <12 months ☐

b) 12 to <18 omnths ☐

c) 18 to 24onths ☐

F. Opportunistic infections

1. Sufferred from:

a)TB ☐ b) PCP ☐ c) candidiasis ☐

d) Cryptococcal meningitis ☐ e) toxoplasmosis ☐

f) Other ☐ Specify……..

g) None ☐

2. Was the child on any prophylaxis for opportunistic infections:

a) Yes ☐ b) No ☐

3. If yes, indicate the type of prophylaxis: tick appropriately.

a) Cotrimoxazole ☐

b) Dapson ☐

c) Fluconazole ☐

d) Isoniazid ☐

e) Other ☐ Specify……..

G. Prevention of Mother-to- Child transmission (PMTCT) of HIV

1. Did the child receive ARVS for perinatal prophylaxis: tick appropriately.

a) Yes ☐ b) No ☐

2. If yes, which ARVS did the child receive: tick appropritely.

a) NVP ☐ b) Other ☐ Specify …….

H. HIV Viral Load

	Tick	**VL**	**Date tested**
a) First HIV Viral Load after at least six months of ART	☐		
b) Second Viral load taken after the first Viral Load	☐		
c) Any other Viral Load taken after the second one	☐		

Appendix II: IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

17th May, 2016

Reference IREC/2015/127
Approval Number: 0001456
Dr. Nyumbile Ndalu Bonface,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
17 MAY 2016
APPROVED
P.O. Box 4606-30100 ELDORET

Dear Dr. Nyumbile,

RE: APPROVAL OF AMENDMENT

The Institutional Research and Ethics Committee has reviewed the amendment made to your proposal titled:-

"Virologic Response to First-Line Antiretroviral Therapy among HIV-Infected Children Attending AMPATH Clinics in Western Kenya".

We note that you are seeking amendments as follows:-

1. Insertion of "convenient sampling will be used to select three centers from the thirty-five main clinics that serve about twenty-six satellite clinics, based on the large number of children attending the clinics.
2. Replacement of the principal investigator together with the research assistant will travel to other AMPATH clinics to review various patient files whenever necessary until the minimum sample size of 354 subject is achieved.
3. Data collection tool: section C- nutritional status i) insertion of what percentage of expected weight is this. ii) Delete "if yes" in number 4 so that it reads "indicate the type of malnutrion; delete "other" in 4 and insert overweight (>110%, no edema), insert 4.e, "underweight" insert 4.f "no malnutrition"

The amendments have been approved on 17th May, 2016 according to SOP's of IREC. You are therefore permitted to continue with your research.

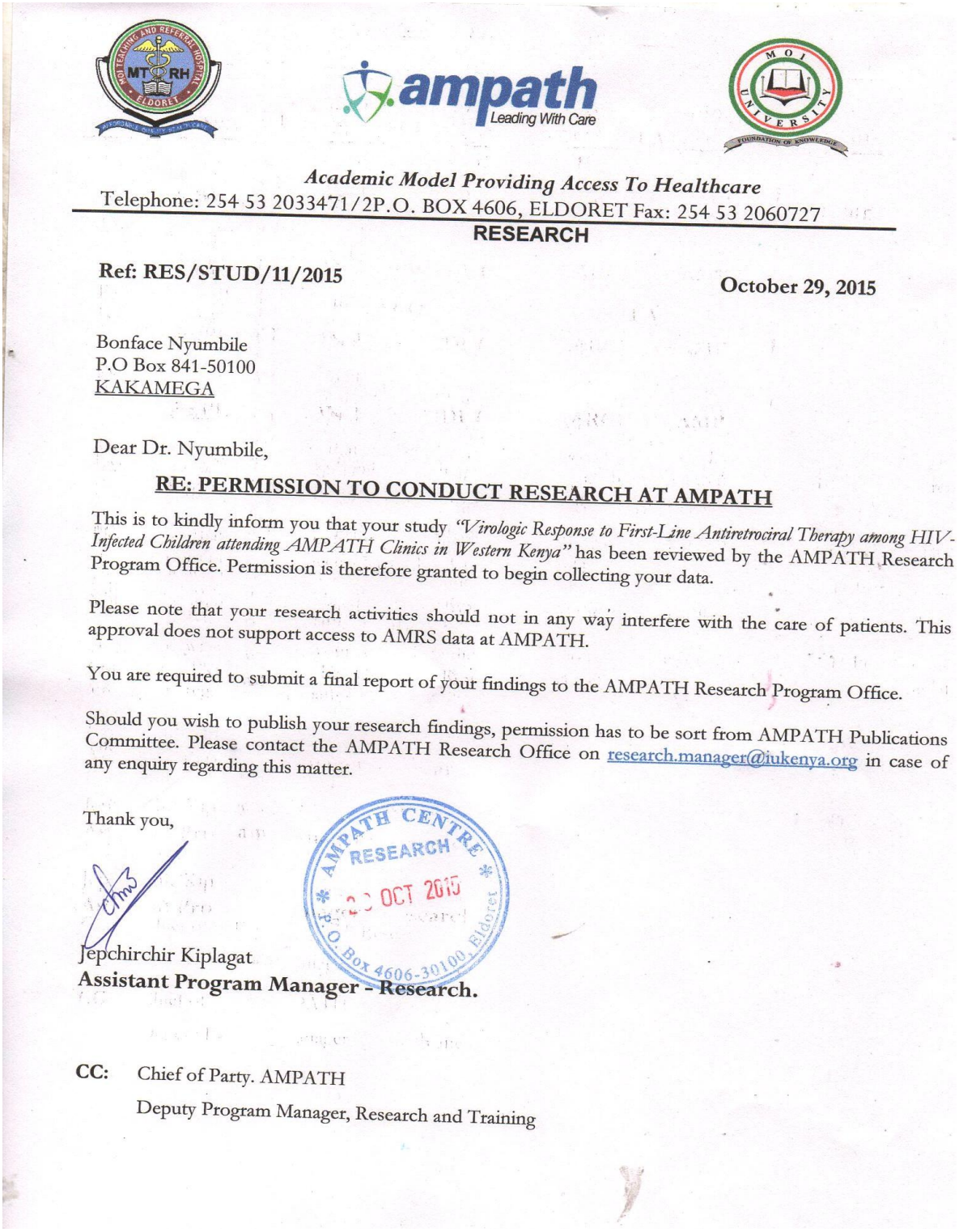
You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH Dean - SPH Dean - SOM
Principal - CHS Dean - SOD Dean - SON

Appendix III:Hospital Approval



Academic Model Providing Access To Healthcare

Telephone: 254 53 2033471/2P.O. BOX 4606, ELDORET Fax: 254 53 2060727

RESEARCH

Ref: RES/STUD/11/2015

October 29, 2015

Bonface Nyumbile
P.O Box 841-50100
KAKAMEGA

Dear Dr. Nyumbile,

RE: PERMISSION TO CONDUCT RESEARCH AT AMPATH

This is to kindly inform you that your study *"Virologic Response to First-Line Antiretrociral Therapy among HIV-Infected Children attending AMPATH Clinics in Western Kenya"* has been reviewed by the AMPATH Research Program Office. Permission is therefore granted to begin collecting your data.

Please note that your research activities should not in any way interfere with the care of patients. This approval does not support access to AMRS data at AMPATH.

You are required to submit a final report of your findings to the AMPATH Research Program Office.

Should you wish to publish your research findings, permission has to be sort from AMPATH Publications Committee. Please contact the AMPATH Research Office on research.manager@iukenya.org in case of any enquiry regarding this matter.

Thank you,

AMPATH CENTRE RESEARCH 2? OCT 2015 P.O. Box 4606-30100, Eldoret

Jepchirchir Kiplagat
Assistant Program Manager - Research.

CC: Chief of Party. AMPATH

Deputy Program Manager, Research and Training