A COMPARISON OF EFFECTIVENESS OF EFAVIRENZ AND NEVIRAPINE - BASED FIRST-LINE HIV TREATMENT IN PATIENTS ATTENDING COAST PROVINCIAL GENERAL HOSPITAL, MOMBASA COUNTY, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PUBLIC HEALTH (EPIDEMIOLOGY AND DISEASE CONTROL) IN THE SCHOOL OF PUBLIC HEALTH OF KENYATTA UNIVERSITY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this work to my family and the people suffering from HIV/AIDS who are always looking at the future with hope.

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ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine amino-transferase
ART	Antiretroviral therapy
ARV	Antiretroviral
CCC	Comprehensive Care Centre
CD4+	Cluster of Differentiation
CPGH	Coast Province General Hospital
CNS	Central Nervous System
CRF	Case Record Form
D4T	Stavudine
DNA	Deoxyribonucleic acid
EDTA	Ethyline diaminetetraacetic acid
EFV	Efavirenz
ELISA	Enzyme-Linked Immunosorbent Assay
FHI	Family Health International
FSC	Forward Scatter
HAART	Highly Active Antiretroviral Therapy
HIV-1	Human immunodeficiency virus (type- 1)
Hb	Hemoglobin

KAPR	Kenya AIDS Progress Report
KDHS	Kenya Demographic Health Survey
KNBS	Kenya National Bureau of Statistics
LFT's	Liver Function Tests
3TC	Lamivudine
MOH	Ministry Of Health
MTCT	Mother to Child Transmission
NASCOP	National AIDS and STI Control Program
NCST	National Council for Science and Technology
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non- Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
OI	Opportunistic Infection
PI	Protease Inhibitor
PMCT	Prevention of Mother –to –Child – Transmission
SAE	Severe Adverse Effects
SDI	Standard Deviation
SGPT	Serum Glutamate Pyruvate Transaminase
SSC	Side Scatter
STI	Sexual Transmitted Infection
UKNEQAS	United Kingdom National External Quality Assurance Scheme
ULN	Upper Limit of Normal
UNAIDS	United Nations Program on AIDS
VCT	Voluntary Counselling and Testing (for HIV)
WHO	World Health Organization.

ABSTRACT

In the Coastal region of Kenya, Mombasa County has the highest HIV prevalence (11.1%) of the estimates based on Kenya AIDS Progress Report of 2014. Limited data exist on effective regimen strategies to manage HIV/AIDS among adults in Kenya. Safe alternative HAART regimens are not currently a viable option for many, especially infected patients in Sub-Saharan Africa. The objective of this study was to compare the effectiveness of Efavirenz and Nevirapine based First-line HIV treatment in patients attending comprehensive care centre in Mombasa. This was a prospective comparative study. The target population was HIV positive adult patients eligible for HAART. A total of 251 patients were enrolled in the study and followed up for 12 months. All ARV naive patients with CD4 count < 350/ul with WHO stage 3 or 4 and eligible for HAART were randomly selected and enrolled into either of the two treatment groups. HIV infected adult patients were randomised into Efavirenz and Nevirapine based regimens (D4T/3TC/EFV and D4T/3TC/NVP) and followed up for a period of twelve months. Laboratory tests were done by collecting 10mls of blood from each patient every three months and tested for Haemoglobin, Liver enzyme test (ALT), Creatinine and CD4 cell count. Clinical examination included physical examination, measurement of body weight and vital signs (Temperature, Blood Pressure and Pulse). Data analysis was done using SPSS Version 17.0 package to perform one way ANOVA comparing more than two means, followed by Post hoc (Student Newman Keul). Independent t-test was used to calculate difference between the two treatment groups. In both statistics, 95% confidence level was used, where $P<0.05$ meant significant difference. The study showed that first-line regimens of Efavirenz and Nevirapine were effective in suppressing HIV/AIDS infection with significant improvement in CD4 count ($P<0.05$). There was also improvement in haemoglobin levels and body weight among the patients on both regimens in the study. However, elevation of ALT and Creatinine were noted in both treatment groups, but this did not warrant drug discontinuation ($P>0.05$). Efavirenz based regimen appeared to be superior to Nevirapine based regimen on CD4+ profiles and renal function ($P<0.05$). There was no significant difference in haemoglobin levels, body weight and ALT enzyme for patients on both treatment groups although these parameters increased with time for patients in both groups throughout the study ($P>0.05$). The findings demonstrated that D4TC/3TC/EFV and D4TC/3TC/NVP combinations were safe, well tolerated and effective in increasing CD4 Cell counts and suppressing HIV progression in advanced HIV infected patients as well as improvement in body weight gain and anaemia. Renal function and Liver function should therefore be monitored on a regular basis in patients with HIV receiving any antiretroviral agent.

CHAPTER ONE: INTRODUCTION

1.1 Background information

According to Global Fund report issued by UNAIDS (2015), more than 38 million people have been infected with Human Immunodeficiency Virus (HIV). Twenty five million people have died of AIDS related illness. AIDS is one of the leading causes of death worldwide. Over 95% of all HIV infected people globally are from developing world, with sub-Saharan Africa alone accounting for over 32% of those infected. Human Immunodeficiency Virus / Acquired Immunodeficiency Deficiency Syndrome (HIV/AIDS) is a rapidly growing public health problem in Kenya. In less than a decade, HIV has evolved from a fatal illness, to what could be considered a chronic illness. The number of people living with HIV in Kenya includes about 2.0 million adults between 15 and 49 years and another 60,000 aged 50 years and over (KDHS, 2008/9 ; UNAIDS, 2015). Urban populations have higher adult HIV prevalence (10%) than do rural populations (6.7%). Results from the Kenya Demographic Surveillance indicate that 6.7% of Kenyan adults are infected with HIV. Some of the interventions currently available in resource limited settings are only partially effective.

HIV/AIDS remains a major challenge in Kenya. Substantial regional variations in HIV infection, low levels of HIV testing, HIV discordance within couples and ongoing epidemics of sexually transmitted infections (STI) are important challenges to overcome in the control and management of the HIV epidemic (KDHS, 2014). The advent of Highly Active Antiretroviral Therapy (HAART) has resulted in a significant reduction

in HIV/AIDs related morbidity and mortality in Sub-Saharan Africa, and has improved the survival and life expectancy in the HIV infected patients by reducing morbidity and mortality (Lahuerta *et al.,* 2013). However, this success has also resulted in the emergence of adverse events, some of which might interrupt antiretroviral therapy adherence. Anaemia, skin rash, fat re-distribution syndrome, peripheral neuropathy and hepatotoxicity are among the most common adverse events following initiation of antiretroviral therapy by HIV infected patients (Kenneth, 2013). HAART has therefore transformed HIV infection from an acute illness to a manageable chronic condition.

However, the remarkable decreases in morbidity and mortality and increase in life expectancy caused by HAART have been accompanied by an increase in several clinical and metabolic disorders, including cardiovascular risks (John *et al.,* 2012). UNAIDS estimates that 2 million of Kenya's 40 million people are currently infected with HIV and that 1.5 million have already succumbed to the disease, resulting in an overall decline in life expectancy of 13 yrs (UNAIDS, 2015). In Coast Province, Mombasa County has a higher Adult HIV prevalence of 11.1% based on Kenya AIDS Progress Report (KAPR, 2014). The wide-spread use since 1996 of HAART, a combination of at least three drugs that typically includes either a protease inhibitor (PI) or a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI) and two nucleoside analogue reverse transcriptase inhibitors (NRTI's) has substantially improved the prognosis of HIV1 infected patients. However, accurate estimates of the probability of clinical progression in treatment of naive men and women of different age categories according to different levels of immunodeficiency and viral replication

are not available at present. Information on prognosis is of obvious importance to patients, and is also required to gain a better understanding of the treatment history of HIV-1 infection to develop treatment guidelines, to monitor and predict the progress of the HIV/AIDS epidemic, and to plan health services in the era of HAART. Such data are also important as a basis for comparisons with treatment outcomes in resource poor setting once HAART becomes more widely available (Rubaihayo *et al.,* 2015).

1.2 Statement of the problem

Antiretrovirals have been widely used in developing countries, yet little has been done to find out the treatment outcome of these drugs by assessing the immunological and virological parameters of an individual's immune system in relation to the clinical response. Hence, it is important to follow the progression of HIV/AIDS patients through laboratory monitoring, as well as to review the acceptability and tolerability of ARV's which contribute to adherence, as this will help in treatment decisions by clinicians as they can decide to continue treatment, change a particular regimen or discontinue therapy at the appropriate time. This decision will often be based on the occurrence of side effects, or increased toxicity or non-adherence to antiretroviral drugs. For a long time, more focus on HIV/AIDS has been on use of Nevirapine in PMCT transmission programs. Secondly, although in the past antiretrovirals were given, very little has been done to assess the impact on treatment outcome and toxicity of these ARV's through laboratory monitoring. The rapid scale-up of HAART programmes in Sub Sahara Africa, a region at the epicenter of the HIV/AIDS epidemic, suggests that the incidences of antiretroviral associated adverse events may also arise. The severe complications of

developing adverse events make the public health impact a particular concern in our setting. ART has significant toxicity that requires monitoring. Laboratory tests performed on a regular basis are usually used to detect severe toxicity, before it becomes clinically apparent and harmful. These tests, however, are costly and require patient visits, phlebotomy and appropriate infrastructure and equipment.

1.3 Justification for the study

Coast Provincial General Hospital was selected as the study area because the centre offered free voluntary Counselling and Testing services as well as antiretroviral therapy. HIV/AIDS was also leading in mortality and morbidity in Mombasa County compared to other counties in the Coastal region. The region which is located alongside the Indian Ocean is popular with many tourists and commercial sex workers. Homosexuality is also common in the Northern part of Mombasa County along the beaches contributing to spread of HIV/AIDS in the County. The centre also served the bulk of population in the county and had a balanced population of both youth and older people. It was also important to identify an effective HAART regimen between the two regimens used in the country as first line treatment, to manage HIV/AIDS infected adults in Kenya. Finally, it was necessary to identify a safe viable regimen that will decrease morbidity and mortality among patients on HAART in Kenya.

1.4 Research questions

i. What is the effect of HAART on CD4+ profiles?

ii. What is the effect of HAART on body weight and HIV/AIDS progression?

iii. What is the effect of HAART on liver and renal functions?

iv. What is the effect of HAART on haemoglobin levels?

1.5 Hypotheses

i. The use of HAART has no effect on immunological response in HIV patients.

ii. HAART use does not have an impact on body weight, hemoglobin and liver enzyme levels.

1.6 Objectives

1.6.1 General objective

To compare the effectiveness of Efavirenz and Nevirapine based first-line HIV treatment on HIV/AIDS patients attending the comprehensive care centre, in Mombasa.

1.6.2 Specific objectives

i. To compare the immunological suppression among patients on Nevirapine and Efavirenz based HAART regimens.

ii. To characterize changes in body weight in HIV patients following administration of HAART.

iii. To establish relationship between use of HAART and haemoglobin levels among the study population.

iv. To determine the effect of HAART on liver enzymes and renal function.

1.7 Significance of the study

The Ministry of Health in Kenya had approved the use of Efavirenz and Nevirapine - based HIV regimens to manage HIV/AIDS among adult patients in Kenya. At the time of the study, the government had received the drugs as a donation from USAID through

Family Health International (FHI) to use on HIV/AIDS patients following increased morbidity and mortality in the country. Due to the nature of the epidemic, a pilot study had not being done on a small population of HIV/AIDS patients in Kenya to ascertain the effectiveness of the drugs. The study therefore aimed at evaluating the two drug regimens in terms of their effectiveness in treating HIV/AIDS in Adult population in Kenya as well as their tolerability in terms of renal function, liver function, Anaemia and HIV/AIDS progression. It was also necessary to do a comparison study of the two regimens so as to inform the health community the suitability of both regimens in managing HIV/AIDS.

1.8 Conceptual framework

In order to understand the comparison of effectiveness of Efavirenz and Nevirapine - based regimens, the study relied on the health behavioural theory as illustrated in Figure 1.1. This theory was developed by Rosenstock and is mainly based on perceived susceptibility (an individual's assessment of their risk of getting the condition), perceived severity (an individual's assessment of the seriousness of the condition and its potential consequences), perceived barriers (an individual's assessment of the influences that facilitate or discourage adoption of the promoted behaviour), perceived benefits (an individual's assessment of the positive consequences of adopting the behaviour), perceived effectiveness (an individual's self assessment of ability to successfully adopt the desired behaviour), and cues to action (external influences promoting the desired behaviour). Therefore, improved health status of an individual suffering from HIV/AIDS, depends on regular HAART uptake and clinic attendance, in

addition to regular laboratory monitoring of immune system, liver and kidney function. Besides these, management of opportunistic infections and balanced dietary intake also have influence on the effectiveness of HAART in HIV infected patients, leading to either improved health status and survival or deterioration of health and death.

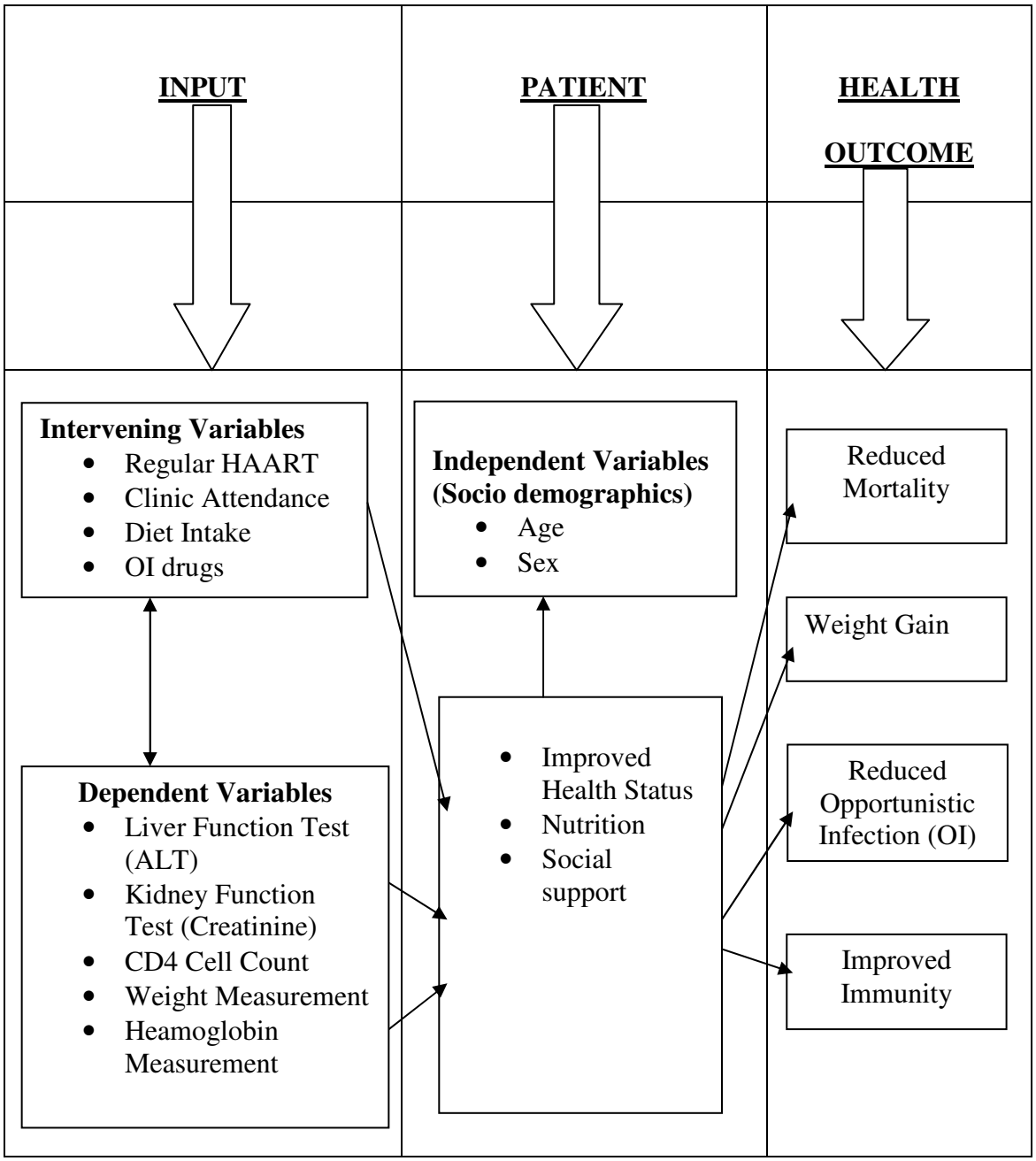

Figure 1.1: Conceptual Frame Work - Modified Health Belief Model (Derived from Health Belief Model (HBM) Concept Huchbaum, Rosenstorn Legals, 1950 and Modified to fit the situation described in this thesis).

1.8 Scope and limitations

The most important limitation in the study was obviously the exclusion of co-morbidities and opportunistic infections like tuberculosis, which can induce important selection bias. Most patients on the NVP treatment arm started off with a higher CD4+ count than their counterparts in EFV group. In particular, physicians might have been more inclined to prescribe EFV to patients perceived to be more compliant and NVP to patients more likely to be non compliant based on clinical assessment and the side effect nature of Nevirapine. It is important to recognize possible limitation of this study in terms of HAART regimen comparisons. Different frequencies in the measurement of liver function for patients on NVP resulted in more frequent ALT monitoring in the NVP based group leading to an increased detection of hepatotoxicity in that group compared to the Efavirenz based group. Thirdly, lack of viral load testing was also another limitation of the study considering that viral load detection in blood is a good indicator of viral suppression and drug effectiveness. Fourthly, another limitation was the lack of calculation of body mass index (BMI) as an important anthropometric measurement to assess nutrition status of patients on HAART.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Global picture of HIV/AIDS

The HIV/AIDS pandemic is affecting all the countries in the world. Joint United Nations Program on AIDS estimates that three million people died from HIV/AIDS and that 5 million more were infected with HIV/ AIDS in 2003 (UNAIDS, 2015). It is imperative to prevent new HIV infections. Globally, the high risk groups for AIDS continue to be intravenous drug users, homosexuals and prostitutes, although in Africa the disease is more widespread among the heterosexual community.

2.2 HIV/AIDS in Africa

Sub-Saharan Africa accounts for the greatest number of deaths in 2003 from HIV/AIDS with around 70% of the total. United Nations Joint Program on AIDS estimates that between 34 and 46 million people live with the disease worldwide with approximately half of them dwelling in sub-Saharan Africa. Adult prevalence of HIV/AIDS is between 7.5 and 8.5 percent of the adult population in sub-Saharan Africa (UNAIDS, 2015).

2.3 HIV/AIDS in Kenya

The National AIDS Control Program (NASCOP) was established as a department in the ministry of health to organize and co-ordinate the country's effort in the fight against HIV/AIDS (MOH, 2004). It was reported that 95% of Kenyans were aware about the modes of spread of HIV/AIDS. The prevalence rate currently stands at 7% down from 14% (KAPR, 2014). The differences in HIV infection in different regions of the country are significant. Nyanza province (15%) , Nairobi (10%) and Coast province (6%) have

the highest prevalence rates while eastern (4%) and north eastern provinces (less than 1%) have the lowest rates. Many other demographic and social characteristics are associated with HIV infection. The wealthiest quintile of the population has the highest prevalence; nearly 10% while prevalence among the poorest is less than 4% (KAPR, 2014). New information from Kenya AIDS Progress Report (KAPR) indicates that 7% of Kenyan adults aged 15-49 are HIV infected. There are significant differences in regional prevalence, and those living in urban areas, those with greater wealth, and those in polygamous marriages are more likely to be infected. Trend information from sentinel surveillance suggests that adult prevalence peaked at a level of 10% in over 200,000 people to approximately 86,000 people but deaths have continued to increase each year. Approximately, 1.3 million adults and 100,000 children are currently infected with HIV (KAPR, 2014).

2.4 Clinical staging of HIV- 1

When CD4 testing is available, it is recommended that HIV clinical staging will be done according to WHO classification based on CD4 count and clinical symptoms. Studies have shown that clinical symptoms of AIDS and a lower CD4 count are correlated with late stage of disease (Appendix VIII , WHO Clinical staging in adults and adolescents 2007 Revision).

2.5 Highly Active Antiretroviral Therapy (HAART)

Highly Active Antiretroviral Therapy (HAART) is the most effective therapeutic intervention for reduction of mortality in people with HIV1 infection. It is increasingly

available in the developing countries where 90% of HIV infected people live, including 64% in Africa (WHO, 2015). However, despite substantial effort, most HIV infected people in Africa who could benefit from HAART do not have access to it. Availability of HAART in many areas is constrained by inadequate numbers of trained health care providers, poorly equipped clinics and distance to health centres (UNAIDS, 2015). In Africa, initial introduction of ART has usually been as HAART. Insight into the effectiveness of HAART in Africa could enable improved decision making by individuals, governments and donor agencies in the selection of potent, tolerable and effective regimens for management of HIV/AIDS (Bendacid *et al*., 2011).

Highly Active Antiretroviral Therapy (HAART) is a combination of at least three drugs, typically including either a protease inhibitor or non-nucleoside analogue reverse transcriptase inhibitor and two nucleoside analogue reverse transcriptase inhibitors (Henry *et al.,* 2012). Highly Active Antiretroviral Therapy consisting of two nucleosides and one protease inhibitor (PI) has been widely used for the treatment of advanced HIV infected patients. The introduction in 1996 of combination therapy, known as 'highly active antiretroviral treatment' (HAART) resulted in great enthusiasm and a 'hit early – hit hard' strategy. Antiretroviral (ARV) therapy has dramatically improved the prognosis of many patients with human immunodeficiency virus (HIV) infection (Ramana, 2014). The available ARV drugs fall into three classes: nucleoside reverse transcriptase inhibitors (NRTI's), non-nucleoside reverse transcriptase inhibitors (NNRTI's) and protease inhibitors (PI's) (Ramana, 2014). Current recommendations for treatment of HIV-1 infection, require a combination therapy with

at least three ARV drugs (British HIV Association, 2014). Although this highly active antiretroviral therapy (HAART) suppresses replication of HIV-1, its effectiveness is often limited by the emergence of ARV drug – resistant variants (Mani *et al*., 2015). The prolonged use of HAART is limited by the toxicity of the drugs. As a result, most recent European guidelines recommend starting treatment only when CD4- counts are below 350 cells per mm^3 and or the viral load is higher than 50,000 copies/ml justifying the initiation of HAART (British HIV Association, 2014).

The use of antiretroviral drugs has been extremely limited in resource poor settings of Africa, Asia, and South America (Bendacid *et al.,* 2011). Therefore, the extent of usage of antiretrovirals to treat HIV- infected patients is not known. Little data exist from these regions of the world on various aspects of antiretroviral therapy, including levels of drug resistance. Antiretroviral therapy for HIV infection has frequently been associated with elevated liver enzymes (Kalyesubula *et al.,* 2011*).* It is important to monitor adverse events from Nevirapine using liver function tests among HIV/AIDS patients (Tansuphaswadikul *et al*., 2007). Prolonged utilization of some antiretroviral drugs in patients infected by HIV can lead to the outbreak of fat redistribution syndrome also known as lipodostrophy syndrome (Domingo *et al*., 2012). When administered on a long term basis, HAART regimen containing Stavudine may result in hyperlipideamia, insulin resistant and fat malabsorption increasing risk of coronary heart disease (Pineda *et al*., 2010). In a study in western India, prevalence of lipodostrophy was 46.1%, all associated with Stavudine use (Shakirat *et al*., 2014).

Effective ART programmes require high adherence to medication, attention to potential drug toxicity, continuing diagnosis and treatment of opportunistic infections (Kenneth, 2013). Ideally, programmes in Africa could provide these services with limited use of physicians and minimum transportation requirements (UNAIDS, 2015). Use of trained lay providers to give ART to HIV infected people at their homes, collecting standard health information and referring patients to clinics for selected symptoms could potentially avoid adherence problems stemming from inadequate transportation to clinics and could further reduce crowding at health centres. Although several studies provide information about survival and changes in immunological and virological markers during ART in patients in Africa, an assessment of ART effectiveness requires a comparison group and a carefully followed up cohort because randomised trials could be unethical (Bendacid *et al*., 2011). Data for effectiveness of highly active antiretroviral therapy (HAART) from developed countries are few because contemporary comparison groups were taking dual treatment at the time HAART was available.

2.5.1 Efavirenz (EFV)

It is a non- nucleoside reverse transcriptase inhibitor that was licensed by regulatory agencies for the treatment of the HIV infection in 1998 and is one of the most prescribed drug (Kryst *et al*., 2015). Peak Efavirenz plasma concentrations are reached by 5 hours following single oral doses in uninfected volunteer (Franco, 2009). The time to peak plasma concentration is approximately 3 to 5 hours and steady state plasma concentrations of Efavirenz are reached in 6-7 days. The bioavailability of a single 600

mg dose of Efavirenz hard capsules in uninfected volunteers is increased by 17%- 22% by food. Efavirenz is highly bound (99.5%-99.75%) to human plasma proteins, predominantly albumin (Avery *et al*., 2013).

2.5.2 Biotransformation of Efavirenz

Efavirenz is converted to inactive Hydroxylated metabolites by the cytochrome P450 system. CYP2B6 is one of the major isozymes responsible for Efavirenz metabolism. Efavirenz plasma exposure is increased in patients with the homozygous G516T genotype of CYP2B6 (Podriguez *et al*., 2006). This is not associated with treatment failure but it can lead to a higher rate of neuropsychiatric adverse events. In this situation, dose reduction is feasible and maintains virological suppression (Gatanaga *et al*., 2007). The G516T genotype is more common in African Americans than in European Americans and this has been reported to cause a greater Efavirenz exposure, although there is considerable overlap between racial and ethnic population. The C1459T polymophism has been reported not to affect Efavirenz exposure. Other alleles of CYP2B6 may also affect Efavirenz metabolism. Exposure to Efavirenz is significantly higher in women than in men having G516T genotype (Burger *et al*., 2006).

2.5.3 Elimination of Efavirenz

Efavirenz has a terminal half life of at least 52 hours after single doses and 40-55 hours after multiple doses. Approximately, 14%-34% of a radio labelled dose of Efavirenz is recovered in the urine and less than 1% of the dose is excreted in urine as unchanged

Efavirenz. The half life of Efavirenz appears to be shorter (approx. 24 hours) when it is given in combination with didanosine and emtricitabine but this combination is effective and well tolerated in long term therapy (Molina *et al*., 2007). The long half-life of Efavirenz makes it suitable for once-daily dosing. The recommended dosage in adults is 600 mg once daily.

2.5.4 Stavudine

Stavudine (d4T) is a nucleoside analogue that has been widely used as part of highly active antiretroviral therapy (HAART). Despite being highly effective, use of stavudine based regimens has been eliminated from resource-rich environments due to the poor side effects profile and high rates of adverse events (Henry *et al*., 2012). In particular, Stavudine regimens have been associated with metabolic complication, such as dyslipidemias lipoatrophy and other mitochondrial toxicities, notably peripheral neuropathy and lactic acidosis (Shakirat *et al*., 2014). This led the World Health Organization to recommend a lower maximum dose of Stavudine for all adults in 2007 and in 2009 to recommend that it will no longer be used for initial treatment of HIV infection due to serious side effects (WHO, 2015). Stavudine has traditionally been prescribed in weight dependent dose of 30 mg for those weighing less than 60 kg and 40 mg for those at or above 60 kg. However, using 30 mg of Stavudine may reduce adverse events and toxicities without reducing efficiency regardless of body weight. In addition to the studies noted, small scale randomized trials have suggested a trend towards a decrease in plasma lipids and improvements in peripheral wasting with lower-dose Stavudine (Milinkovic *et al*., 2007).

2.6 Adverse effects of antiretroviral therapy

Adverse events are studied to characterize drug safety and tolerability. Events that are rare, delayed in onset, or fairly unique to one or more population are considered unexpected in that they are usually identified after marketing, in part because pre-licensing studies are too few, too short, or because specific patient groups are under-represented. The only adverse events that have to be studied to gain regulatory approval relate to essential organs (CNS, cardiovascular and respiratory) (Henry *et al*., 2012). The toxicity and tolerability of HAART, however, are increasingly important factors in the decision to prescribe one of the many potential regimens because side effects are frequent (74% of adults in the largest survey) and because long term benefits depend on near perfect (> 95%) and lifelong adherence (Kenneth *et al*., 2013) which in turn is affected by tolerability and human factors.

A potent but intolerable regimen is therefore doomed to fail. Near perfect and lifelong adherence to treatment is not required for any other chronic infectious disease and so development of drugs that allow for such adherence has not been previously attempted. Several factors have combined to increase the attention on the toxicity of HAART since HIV-1 eradication seems unlikely with current therapy (Reto, 2006). HAART will need to be indefinite for clinical benefits to be preserved. Secondly, the severity of the HIV epidemic led to accelerated licensing of many antiretroviral agents, often with little known about long term safety. Thirdly, the sustained benefits of HAART have led to far greater numbers of HIV-1 infected patients receiving at least three drugs for greater period of time. Moreover, drug related toxicity is being increasingly recognised because

of the declining incidence of HIV-1 associated opportunistic disease. Lastly, there are now 15 antiretroviral drugs available in four drug classes and so the number of possible HAART combinations is huge. Choosing between many of these combinations is therefore, increasingly dependent upon knowledge of antiretroviral toxicities (Kalyesubula *et al*., 2011).

2.7 Mitochondrial toxicity

Nucleoside Reverse Transcriptsae Inhibitors (NRTI's) and mono-phosphorylated nucleotide- analogue reverse- transcriptase inhibitors are both phosphorylated intracellularly to active triphosphate forms and are then incorporated into new DNA strands synthesised by HIV reverse Transcriptase (Apostolova *et al*., 2011). The lack of a 3'hydroxyl in NRTIs and NtRTs results in HIV DNA chain termination. The major toxicities of NRTI and NtRTI therapy, particularly over the medium term to long term are thought to be secondary to inhibition of mitochondrial DNA polymerase - gamma (γ), resulting in impaired synthesis of mitochondrial enzymes that generate ATP by oxidative phosphorylation (Matute *et al.,*2013). These include myopathy (zidovudine), neuropathy (stavudine, didanosine, zalcitabine), hepatic steatosis and lactic acidaemia (didanosine, stavudine, zidovudine) and possibly also peripheral lipoatrophy (possibly all NRTIs, although predominantly with stavudine) and pancreatitis (didanosine). The most serious mitochondrial toxicities are lactic acidosis and pancreatitis. NRTIs and NtRTIs active against other viruses can also exert mitochondrial toxicity (fialuridine, Ganciclovir, aciclovir and cidofovir at least *in vitro* (Matute *et al.,* 2013).

Mitochondrial toxicities at the clinical level are generally gradual in onset and offset, but may occur within days of the start of therapy. Overall, their prevalence and severity increases with more prolonged therapy, some such as peripheral neuropathy and renal tubular acidosis may worsen for several weeks after drug cessation (the so called "coasting" phenomenon). Similarly, the capacity for tissue to recover after cessation of reverse transcriptase inhibitors varies (Apostolova *et al*., 2011). This capacity may be dependent upon tissue degenerative capacity, the duration and severity of the toxicity and the duration of therapy. For example, didanosine induced pancreatitis usually resolves rapidly and completely although it is not known what occurs at the tissue level. In contrast, peripheral neuropathy improves slowly and there may be a permanent deficit, especially if cessation of therapy is delayed. Another striking feature of these toxicities is their relative tissue specific and drug specific nature.

The "pol-gamma (γ) hypothesis" suggests that this specificity may be due to tissue specific drug penetration and metabolism to the triphosphate form, to tissue specific polymorphisms in mitochondrial DNA polymerase-γ, to the target tissue's stores of natural nucleotides and to the dependency of a given tissue upon mitochondrial function. For example, the proximal renal tubular toxicity of adefovir might be due to its selective accumulation within proximal renal epithelia by the protein organic anion transporter 1 (Robert *et al*., 2015). Although the weight loss seen with adefovir therapy is unexplained, it was noted that this transport molecule is highly expressed in skeletal muscle. Diagnosis of mitochondrial toxicity is difficult only if patients are receiving other drugs with overlapping toxicities.

Furthermore, no diagnostic (metabolic or serological) assay predicts who will develop toxicity. In particular, plasma NRTI concentrations do not reflect intracellular NRTI - triphosphate concentrations. Measurement of the latter is difficult and time consuming and may only be relevant if the target organ is sampled. Most patients treated with NRTIs or NtRTIs do not develop mitochondrial toxicity. Factors that may contribute to toxicity include underlying organ dysfunction like chronic liver disease and NRTI associated hepatic steatosis, prior pancreatitis and didanosine, prior NRTI associated neuropathy and stavudine) , concomitant HIV-1 opportunistic disease and particularly the administration of other drugs with similar toxicity profiles including peripheral neuropathy, Vinca alkaloids and Zalcitabine or didanosine (Flavia *et al*., 2009).

The management of mitochondrial toxicities is generally limited to cessation of the causative drug and sometimes of other drugs that might exacerbate the condition. Co-administration of other drugs including other antiretroviral agents with potentially addictive or synergistic toxicities should of course be avoided. Given that toxicity can be of late onset, clinical screening for drug toxicity should be done throughout therapy. Three categories of highly active antiretroviral therapy (HAART) associated major toxic effects have been identified as nucleoside related toxic effects like neuropathy, myopathy pancreatitis, hepatic steatosis, lactic acidosis and possibly lipoatrophy metabolic complications including osteopenia and or osteoporosis. The toxic effects caused by nuecleosides are hypothesized to be a result of mitochondrial injury and include myopathy, pacreatitis, liver failure and lactic acidosis. Alteration in lactic acid

metabolism, range from common instances of asymptomatic lactic acidemia to rare occurrences of life threatening lactic acidosis with hepatic steatosis (Grait *et al*., 2010). A metabolic syndrome consisting of lipodystrophy like fat redistribution, hyperlipidemia and insulin resistance has been observed particularly with protease inhibitor treatment. Some additive interaction between protease inhibitors and nucleosides has also been described. The potential relationship of these metabolic abnormalities to increased risk of cardiovascular disease and diabetes has broad implications on patient management. Lipodystrophy associated with HAART is generally accompanied by potentially serious abnormalities including dyslipidemia like hypercholesterolemia and hypertriglyceridemia and altered glucose metabolism like. insulin resistance. Regimens of HAART may have adverse effects on bone metabolism as indicated by emerging reports of osteopenia, osteoporosis and avascular necrosis (Marco *et al*., 2009).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

The study was carried out in Mombasa County, over a twelve month period at the Coast Provincial General Hospital (CPGH). The County has approximately 939,370 people (KNBS, 2009). It is a Cosmopolitan Centre with people of different races. The area has one County hospital, one sub county hospital, five clinics and over ten health centres at Changamwe, Kisauni, Likoni and Mombasa mainland. It has a balanced population of both the youth and older people. The Centre was selected because it offered free services sponsored by Family Health International (FHI) and Ministry of Health (MOH) to all willing clients for Voluntary Counseling and Testing Services, and also that it formed the bulk of population of Mombasa District.

3.2 Target population

The target population was all HIV positive adult patients both male and female who were eligible for HAART. The number of people enrolled for the study was proportional to the population of the study area i.e. Mombasa County.

3.3 Research design

A Prospective comparative cohort study of ARV naïve HIV infected adult patients randomised into Efavirenz and Nevirapine based regimens (D4T /3TC / EFV and D4T /3TC / NVP) and followed up for a period of twelve months at the Coast Provincial General Hospital in Mombasa County. Screening of patients was done at the Comprehensive Care Centre (CCC) after patients were referred from VCT clinics,

outpatient clinics and the wards. Patients were screened for eligibility criteria which included a baseline laboratory examination for HIV test using ELISA Murex method, Liver enzyme (ALT) and Creatinine test using done using photometer by Boeringher Mannheim (Germany), Hemoglobin measurement using Coulter Counter and CD4 Cell count measurement using Partec Cyflow machine. The baseline data was used as part of the study data for the patients who were enrolled in the study. Questionnaires were used to obtain primary data from patients by recording personal and clinical history, medication history, opportunistic infections and adverse events among others. Enrolment of patients was done based on eligibility criteria and those who were eligible for HAART based on their CD4 count, were enrolled into the study.

Random sampling was used to select patients who were allocated to two different treatment arms. All patients with CD4 count < 350 Cells / ul with WHO stage 3 or 4 were selected as eligible. Every patient who was HIV positive with CD4 <350 Cells / ul and eligible for the study was selected randomly and allocated into a treatment group. The first patient was enrolled into one regimen and the subsequent patient was assigned to a different regimen. This was done to avoid selection bias. Patients who met the criteria for initiation of HAART received adherence counseling and screening for opportunistic infections prior to initiation of therapy. Patients initiated on HAART were required to attend clinic visit every three months for laboratory monitoring of CD4 cell count, Creatinine, ALT, Hemoglobin and Body weight. Clinical assessments on these subsequent visits were done to check for adherence, medication history and adverse

events. Patients were then traced by a healthcare worker and referred to HIV support group for the entire period of the study.

3.4 Variables

3.3.1 Dependent variables

Dependent variables were HAART regimen, body weight, CD4 count, Liver enzyme test (ALT), Creatinine and Hemoglobin.

3.3.2 Independent variables

Independent variables were Age and Sex.

3.5 Inclusion and exclusion criteria

3.5.1 Inclusion criteria

In order to be eligible for enrolment to the study, patients were to be confirmed to be HIV positive at the study site irrespective of their CD4 Cell count, consent for blood withdrawal, aged above 18 yrs old, residing within the defined catchment area, no previous history of taking ARV's or previous enrollment to ART program and willing to adhere 95% to ART treatment.

3.5.2 Exclusion criteria

Patients were excluded from the study if they were found to be HIV negative , had history of taking ARV's or previous enrollment to another ART program, did not Consent for study, aged below 18 yrs old and not residing within a predefined site specific catchment area.

3.6 Sample size

This was determined using the formula used by Fisher *et al*. (1998) where;

$$\text{Sample size } n = \frac{Z2\ PQ}{d2}$$ Where;

n= desired sample size for population greater than 10,000.

Z= standard normal deviate (set at 1.96) at 95% confidence interval level.

P= the proportion in the target population. (7.0%)

Q = 1.0 – P

d = degree of accuracy desired, in this case is 0.05

$$\frac{1.96^2 \times 0.07(0.93)}{(0.05)^2} = 100$$ patients for each arm of treatment.

A minimum of 100 patients were required for each treatment regimen. However, 251 patients were selected to account for loss to follow up.

3.7 Measurement of liver enzyme alanine aminotransferase (ALT)

Alanine Aminotransferase was measured using reflectance spectrophotomerty 5010 (Boeringer Mannheim). Briefly, a patient's plasma sample of 100 µl was mixed with 1000 µl of aspartate reagent solution in a test tube. The mixture was then poured into a cuvette at 37^0C. The rate of oxidation of the enzyme was then measured by reflectance spectrophotometry at 340 nm at 37^0C. The rate of change in reflection density measured in a linear region was then read in international units (U/L).

3.8 Measurement of creatinine

Creatinine was measured using reflectance spectrophotometry 5010 (Boeringer Mannheim) as per protocol whereby a patient's plasma sample of 100 µl, was mixed with 1000 µl of picrate reagent solution in a test tube. The mixture was then poured into a cuvette at 37 ^{0}C. The rate of formation of the complex was then measured by reflectance spectrophotometry and the rate of complex formation was proportional to the creatinine concentration.

3.9 Measurement of hemoglobin

Hemoglobin levels were measured using Coulter counter analyser. Briefly, a patient's whole blood sample was collected in an EDTA vacuitaner and mixed gently before cycling it by inverting the sample at least eight times. After removing the vacuitaner lid, the sample was then held up to the aspirating probe of the machine until the probe was sufficiently immersed in the blood sample. After pressing the aspiration knob, the probe was then allowed to aspirate the sample and to move up into the instrument. The sample tube was then removed from the probe once the audible alarm of aspiration was heard. The coulter machine was then allowed to analyse and give the results on the digital screen.

3.10 Measurement of CD4+ cell count

CD4+ cell count was measured using Partec cytoflow machine. In the test, a partec tube was labeled with the patient's number. A patient's whole blood sample was collected in an EDTA Vacuitaner tube and 50µl was put in tube. In tube , 10 µl of CD4 PE reagent was added and mixed gently, covered with an aluminium foil and incubated for 15 minutes. After incubation, 800µl of dilution buffer was added into the tube, shaken and mixed gently by a vortex machine. A count analysis was then done using the Cytoflow sytem and CD4+ cells were enumerated and recorded.

3.11 Testing of HIV by Enzyme linked immunosorbent assay (ELISA)

HIV ELISA testing was done using Murex HIV 1.2.0 pre coated test. Briefly, a volume of 50 µl of diluent was added to each well of a microwell plate using a multichannel pipette. This was then followed by 50 µl of sample or control to the appropriate well. Wells A to E were reserved in the first column for controls. Controls were then added to the designated wells after dispensing the samples. Samples were added starting from well G1. A volume of 50 µl of the negative control was added into each of the three wells A1, B1 and C1, while 50 µl of the anti-HIV-1 and anti-HIV-2 positive controls were added into wells D1 and E1 respectively. The plate was then covered with a lid and incubated at 37 ^{0}C in the dark.

At the end of the incubation time, the plate was washed using an automated washer. Once washing was complete, the plate was inverted and firmly tapped on a clean paper towel to remove excess wash fluid. A volume of 50 µl of conjugate solution was then

added to each well using a multichannel pipette and the wells were covered and incubated at 37 ^{0}C for 30 minutes. At the end of the incubation time, the plate was then washed as described before. This was followed immediately by adding 100 µl of substrate solution to each well using a multichannel pipette. The wells were then covered and incubated at 37 ^{0}C for 30 minutes and kept away from direct sunlight. After the incubation, 50 µl of stop solution (0.5M to 2M sulphuric acid) was added to each well using a multichannel pipette. The absorbance was then read within 15 minutes at 450 nm.

3.12 Data management and analysis

Data quality was done by pre testing questionnaires before use and using double entry system in entering data in the computers. This was supervised by the collaborating partners sponsored by FHI/USAID. Queries were raised and resolved as soon as they were identified. Quality control was done to ensure reliability and validity of data. Data collected from patients at the Comprehensive Care Centre (CCC) were captured in the electronic data base and exported to excel work book 2010. Data analysis was done using SPSS Version 11.0 statistical software. Basic characteristics of the study samples were summarised using simple proportions and means, median and inter quartile ranges.

Further analysis was done to perform one way ANOVA comparing more than two means followed by Post hoc Student Newman Keul for multiple comparisons . Independent t-test was used to calculate if there was any significant difference between the two treatments. Study subjects were followed from HAART initiation to the earliest

of death, loss to follow up, development of toxicity or end of twelve months. Data were presented by use of frequency distribution tables, bars and graphs. In both statistics, 95% confidence level was used and all statistical tests were considered significant at $P < 0.05$. The data were presented qualitatively and quantitatively by way of narrative, description, tabulation and discussion.

3.13 Ethical considerations

Permission was sought from Kenyatta University graduate school whereas research permit was obtained from National Council for Science and technology (NCST). Clearance was obtained from the medical superintendent at Coast Provincial General Hospital- Mombasa. Consent was received from participants and confidentiality was observed all the time.

CHAPTER FOUR: RESULTS

4.1 Baseline characteristics and treatment outcome

4.1.1 Baseline characteristics of study participants

The majority (121) of patients in the study cohort were in the age group of 29-39 years whereas the smallest number of patients were in the age group of 51-61 years. The age-group and frequency of the study clients are summarized in Table 4.1 (a) below.

Table 4.1a: Number of Patients enrolled per age group

Age group (years)	Frequency	Percentage (%)
18-28	30	12.0
29-39	121	48.2
40-50	70	27.8
51-61	30	12
TOTAL	251	100%

Forty-nine percent (49%) of the study group were males and fifty one percent (51%) were females. The mean age for the patients on EFV based HAART was 37.8 years (95% CI: 36.7 - 38.9) while the mean age for the patients on NVP based HAART was 37.3 years (95% CI: 36.1-38.2). The treatment arm of D4T/3TC/EFV had 35.46% males and 27.49% females whereas D4TC/3TC/NVP had 13.55% males and 23.51% females. The D4T/3TC/EFV treatment arm had more patients (159) compared to the D4TC/3TC/NVP which had 92 patients (Table 4.1b).

Table 4.1b: Demographic characteristics of patients enrolled per treatment group

Variable	D4T/3TC/EFV- Patients n=159	D4T/3TC/NVP - Patients n=92
Mean Age (95%CI)	37.8 (36.7 – 38.9)	37.3 (36.1 – 38.2)
Sex: Males 49.0 %	35.46%	13.55%
Sex: Females 51.0 %	27.49%	23.51%
Total Patients	159 (63.4)	92 (36.6)

4.1.2 Characteristics of participants at endpoint

At the end of the study period, a total of 233 patients were still participating in the study. Seven patients had their drug regimen changed. Eleven patients including those who had their drug regimen changed were lost to follow up at the end of the study. Patients were considered lost to follow up if they missed their last scheduled appointment by more than 30 days since their last visit. In total there were about 10% of the patients in this cohort who were either lost to follow up or died by the end of the study period (Table 4.1c). More than half the patients had at least one opportunistic infection. The antiretroviral treatment given was the first line and consisted of a combination of 2 NRTI's +1 NNRTI.

Table 4.1c: Mortality outcomes for patients enrolled in the study at end point

Category	**Frequency**	**Percentage (%)**
Completed	233	92.8
Died	11	4.4
Drug change / Lost to follow up	7	2.8
Total	**251**	**100**

4.2 Characteristics of patients body weight

Body weight increased for both of the two treatment groups after initiating antiretroviral therapy for the first six months (Table 4.2) and tapered over time between 6 months and 9 months period for the two treatment groups. The mean weight increased between 9th month and 12th month (1 year) period with a mean of 62.1 kg (95% CI 60.6 - 63.5) compared to baseline which was 55.6 kg (95% CI 54.1- 57.1. The average increase in body weight for patients on EFV over the 12 months period was 2.2 kg whereas for patients on NVP was less than 2 kg. There was no significant difference on the body weight for both patients on EFV and NVP groups ($p > 0.05$) as measured at 6 months. However, patients in both treatment groups experienced slight increase in weight between the 9th month and 12th month follow up period respectively.

HAART had modestly favourable effects on body composition, particularly in patients with greater pretreatment immunosuppression and virological suppression. However, body weight was found to have subject variability with consistent mean body weight increases among the NVP based and EFV based treatment arms in the study period.

Table 4.2: Body weight in kilograms of patients in the EFV and NVP study groups

Time in months	Efavirenz	Nevirapine	*p* - value
0 months	56.21±12.59	54.75±10.38	0.347
3 months	57.49±12.29	56.48±10.18	0.504
6 months	59.18±12.31	58.80±10.00	0.797
9 months	59.49±12.29	58.30±10.29	0.432
12 months	62.49±12.29	61.30±10.29	0.432

4.3 CD4+ T lymphocyte counts

Immunological variables were measured on a maximum of 5 occasions. There were significant differences in CD4 lymphocyte subsets between patients on EFV and patients on NVP treatment arm ($p < 0.01$) at the 6^{th}, 9^{th} and 12^{th} month. The means of the differences between changes for EFV and NVP were significantly different ($p < 0.001$). The data on CD4 count confirm that patients at enrolment had lower levels of CD4 cell count at the onset of the study for both groups. In addition, the data indicate that the mean numbers of CD4 count were slightly higher for the patients on NVP regimen compared to EFV regimen at the baseline with means of 165.11 (103.78 – 226.44) and 133.75 (41.77 – 225.73) respectively.

The mean CD4 cell count for patients on both regimens increased to the 12^{th} month period suggesting positive immune response following HAART therapy. During the first 6 months of ART, the number of CD4+ T cells typically increased by 30 cells to 60 cells/mm^3 of blood. This was then followed by a second, faster phase of T cell

repopulation with an average rate increase of 70 cells / mm^3 in the 9^{th} month of ART (Table 4.3).

Table 4.3: Mean CD4 + cells for patients in the study CD4 + Cells/mm^3

Time in months	Efavirenz	Nevirapine	*p*-value
0 months	133.75±91.98	165.11±61.33	0.001
3 months	171.32±103.38	139.74±63.65	0.003
6 months	200.07±114.28	161.39±68.33	0.001
9 months	270.07±114.28	231.39±68.33	0.001
12 months	365.07±114.28	326.39±68.33	0.001

4.4 Hemoglobin levels during the study period

Hemoglobin levels increased for patients in both arms of treatment (EFV and NVP) groups up to the 6^{th} month. However, there was a sudden decline of hemoglobin levels towards the 9^{th} month. Hemoglobin levels increased further from 9^{th} month to 12^{th} month during the study. There was no significant difference in haemoglobin levels of patients on EFV and NVP regimens throughout the study ($p > 0.05$) (Table 4.4).

Table 4.4: Mean of Hemoglobin (g/dl) during the 12 months study period

Time in months	Efavirenz	Nevirapine	*p*-value
0 months	10.49±2.40	10.03±2.29	0.135
3 months	11.18±2.39	10.92±2.30	0.406
6 months	11.88±2.52	11.82±2.39	0.858
9 months	11.58±2.39	11.33±2.33	0.145
12 months	11.98±2.39	11.73±2.35	0.420

Reference ranges for Hemoglobin: Adult Male: 13.0g/dl – 18.0 g/dl and Adult Female: 12.0g/dl – 15.0g/dl

4.5 Liver enzymes (ALT) before and after initiation of HAART

The monitoring of patients through the two ARV regimens allowed the analysis to be done for toxicity. There was no significant difference in the mean values of ALT enzymes for patients on EFV based regimen and NVP based regimen from baseline to the 12^{th} month ($p > 0.05$). Nevertheless, the hepatic biological tolerance during EFV or NVP based regimen was generally good in months 3 and 6. The majority of the mean values of ALT enzymes studied were above normal ranges except for those at baseline.

The comparison of ALT enzymes after initiation of HAART has shown that changes in these parameters were similar in both HAART regimens. The early hepatic biological tolerance during EFV or NVP based regimen was generally good and similar. However, increases in the enzyme values for ALT were observed with the NVP based regimen when compared with EFV regimen (Table 4.5).

Table 4.5: Mean Liver Enzymes ALT (U/L) for patients during the study

Time in months	Efavirenz	Nevirapine	*p*-value
0 months	49.81±3.48	44.83±4.40	0.379
3 months	67.86±5.13	62.39±5.29	0.485
6 months	85.80±9.21	90.38±14.55	0.780
9 months	126.57±12.13	137.89±21.40	0.620
12 months	165.99±18.30	183.32±31.09	0.608

Reference ranges for ALT enzyme: Adult Male and Female: 7 U/L – 55 U/L.

4.6. Creatinine trends of patients in the study

The Creatinine levels increased minimally for all patients from onset of ARV's up to a maximum of 154 umol / L at the end of the follow up**.** There was significant difference in Creatinine levels for the two regimens with Nevirapine giving higher values than Efavirenz regimen ($p < 0.001$). At endpoint patients on EFV regimen had Creatinine mean of 135.61±0.89 compared to NVP mean of 151.04±3.30 (Table 4.6). However, none of the patients reached a Creatinine level of >300 umol / L to warrant drug discontinuation or regimen change.

Table 1: 4.6: Mean Creatinine levels (umol/L) for patients on EFV and NVP

Time in months	Efavirenz	Nevirapine	*p* -value
0	74.16±2.60	75.19±3.37	0.808
3	91.47±1.51	94.49±3.29	0.405
6	115.83±1.21	123.15±3.23	0.036
9	128.81±1.02	137.08±3.11	0.003
12	135.61±0.89	151.04±3.30	< 0.001

Reference ranges for Creatinine: Adult Male: 80.0 U/L – 115.0 U/L and Adult Female: 53.0 U/L – 97.0 U/L

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Effect of HAART on body weight changes

Given measurements of CD4 cell counts are not routinely available in resource poor settings, and because body weight is an independent predictor of HIV progression, body weight was assessed as one of the clinical outcomes (Barnejee *et al*., 2010). The current study found that mean body weight gains mirrored CD4 cell increase immediately following antiretroviral therapy initiation and this correlated with the findings of Barnejee *et al.* (2010) which showed that HAART had a positive effect on growth in HIV 1 infected individuals. Decrease in body weight and body mass are common problems in HIV infected persons. Depletion of body cell mass may occur early in asymptomatic HIV infected patients before progression to AIDS.

In a previous study HIV-1 infected individuals and especially children experienced a continued gain in body weight after starting HAART (Denue *et al.,* 2013). The substantial variability in weight could be attributed to patients' inability access food consistently. However, the positive correlation between weight increase and increase in CD4 cell count indicates that at least some of the variability in body weight reflects variations in clinical status. Therefore, in the absence of reliable food supply, weight should be used with caution in assessing individual responses to antiretroviral therapy.

5.1.2 Effect of HAART on CD4 profiles

This study has demonstrated clinical benefits in terms of CD4 cell count and weight increase well into the end of first year of follow up. The finding of a rapid rise in CD4 cell counts during the initial few weeks of therapy followed by a slower rise for the patients in the current study are consistent with earlier reports on CD4 cell kinetics conducted in the USA. In addition, the mean CD4 cell increase seen in patients in this study at one year after antiretroviral therapy initiation is similar to the response seen in studies conducted in Europe (Lifson *et al.*, 2011). As such, the mean CD4 cell increase seen at later time periods reflect responses to both primary and secondary regimens (about 10% of the patients in the study switched to second line therapy). Alternatively, individuals in the current study may have different hepatic regeneration capacities at the initiation of ART. For this study population, $CD4^+$ cells had continued to increase up to 12th month period. In fact, even among highly advanced patients who initiated HAART with CD4 cell counts below 200 cells/mm3, subsequent increase in CD4 cell count was most strongly predicted by a advance treatment to HAART. Previous studies showing that CD4 cell counts <350 cells/mm3 may preclude a CD4 cell count response may have been confounded by patient non adherence (Kenneth *et al*., 2013).

It has been suggested that HIV-1 infection through a process of chronic immune activation and increased T cell differentiation accelerates immune aging leading to a reduction in T cell renewal and an accumulation of terminally differentiated $CD4^+$ cells (Madhu *et al.*, 2013). The main immunologic outcome observed was the change in CD4

cell count over time. A switch in therapy occurred if the ART regimen recorded at follow up was different from the regimen initially started. Any individual drug substitution or regimen change was considered a switch. Dose reduction however, was not considered an ART switch. The time of the first switch defined the time of reaching the outcome. If the physician recorded a symptom during a clinic visit believed to be attributed to ART, a toxicity event was considered to have occurred. While the study reflects what happens in real clinical setting, the results should be interpreted with some caution considering a number of limitations. Loss to follow up is a common limitation in observation control studies and may introduce bias to estimate if the individual lost to follow up had a different pattern of exposure variables and severe hepatotoxicity from those retained in the cohort.

5.1.3 Effect of HAART on renal function

Acute renal failure is frequently caused by the toxic effects of antiretroviral therapy or nephrotoxic antimicrobial substances used in the treatment of opportunistic infections. In the present study, none of the patients had renal failure but the study showed that closer monitoring of renal function is essential in all HIV patients on HAART. This result is consistent with the study of Mainasara *et al*. (2014) which showed that HAART of Stavudine, Lamivudine and Nevirapine improve renal creatinine clearance functions among the HIV positive patients. Similar results were also obtained by Robert *et al*. (2015) on the study of predictors of renal outcome in HIV associated nephropathy.

HAART and other medical therapies for HIV related infections have been associated with both short and long term toxicities including nephrotoxicity. Antiretroviral therapy can contribute to renal dysfunction directly by inducing acute tubular necrosis, acute interstitial nephritis, crystal nephropathy and tubular disorders or indirectly via drug interactions (Robert *et al*., 2015). Infection with human immunodeficiency virus (HIV) has also been associated with many types of renal diseases including acute renal failure; acute tubular necrosis and HIV associated nephropathy (Emmanuel, 2010). HIV related renal impairment can present as acute or chronic kidney disease; it can be caused directly or indirectly by HIV and / or by drug related effects that are directly nephrotoxic or lead to changes in renal function by inducing metabolic vaculopathy and renal damage.

Acute renal failure is frequently caused by the toxic effects of antiretroviral therapy or nephrotoxic antimicrobial substances used in the treatment of opportunistic infections. Chronic renal disease can be caused by multiple pathophysiological mechanisms, leading to HIV associated nephropathy, a form of collapsing focal glomerulosclerosis, thrombotic microangiopathy and various forms of immune complex glomerulonephritis (Robert *et al*., 2015). Studies have shown that HAART has the capability of increasing the risk of kidney disease or renal failure which is associated with disease progression and death in HIV infected patients. Nephropathy is a common finding in patients infected with HIV, and it necessitates increased surveillance and adaptation of dosages of HIV drugs (Robert *et al*., 2015). Direct effects of HIV seem to play a major role in the development of HIV-associated nephropathy (HIVAN) and thrombotic

microangiopathy. Improved survival among patients with HIV infection is anticipated to result in an increase in the long-term development of HAART-associated metabolic complications, such as diabetes and dyslipidemia, which, in turn, can be contributed to vascular changes and decreased renal function (Boua *et al*., 2012). Many antiretroviral agents are eliminated at least partly by the kidneys and require dosage adjustments in patients with HIV-1 infection. With HAART multiple medications are prescribed for treatment of HIV infection. Patients are also prescribed other medications for prevention or treatment of opportunistic infections and for management of their other medical conditions. Therefore the potential for drug –drug interactions is significant (Henry *et al*., 2012).

Since the introduction of HAART, varieties of renal side effects and adverse drug reactions have been recognized and vary. Acute renal failure in HIV-infected persons can be caused by the same mechanisms that cause it in HIV uninfected patients (Mitchelle *et al*., 2010). The prevalence of chronic kidney disease in the various stages of HIV infection and treatment is difficult to assess. Proteinuria and elevated creatinine level have been found in 7.2% to 32% of HIV seropositive patients (Mitchelle *et al*., 2010). The expected increase in the incidence of renal disease and end- stage renal failure might to some extent be prevented by close monitoring of renal function.

5.1.4 Effect of HAART on Liver enzyme Alanine aminotransferase

The overall effect of HAART on the liver is the result of the balance between hepatotoxicity and the consequences of immune-reconstitution on the evolution of HIV – associated liver diseases. HAART may lead to the emergence of acute toxic hepatitis, steatosis, steatohepatitis, liver fibrosis and non-cirrhotic portal hypertension (Pineda *et al*., 2010). In reality, all HAART drugs have the potential of causing severe hapatotoxicity (Henry *et al*., 2012). The results of this study should therefore be inferred while aware of the fact that all HAART drugs have a potential of causing severe hepatotoxicity (Adikwu *et al*., 2013). Antiretroviral drug induced liver injury (ARLI) is defined by elevations in liver enzymes in serum, with alanine aminotransferase (ALT) characteristically greater than aspertate aminotransferase (AST). It is one of the greatest causes of treatment discontinuation in HIV infected patients (Lucien *et al*., 2010). A pattern of drug injury with nevirapine use had emerged, with onset of liver enzyme elevations occurring beyond 16 weeks of therapy, consistent with direct or idiosyncratic host mediated liver injury. HAART related drug reactions should be considered as host factors that may determine and influence HIV- 1 disease progression (Ramana, 2014).

Non-nucleoside reverse transcriptase inhibitors like NVP and EFV are often used in combination with other ARVs for the treatment of HIV-1 infection and contribute to significant hepatotoxicity. The toxic effects of antiretroviral therapy play a key role in the pathogenesis and progression of HIV disease. This was observed with their use in clinical trials in practice (Ramana, 2013). In the current study, the initiation of ALT

biochemical parameter from month 0 to month 6 is variable with EFV based regimen. Liver Function enzyme tests for both patients on EFV and NVP were generally normal suggesting good hepatic biological tolerance by patients on the two treatment regimens. These results tie with those obtained by another study by Lucien *et al.* (2010) which showed that HAART was associated with low level hepatotoxicity at therapy initiation, regardless of drug class. The lack of significant difference in the proportion of patients who experienced an increased enzyme activity between these two ARVs regimens could be due to the fact that Stavudine toxicity present on both arms of treatment could have masked the actual toxicity experienced by NVP. In another related study, patients who have been on HAART had significantly elevated ALT and AST levels but mild toxicity (Shakirat *et al*., 2014). The current study was also comparable with that of Kalyesubula *et al*. (2011) who in their retrospective cohort study determined the incidence of hepatotoxicity associated with (NNRTI) in a group of HIV infected patients who received EFV and NVP and the rate was similar among the treatment groups.

It has been reported in another study by Lucien *et al.,* (2010) that some patients who experienced serious liver toxicity with NVP did not develop hepatotoxicity during subsequent treatment with EFV, suggesting that toxicity was related to ARV and not to specific class (Lucien *et al.,* 2010). It was also noted in the current study that hepatotoxicity with NVP or EFV does not appear to increase the risk of developing liver damage from exposure to NNRTIs as noted in the study by Manosuthi *et al*. (2006). The high incidence rate of severe hepatotoxicity in the first 3 months of initiating ART

necessitates the need for more frequent and careful monitoring of ALT levels early during therapy. The present study showed that patients on nevirapine based regimen had a higher risk of developing severe hepatotoxicity when compared to their counter parts on an efavirenz based regimen, a result consistent with findings from previous studies (Dart trial team, 2010).

Significant toxicity of ART was frequently observed in the study including mortality which was attributed to drug toxicity. The incidence of laboratory adverse events was higher in this study compared to industrialised countries but similar to investigations in other resource limited settings (Robert *et al*., 2015). One reason for this could be the lack of therapeutic alternatives. Many patients had to continue their treatment despite low grade toxicity. ART was frequently interrupted due to ALT elevation. Several adverse events were reported in the study including vomiting, rash, acute hepatitis, hypersensitivity reaction, peripheral neuropathy, asthemic, anemia, pancreatitis, lactic acidosis and psychiatric mood disorder.

5.1.5 Effect of HAART on Haemoglobin levels

The present findings show that there is an association between anemia, decreased survival, and increased disease progression in patients with HIV infection. Even with use of HAART anaemia remains strongly and consistently associated with HIV disease progression. As hemoglobin levels decrease, the risk of disease progression increases (Simbarashe *et al.,* 2013). HAART has the capability of reducing the incidence of anaemia which is associated with disease progression and death in HIV infected

patients. In these study low platelets counts resolved in patients on ART and were probably not drug related. Thus monitoring of hemoglobin would have been enough to detect nearly all of the significant cases of anaemia. Metabolic abnormality associated with potent antiretroviral regimens including NVP may revert at least partially with time and after replacing NVP by EFV as observed in this study. In a different study haemoglobin changes following HAART varied by sex and age, but remained significantly associated with prognosis in both sexes (Florence *et al.,* 2011). Also studies from other developed countries suggest that use of HAART reduces the risk of anemia in patients with HIV infection and improves hemoglobin values in many patients who are already anemic at the time of HAART initiation (Simbarashe *et al.,* 2013).

Anemia has been shown to be the most frequent hematological abnormality in HIV infected patients globally. Recent reports suggest that hemoglobin levels improve with ART (Simbarashe *et al.,* 2013). However, few studies have documented the evolution of hemoglobin levels among patients on ART in resource limited settings, and whether the effects on hemoglobin levels vary by ART regimen. The current study showed decreased anemia with HAART use, which supports data from prior studies which showed that patients on HAART had improved hemoglobin levels and less incidence of anaemia (Chauhan *et al*., 2011). Similarly, studies by Zelalem *et al*. (2014) found that HAART was an effective treatment for anemia of HIV infection, and the potential mechanisms that might be involved included a reduction in opportunistic infections and the anemia of chronic disease, and an improvement in nutritional status. Lower body

mass index was also associated with a high risk of anemia. This association may be caused by deficiencies of many micronutrients, including iron, folate, B12, and vitamin A, which contribute directly to anemia.

It is of concern that adequate treatment of anemia is not always considered in developing countries because most attention is paid to HIV infection and the frequent complications such as opportunistic infections. Previous studies suggest that recovery from anemia is associated with improved survival among persons with HIV infection (Zelalem *et al*., 2014). If recovery from anemia is shown to directly increase survival, screening for anemia should receive more attention and the patients with anemia should be managed properly. Diagnosis of anemia is made by measurement of hemoglobin, which is one of the simplest techniques in the laboratory.

5.1.6 Effect of HAART on HIV/AIDS progression

Previous studies have shown that toxicities due to HAART is associated to progression to AIDS and death (Paolo *et al*., 2012).The probability of reaching a combined treatment failure end point, which included treatment discontinuation (due to adverse events or losses to follow up) and treatment change, was higher for NVP HAART than for EFV HAART. The superior antiviral activity of EFV HAART over NVP HAART was consistently demonstrated by switching of NVP based HAART to more consistent regimen because of intolerance which are common clinical practices. In this study, 19% of patients treated with NVP HAART suffered from adverse events that prompted a switch from NVP to EFV for reasons of intolerability. Morbidity rates of HIV infected

patients from the study at Coast Provincial General Hospital fell substantially within the first few months of HAART.

Patients in Mombasa settings started treatment with considerably more advanced immunodeficiency than those from western countries (The UK collaborative Committee, 2010) but immunological responses to HAART were similar in both settings, a finding that tallies with results from a recent collaborative analysis of treatment sites (Annison *et al.,* 2013)

High mortality was observed in patients who had low baseline CD4 counts which was consistent with a study conducted by Addisu *et al.* (2015) which showed that consideration should be given to initiation of HAART at a CD4 cell count > 350 cells /ul to achieve better immune recovery. The current study showed similar co-morbidities were present in many patients starting HAART including tuberculosis and invasive bacterial and fungal infections which might have increased mortality as also indicated and observed in other studies (Achkar *et al*; 2011). There was generally low mortality throughout the study. However, even the first months of HAART, mortality was lower than previously noted in untreated patients suggesting an early beneficial effect of HAART. This is comparable with a study which showed advanced immunodeficiency was associated with subclinical and high disseminated infections with high mycobacterial antigen load and rapid improvement of immune function during HAART (Achkar *et al*., 2011). Therefore, eligibility for antiretroviral therapy and the need for treatment of the tuberculosis should be determined earlier and HAART should be started before serious comorbidities develop (Addisu *et al*., 2015).

Efavirenz has been shown to be highly efficacious regardless of initial CD4 count. While this study has compared the response rates to nevirapine for different starting CD4 counts, the data reveal similar success rate for both the NNRTIs, suggesting a good response to both Efavirenz and Nevirapine at low CD4 counts. There was a trend towards poorer CD4 count response to NVP based regimen but these no longer constitute a reasonable choice for first line therapy. Patients on ART in this current study with poor CD4 recovery were at a greater risk of progression to AIDS similar to those patients in a study conducted by Simbarashe *et al*. (2013).

5.2 Conclusions

i. The one year (52 weeks) results from this study demonstrated that D4TC/ 3TC/ EFV and D4TC/ 3TC/ NVP combinations were safe, well tolerated and effective in increasing CD4 Cell counts and suppressing HIV progression in advanced HIV infected patients. Majority of patients who died were on NVP regimen in addition to having a history of elevated liver enzymes. There were significant clinical and immunological changes in the patients that appear to persist for at least a year after initiation of antiretroviral therapy.

ii. The study has shown that body weight changes increased significantly for patients in the two treatment regimens after initiation of ART. However, weight was found to have substantial subject variability.

iii. The study also showed that haemoglobin levels increased significantly for both patients in the two treatment regimens after initiation of ART. HAART produces

an improvement of hemoglobin levels. The findings showed that anemia is a frequent complication of HIV/AIDS infection in female patients.

iv. The study has shown that D4T/3TC/EFV was a superior regimen compared to D4T/3TC/NVP. Creatinine levels had increased for patients in both treatment regimens but were not >300 U/L to warrant regimen change or drug discontinuation. There was no significant difference observed in the liver function of patients on the two treatment groups although increase in individual liver enzymes (ALT) were observed in both treatment groups towards the end of the study period. The ALT elevations were also found to have substantial subject variability.

5.3 Recommendations

i. The CD4 cell count serves as the major laboratory indicator of disease progression. The baseline and subsequent CD4 cell counts also act as the strongest predictor of disease progression, survival and treatment. It is one of the key factors in determining both the urgency of antiretroviral therapy initiation and the need for prophylaxis of opportunistic infections. Patients with HIV should have their CD4 cell count monitored regularly before and after commencement of HAART before they reach immunological failure.

ii. The findings support the ongoing feasibility of early ART roll out in the country. Early commencement of HAART leads to improvement of healthcare for the HIV infected patients. This improvement has been noted in terms of weight gain

and delay in disease progression. Therefore, efforts should be made to maximize HAART accessibility, adherence and tolerability to patients who require HAART by availing durable and tolerable regimens so as to minimize the number of patients switching therapy or withdrawing from these important life saving programs. These endeavors may include the use of more tolerable first line HAART, ensuring unlimited HAART provision and consideration of alternative treatment options.

iii. The treatment of patients with anemia should target its cause, but it is important to know that early start of HAART may prevent anemia or reduce its severity. Our study had a small sample size. A wider study that includes several HAART regimens and a long-term follow-up is recommended.

iv. Therapeutic monitoring may be a useful tool for the administration of HAART in the future. In the future, closer monitoring of renal function and adverse effects of HAART should be enhanced as these parameters appear elevated with time as patients continue to use the drugs. Renal function and Liver function should therefore be monitored on a regular basis in patients with HIV receiving any antiretroviral agent. With the increase in HAART use, clinicians must screen patients for the development of kidney disease especially if the regimen employed increases risk of kidney injury.

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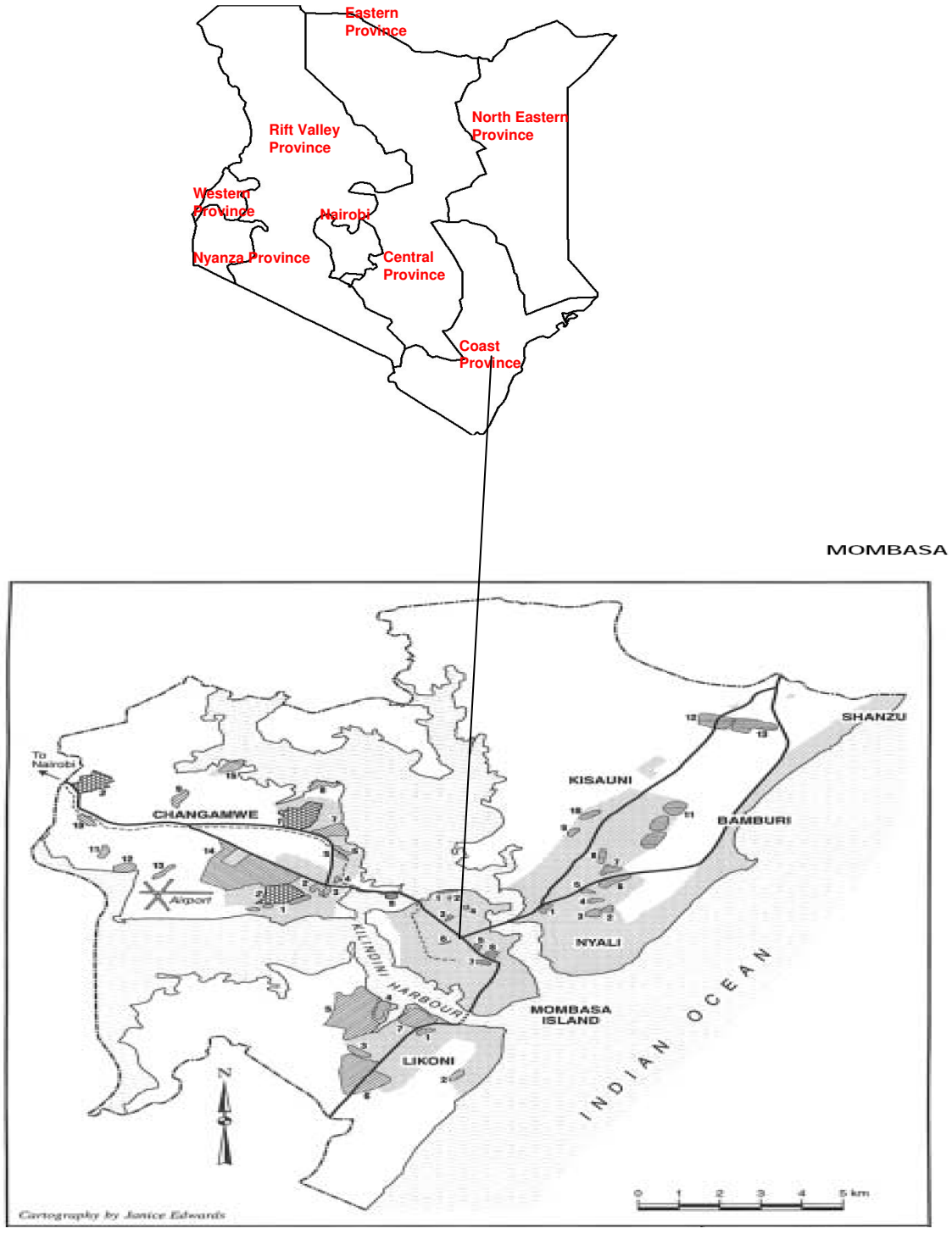
APPENDICES

Appendix I: Map of Kenya Showing Coast Province



Appendix II: Participant Questionnaire Forms

Personal history

All the following questions are to be asked directly to the participant

1. (a) What month and year were you born?_____ *If unknown, code 99 99*

   (b) How old are you_____ (if unknown, give estimate)? Years

   Compare and correct Q1a or Q1b if inconsistent.

2. Education

   (a) Have you ever attended school? 1 = Attended 2 = Never attended **[□ Q3d]**

   (b) What is the highest level of education you reached? 1 = Primary 2 = Secondary 3 = Higher

   (c) How many years did you complete at that level (excluding repetition)?_____

   (d) Have you ever attended technical training or adult education? 1 = No 2 = Yes

4. Occupation

   (a) Are you currently studying? 1 = No 2 = Yes, part-time 3 = Yes, full-time

   (b) Apart from your own housework, are you currently working? 1 = Working 2 = Not working**[□ Q5]**

   (c) What is your main occupation? 1 = Salaried job 2 = Self-employed professional 3 = Casual worker 4 = Kiosk owner 5 = Hawker 6 = Artisan 7 = Commercial farmer (i.e. coffee, tea, sugar cane, dairy) 8 = Subsistence farmer 9 = Other, specify _____ _____

5. Marital status

(a) What is your current marital status? 1 = Married monogamous [**Q5e**] 2 = Married polygamous [**Q5b**] 3 = Not married, regular partner [**Q5e**] 4 = Not married, no regular partner [**Q7**] 5 = Divorced or separated, no regular partner [**Q5c**] 6 = Widowed, no regular partner [**Q5d**]

Appendix III: Screening Interview (SCI)

General Interview Date of visit_____

1. (a)From which clinic was the potential participant referred? _____

2. (a) HIV-1 infection documented? Yes □ No □

(b) HIV counselling received? Yes □ No □

3. At least one preparatory visit completed? Yes □ No □

4. (a) Does the potential participant currently take any drugs? Yes □ No □

If 'no', *go to Q8 if yes proceed with Q5.*

5. Are those drugs:

(b) Antiretroviral drugs? Yes □ No □

(c) Corticosteroids (more than 7 days), benzodiazepines, anticoagulants, magnesium sulphate or rifampicin? Yes □ No □

6. Is the participant expected to require corticosteroids, benzodiazepines, anticoagulants, magnesium sulphate or rifampicin during the study period? Yes □ No □

7. Is the participant known to be allergic to Stavudine, Lamivudine, Efavirenz or benzodiazepines? Yes □ No □

8. (a) Has the participant already participated in this study before? Yes □ No □

(b) Has the participant ever taken part in an HIV vaccine trial? Yes □ No □

If yes to any part of Question 6-8 then exclude participant from the study and refer to appropriate clinic

9. Is the participant living within the catchment area of the study clinic and expects to remain there for at least one year? Yes □ No □

10. Is the participant willing to take HAART regimen? Yes □ No □

11. (a) Is the participant able to give informed consent to be screened? Yes □ No □

(b)Has the participant signed the screening consent form? Yes □ No □

Eligibility

If *the potential participant is HIV positive, has a CD4 count lower than 350 cells/µl or in WHO stage 3 or 4 has been counseled and has consented to join the study he/ she should be offered long-term HAART and included in the cases arm of the study. If not eligible for HAART according to WHO clinical staging and CD4 count greater than 350 cells/µl, the participant should receive counseling be allocated to the Control arm of the study and referred to the usual follow up clinic. Female participants if positive for pregnancy test should be referred to PMCT Clinic.*

12. Based on this interview, is the potential participant eligible to continue?

Yes □ No □

Remarks _____

Name _____ Signature _____

Day Month Year ____ / ___ / _____

Appendix IV: Screening – Laboratory Form

Samples Day Month Year

1. Participants name/ ID:_____ Date sample collected ____ / ____ / _____

   Collected by: Name_____ Received by: Name_____

Blood test

2. (a) HIV confirmation test 1 = Negative 2 = Positive 3 = Indeterminate

   (b) Pregnancy test (female participants) 1 = Negative 2 = Positive 3 = Indeterminate

3. Haematology (a) (i) Haemoglobin _____g/dL

   (ii) Packed Cell Volume _____

   (b) (i) Leukocytes _____cells/mm3

   (ii) Neutrophils _____cells/mm3

   (iii) Lymphocytes _____cells/mm3

   (c) Platelets _____X 1,000/mm3

4. Flowcytometry (a) (i) CD4 _____% (ii) CD4 count_____ cells/mm3

   (b) (i) CD8 % _____ (ii) CD8 count _____ cells/mm3

5. Biochemistry enzymes ULN Result

   (a) Creatinine_____ mg/L OR µmol/L _____

   (b)LFT's: Normal □ Abnormal □

Eligibility

6. *Review laboratory test results Tick relevant box*

(a)HIV-positive test confirmed Yes □ No □

(b)Haemoglobin > 18.0 g/dl Yes □ No □

(c)Neutrophils > 750 cells/mm3 Yes □ No □

(d)Creatinine < 2xULN Yes □ No □

(e)LFT's <1xULN Yes □ No □

If any response falls in a No box, *the potential participant is not eligible for the study.* ***If all responses fall in Yes boxes,*** *the participant is eligible to continue.*

7. Based on this clinical assessment, is the potential participant eligible to continue?

1 = Eligible 2 = Temporarily not eligible 3 = Pending test results 4 = Definitely not eligible

If 'definitely not eligible' *the participant should receive counselling and be referred to the usual ART for follow up.* ***If 'temporarily not eligible'*** *or* ***'pending tests results'****, schedule another screening appointment two weeks later.*

Name _____ Signature _____

Day Month Year ____ / ____ / _____

Appendix V: Screening Clinical Assessment (SCA)

WHO Clinical Staging

General

	Day	Month	Year
1. Date staging conducted	_____ /	_____ /	_____

Opportunistic diseases

2.	Stage Status	Diagnosis	Date onset	Last episode
	_____	_____	_____	_____

Details of investigations

A. General

1. Persistent generalised lymphadenopathy

2. Unexplained prolonged fever (intermittent or constant) for more than 1 month

3. Wasting syndrome (weight loss more than 10% + chronic diarrhoea or chronic weakness and unexplained prolonged fever [> 1 month]) 4

B. Skin

1. Minor cutaneous manifestations (e.g. seborrheic dermatitis, prurigo, fungal nail infections)

2. Herpes zoster in the last 5 years 2

C. Head and neck

1. Minor oral manifestations (e.g. recurrent oral ulceration, angular cheilitis)

2. Recurrent upper respiratory tract infections (e.g. bacterial sinusitis) 1 = Never 2 = Yes, not currently 3 = Yes, currently 1 = Presumptive 2 = Definitive If unknown, code 99 9999.

3. Oral candidiasis

4. Oral hairy leukoplakia

D. Chest

1. Pulmonary tuberculosis within the previous year

E. Abdomen

1. Chronic diarrhoea (> 1 month)

2. Cryptosporidia with diarrhoea (> 1 month)

F. Neurological

1. Toxoplasmosis of the brain.

2. Progressive multifocal leukoencephalopathy

3. HIV encephalopathy

G. Other (infection)

1. Extra pulmonary cryptococcosis

2. Cytomegalovirus disease (outside spleen, liver or lymph nodes)

1 = Never 2 = Yes, not currently 3 = Yes, currently 1 = Presumptive 2 = Definitive. Write the name and result of each investigation. Write 'pending' if the investigation is awaiting result

3. Herpes simplex virus infection, mucocutaneous (> 1 month) or visceral of any duration

4. Any disseminated endemic mycosis (e.g.histoplasmosis, coccidioidomycosis)

5. Candidiasis of the oesophagus, trachea, bronchi or lungs

6. Severe bacterial infections (e.g. pneumonia, pyomyositis)

7. Atypical mycobacteriosis, disseminated

8. Non-typhoid *Salmonella* septicaemia

9. Extrapulmonary tuberculosis

H. Other (non-infection)

1. Lymphoma

2. Kaposi's sarcoma

3. Cervical cancer

1 = Never 2 = Yes, not currently 3 = Yes, currently 1 = Presumptive 2 = Definitive. Write the name and result of each investigation. Write 'pending' if the investigation is awaiting result.

4. Weight loss 1 = None 2 = Less than 10% of body weight (Stage 2) 3 = More than 10% of body weight (Stage 3)

5. Current performance level 1 = Normal activity - Asymptomatic (Stage 1) 2 = Normal activity - Symptomatic (Stage 2) 3 = In bed less than 50% of the day during the last month (Stage 3) 4 = In bed more than 50% of the day during the last month (Stage 4) 5 = In bed due to pregnancy-related problem only

Staging *Review the answers to Q3, Q4 and Q5. Determine which pathology (in the past or currently) corresponds to the highest stage. Report this stage as the current HIV clinical stage.*

6. Current HIV clinical stage ***If investigations not complete,*** *wait until results available (maximum 2 weeks).* ***After 2 weeks,*** *if waiting for investigations which might modify current disease stage.*

Remarks: Name _____Signature _____Date __ / ___ / _____

Tick relevant boxes

1. Does the potential participant currently have any of the following clinically-significant condition(s) likely to require specific care arrangements and which may interfere with study interventions?

(a) Obstetric	Yes □	No □
(b) Cardiac	Yes □	No □
(c) Respiratory	Yes □	No □
(d) Active tuberculosis	Yes □	No □
(e) Hepatic	Yes □	No □
(f) Gastrointestinal	Yes □	No □
(g)Endocrine	Yes □	No □
(h)Renal	Yes □	No □
(i) Haematological	Yes □	No □
(j) Psychiatric	Yes □	No □
(k) Neurological	Yes □	No □
(l) Allergic	Yes □	No □
(m)Other chronic disease/condition	Yes □	No □

If 'yes', specify _____

2. (a) Does the participant has a cough for more than 3 weeks? ***If 'yes',*** *schedule sputum tests and a chest X-ray.*

   (b)Does the participant present any enlarged lymph node suggestive of extra-pulmonary tuberculosis? ***If 'yes',*** *schedule a fine needle aspirate.*

3. Is she known to have clinical AIDS or CD4 count < 350?

Eligibility

Any significant condition *(any ticked yes box in Q1 or Q2) Not eligible.* ***Clinical AIDS or CD4<350?*** *(Review Q2) Eligible.* ***Suspected tuberculosis?*** *(review Q2a or b) Not Eligible Pending results of TB tests.*

Based on this clinical assessment, is the potential participant eligible to continue?

1 = Eligible 2 = Temporarily not eligible 3 = Pending TB results 4 = Not eligible

[end questionnaire] If ***not 'eligible',*** *the participante should receive counseling and be referred to the usual follow up TB clinic.* ***If 'temporarily not eligible'*** *schedule another clinical assessment visit after two weeks. Go to Q6.* **Specimens** ***If 'eligible' or 'pending TB results'*** *take blood sample.*

4. Was a blood sample collected? 1 = Collected 2 = Not collected

Date for enrolment visit Day____ Month_____ Year_____

5. Scheduled date for review of test results and study enrolment

End of questionnaire

Date for repeat screening visit (if needed) Day Month Year

6. Scheduled date for repeat clinical assessment

Remarks Name _____ Signature _____

Date __ / __ / __ Day Month Year

HIV support

7. (a) Have you asked any person, organisation or support group for help and/or support to cope with your HIV disease or related issues (e.g. psychological, medical, legal support)? 1 = No **[□ Q7c]** 2 = Yes

(b) Provide information on anyone contacted *More than one answer possible. If the same person/organisation/support group provides different kinds of help and/or support, report one per line.* Name of person/organisation/support group Type of help/support requested Will you continue contact for this particular support?

1. _____

2. _____

3. _____

4. _____

5. _____

1 = Further counselling or information or psychosocial support 2 = Financial 3 = Income generating activities 4 = Drugs 5 = Food / Nutritional 6 = Administrative / legal 7 = Spiritual 8 = Other 9 = Not specified 1 = No 2 = Possibly 3 = Yes 9 = Don't know

HIV support (continued)

(c) (i) Do you feel you need more help and/or support in coping with your HIV disease or related issues? 1 = No **[□ Q8]** 2 = Yes

Ask the following question and report the answer(s) given. Do not prompt, except to ask "Do you need any other kind of support?" Mark up to 3 answers.

(ii) What kind of help and/or support do you need (report up to 3 main needs)?

1 = Further counselling or information or psychosocial support 2 = Financial 3 = Income generating activities 4 = Drugs 5 = Food / nutritional 6 = Administrative / legal 7 = Spiritual 8 = other _____ 9 = Not specified

Medication history

8. (a) Have you ever taken any antiretroviral medicines for HIV? 1 = No **[□ Q9]** 2 = Yes 9 = Don't know **[□ Q9]**

(b) Provide information on antiretroviral medicine(s) for HIV

Name of drug Code Reason Date started Date stopped

Month Year Month Year 1. _____

2. _____

3. _____

4. _____

1 = HIV treatment 2 = MTCT prevention 3 = Post-exposure prophylaxis 4 = Other *If unknown, code 99 9999. If 'ongoing', code 88 8888. If unknown, code 99 9999.*

(c) Please provide details on the health facility (name and location) and provider who prescribed antiretroviral drugs _____

9. (a) Have you ever taken any traditional/herbal medicine for your HIV infection? 1 = No **[□ Q10]** 2 = Yes 9 = Don't know **[□ Q10]**

(b) Provide information on the traditional/herbal medicine(s) for HIV

Name of traditional/herbal medicine Code

1. _____

2. _____

10. Are you currently taking any medicines or supplements other than those prescribed by our study clinic?

1 = No 2 = Yes 9 = Don't know

Medication history (continued)

11. (a) Have you ever had an allergy to any medication? 1 = No **[□ Q12]** 2 = Yes 9 = Don't know

[□ Q12]

(b) What medications caused you to react and what reactions did you develop?

Name of drug Reaction(s)

1. _____ _____

2. _____ _____

3. _____ _____

Continuation

12. Is the participant continuing in the study? 1 = Continuing 2 = Not continuing

Remarks Name _____ Signature _____

Day Month Year __ / __ / __

Appendix VI: Serious Adverse Events Report Form (SAE)

Adverse event characteristics

1. Is this adverse event a new HIV Stage 3 or Stage 4 condition?

   1 = No 2 = Yes.

2. Current status or outcome 1 = Complete recovery 2 = Not yet resolved (partial recovery) [□ **Q5]** 3 = Not yet resolved (no improvement) [□ **Q5]** 4 = Not yet resolved (deterioration) [□ **Q5]** 5 = Chronic condition or sequelae 6 = Death [□ **Q4]** 9 = Unknown [□ **Q5]**

3. (a) Date adverse event ended *Unknown, code 99 99 99*

   (b) **If duration less than 24 hours**, record duration in hours (*<1 hour, code 00)*

4. (a) **If death,** date of death

   (b) Cause of death _____

   (c) Mode of determination 1 = Medical record extraction 2 = Verbal autopsy 3 = Autopsy 4 = Other, specify _____

Health facility

5. Has the participant been examined in or admitted to a health facility, for issues related to this adverse event? 1 = No [□ **Q7**] 2 = Examined (outpatient) in a study clinic [□ **Q7**] 3 = Examined (outpatient) in a non-study clinic. 4 = Admitted to study hospital.5 = Admitted to other health facility/hospital.

6. Name of clinic/hospital/Department _____

Investigations

7.(a)Any new abnormal result available today? 1 = No [□ **Q8**] 2 = Yes

(b)For each abnormal result, specify

Action taken with treatment

8. **New report:** Record information on any drug (including study treatment) taken at or within 30 days of onset of this event

Follow-up report: Record information on any drug for which: - Effect on symptoms was 'not yet known' during last assessment - Was re-introduced since last assessment - A new action has been taken since last assessment.

Drug name	Treatment duration	Date started	Date stopped	Action taken related to SAE Effect
a. _____	_____	_____	_____	_____
_____				
b. _____	_____	_____	_____	_____
_____				
c. _____	_____	_____	_____	_____
_____				
d. _____	_____	_____	_____	_____
_____				
e. _____	_____	_____	_____	_____
_____				
f. _____	_____	_____	_____	_____
_____				
g. _____	_____	_____	_____	_____
_____				

h. _____ _____ _____ _____

_____

Unknown, code 99 If ongoing, code 88 88 88 Unknown, code 99 Ensure all drugs have been reported in the 'Concomitant Medication Log' (CML) In case of ARV change (dosage or drug) complete an 'Antiretroviral Change' (ARC) form 1 = No change - drug continued 2 = Dose reduced 3 = Drug temporarily stopped 4 = Drug permanently stopped 5 = Reintroduced 6 = Used to treat the SAE 7 = Not applicable 1 = Improvement 2 = No change 3 = Deterioration 4 = Not yet known 5 = Not applicable 1 = Not related 2 = Unlikely 3 = Possible 4 = Probable 5 = Definitely 6 = Not assessable

Case summary

In case of follow-up, report relevant additional data

9. Description of event: _____

10. Relevant medical and surgical history and possible history of allergy:

_____

_____

11. Other relevant details on medications (traditional, herbal, homeopathy): _____

12. Other relevant details: _____

_____

Follow-up

13. Is the participant continuing in the study? 1 = Continuing 2 = Not continuing or released from study.

14. (a) Any follow-up visit required for this SAE? 1 = No [□ **Q15**] 2 =Yes

(b) Date next SAE follow-up visit

Causality

15. Physician's assessment:

(a) (i) Relation to study drugs 1 = Not related [□ **Q15b**] 2 = Unlikely 3 = Possible 4 = Probable 5 = Definitely 6 = Not assessable [□ **Q15b**]

(ii) Name of drug: a. _____

b. _____

(iii) Expected adverse event? 1 = Not expected 2 = Expected

(b) Relation to other causes 1 = Not related 2 = Unlikely 3 = Possible 4 = Probable 5 = Definitely 6 = Not assessable

.**Physician** Name _____ Signature _____

Date __ / __ / __ Day Month Year

Appendix VII: WHO Clinical Staging of HIV Disease (2006)

Clinical Stage I:

- Asymptomatic
- Persistent generalized lymphadenopathy
- Clinical Stage II:
- Moderate unexplained* weight loss (under 10% of presumed or measured body weight)**
- Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)
- Herpes zoster
- Angular chelitis
- Recurrent oral ulceration
- Papular pruritic eruptions
- Seborrhoeic dermatitis
- Fungal nail infections

Clinical Stage III:

- Unexplained* severe weight loss (over 10% of presumed or measured body weight)**

- Unexplained* chronic diarrhoea for longer than one month
- Unexplained* persistent fever (intermittent or constant for longer than one month)
- Persistent oral candidiasis
- Oral hairy leukoplakia
- Pulmonary tuberculosis
- Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
- Unexplained* anaemia (below 8 g/dl), neutropenia (below 0.5 billion/l) and/or chronic thrombocytopenia (below 50 billion/l)

Clinical Stage IV:***

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)
- Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
- Extrapulmonary tuberculosis
- Kaposi sarcoma
- Cytomegalovirus infection (retinitis or infection of other organs)
- Central nervous system toxoplasmosis

- HIV encephalopathy
- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Chronic cryptosporidiosis
- Chronic isosporiasis
- Disseminated mycosis (extrapulmonary histoplasmosis, coccidiomycosis)
- Recurrent septicaemia (including non-typhoidal Salmonella)
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

Appendix VIII: National Council for Science and Technology Research Permit

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telegrams: "SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123.
Fax: 254-020-2213215, 318245, 318249
When replying please quote

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/RRI/12/1/MAS/140/3**

Date: **29th June 2010**

Mr. Philip Kasawa Naluande
Kenyatta University
P. O. Box 43844 - 00100
NAIROBI

Dear Sir,

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"The effectiveness of Stavudine, Lamivudine and Efavirenz on prognosis of HIV/AIDS among ARV Naïve patients in Mombasa District, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Mombasa District** for a period ending ***31st December 2010.***

You are advised to report to **the District Commissioner and the District Education Officer, Mombasa District** before embarking on the research project.

On completion of the research, you are expected to submit two copies of the research report/thesis to our office.

P. N. NYAKUNDI
FOR: SECRETARY/CEO

Copy to:

The District Commissioner
Mombasa District
The District Education Officer
Mombasa District