

**THERAPEUTIC DRUG LEVELS AND TREATMENT
RESPONSIVENESS AMONG PATIENTS FROM HOMA BAY
AND KISUMU COUNTY INFECTED WITH HIV TYPE 1**

MAUREEN JEBICHII KIMULWO

MASTER OF SCIENCE

(Medical Virology)

**JOMO KENYATTA UNIVERSITY OF
AGRICULTURE AND TECHNOLOGY**

2018

**Therapeutic Drug Levels and Treatment Responsiveness among Patients
from Homa Bay and Kisumu County Infected with
HIV Type 1**

Maureen Jebichii Kimulwo

**A thesis submitted in partial fulfillment for the degree of Master of
Science in Medical Virology in the Jomo Kenyatta University of
Agriculture and Technology**

2018

DECLARATION

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

Signature..... Date.....

Maureen Jebichii Kimulwo

This thesis has been submitted to the University for Examination with our approval as supervisors:

Signature..... Date:

Prof. Washingtone Ochieng'

Kenya Medical Research Institute (KEMRI), Kenya

Signature..... Date.....

Prof. Anne W. T. Muigai

JKUAT, Kenya

DEDICATION

This work is dedicated to my loving husband Dennis Mung'oma Oware and my parents Faith and Josphat Kimulwo for the love, courage and inspiration that I drew from them during the entire course of my study. This work is also dedicated to my brothers Felix, Robert and Daniel from whom I drew strength to move on.

ACKNOWLEDGMENT

I wish to thank my supervisor Prof. Washington Ochieng for his guidance and support throughout the entire project.

I wish to thank my supervisor Prof. Anne Muigai for her acceptance, guidance and assistance from the start of the project until its completion.

I extend my sincere gratitude to Ahero Sub-district Hospital and Homa Bay district hospital for allowing me to conduct my research in their facilities.

Special thanks go to the Center for Research in Therapeutic Sciences, Strathmore University, and the Kenya Medical Research Institute (KEMRI) for the training and support throughout the entire project.

Finally, yet importantly, I am thankful to the study participants who gave consent for their samples to be collected and used for the study.

May God bless you all.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
CCC	Comprehensive Care Center
CD4	Cluster of Differentiation 4
cNVP	Plasma Nevirapine Concentrations
CPS	Community Peer Support
CREATES	Center for Research in Therapeutic Sciences
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
KAIS	Kenya AIDS Indicator Survey
KEMRI	Kenya Medical Research Institute
NASCOP	National AIDS and STI control Programme
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NVP	Nevirapine
PI	Protease Inhibitors
SIV	Simian Immunodeficiency Viruses
TDL	Therapeutic Drug level

TDM	Therapeutic Drug Monitoring
VF	Virological Failure
VL	Viral Load
WHO	World Health Organization

ABSTRACT

Treatment failure is a key challenge in the management of HIV-1 infection, with multiple viral and host dependent variables as well as socio-demographic factors variously influencing treatment outcome. A mixed-model survey of plasma Nevirapine (NVP) concentrations (cNVP) and viral load was conducted to examine associations of cNVP, with treatment and adherence outcomes among Kenyan patients on prolonged antiretroviral therapy (ART). Blood plasma was collected at 1, 4 and 24 hours post-ART dosing from 58 patients receiving NVP-containing ART and used to determine cNVP and viral load (VL). The median duration of treatment was 42 (range, 12-156) months, and 25 (43.1%) of the patients had virologic failure. cNVP was significantly lower for virologic failure than non-virologic failure at 1hr (mean, 2,111ng/ml vs. 3,432ng/ml, $p=0.003$) and at 4hr (mean 1,625ng/ml vs. 3,999ng/ml, $p=0.001$) but not at 24hr post-ART dosing. Up to 53.4%, 24.1% and 22.4% of the patients had good, fair and poor adherence respectively. cNVP peaked and was $\geq 3\mu\text{g/ml}$ at 4 hours in a majority of patients with good adherence and those with virologic success. Using a threshold of $3\mu\text{g/ml}$ for optimal therapeutic drug level of NVP, 74% (43/58), 65.5% (38/58) and 86% (50/58) of all patients had sub-therapeutic cNVP at 1, 4 and 24 hours respectively. cNVP at 4 hours was associated with adherence ($p=0.05$) and virologic response outcome ($p=0.002$) in a chi-square test. Mean cNVP differed significantly in non-parametric tests between adherence categories at 1hr ($p=0.005$) and 4hrs ($p=0.01$) and between ART regimen categories at 1hr ($p=0.004$) and 4hrs ($p<0.0001$), as well as correlated inversely with VL ($p<0.002$). Nevirapine plasma levels correlated inversely with VL and positively with adherence behavior. Hence, the concentrations of NVP and other ART drugs should be considered for structured monitoring in a clinical setting, particularly in patients not suppressing VL after months of initiating HAART.

CHAPTER ONE

INTRODUCTION

1.1 Epidemiologic Trend of HIV Infections in Kenya

Human Immunodeficiency Virus (HIV) infection was first reported in Kenya in the year 1984 and by the year 1990, the prevalence reached a peak average of 10.5% in 1995-96, before declining by about 40% to about 6.7% in the year 2003 (Onsomu *et al.*, 2013). Since then the prevalence has remained relatively stable. The decline in the prevalence coincides with the scale-up of antiretroviral therapy (ART) as well as increased awareness which was led to behavioral changes as well as a reduction in the number of new infections (Amico and Orrell, 2013).

According to Kenya AIDS Indicator Survey (KAIS), the prevalence of HIV as at 2012 among the general population was 5.6%. This represented a steady reduction from the 6.4% prevalence data observed in 2008 (Kenya AIDS Indicator Survey, 2014). According to the same KIAS 2014 report, the prevalence among women was high (6.9%) as compared to men (4.2%). Similarly, among key populations the prevalence of HIV appears to be highest among commercial sex workers (29.3%), Men having sex with men (18.2%) and intravenous drug users (18.3%) and in malarial endemic counties of Homabay (27.1%) and Kisumu (18.7%), Siaya (17.8%), Migori County (13.4%) and Kisii (8.9%) (Alemu *et al.*, 2013). In these counties and demographics, the KAIS data reported HIV prevalence that was at the time, significantly above the 5.6% prevalence levels of the general population.

The scale-up of ART has seen the annual AIDS-related deaths decline steadily from 167,000 in 2003 to 58,465 in 2013 (Oti, 2013). However approximately 17.1% associated fatalities, HIV/AIDS remains the leading cause of deaths in Kenya especially among older persons (Negin, *et al.*, 2010). Center for Disease Control (2012) reports that deaths due to HIV are high up to 15% -higher than malaria (3%), Tuberculosis (3%) and lower respiratory infections (12%) (CDC, 2012). Some of the reasons for high mortalities

associated with HIV/AIDS include non-compliance with antiretroviral (ARV) treatment or failure to use ARV for HIV management (Odhiambo *et al.*, 2014).

1.2 Antiretroviral Treatment and Coverage

Kenya developed a national HIV/AIDS strategic plan that aims at reducing HIV transmission, morbidity, and mortality in part through increasing the number of treatment-eligible persons receiving ART (Odhiambo *et al.*, 2014). Because of this, the number of people receiving ARV increased over the years from 184,000 in 2007 to 940,000 adults and 60,000 children in 2016, which is approximately 65% coverage and currently a 75% coverage in adult and 82% coverage in children by the year 2017 (Avert, 2018)

In Kenya, ART is available to all people living with HIV irregardless of their CD4 count, WHO clinical stage, pregnancy status, comorbidity and age (NASCOP, 2016). In Kenya, the recommended first-line therapy for individuals above the age of 15 years is Tenofovir (TDF) + Lamivudine (3TC) + Efavirenz (EFV)/ Nevirapine (NVP) or Zidovudine (AZT), lamivudine (3TC) + Efavirenz (EFV)/ Nevirapine (NVP) (NASCOP, 2016). The description of these drugs and their classes is shown in table 1.1.

Table 1.1: Current Antiretroviral Agents recommended for first line and second line treatment of HIV in Kenya

1. Nucleoside Reverse Transcriptase Inhibitors
Zidovudine * Stavudine* Didanosine Lamivudine* Abacavir * Emitricitabine* Zalcitabine NtRTI Tenofovir Disoproxil Fumarate*
2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
Efavirenz* Nevirapine* Etravirine Ralpivirine
3. Protease inhibitors (PIs)
Indinavir Nelfinavir Ritonavir* Saquinavir Lopinavir * Atazanavir Fosamprenavir Tipranavir Darunavir
4. Entry inhibitors
Fusion inhibitor; this includes CCR5 inhibitors and CXCR4 Inhibitors Enfuvirtide Integrase Strand Transfer Inhibitors Dolutegravir Raltegravir

* Drugs that are recommended for use as first or second-line in Kenya; drugs in bold are the most commonly used ARV agents in Kenya (NASCOP, 2018; NASCOP, 2011).

Despite the documented effectiveness of antiretroviral drugs, up to 57% of HIV patients in Kenya were shown not to respond to treatment (Hassan *et al.*, 2014). Several factors have been determined to associate with this treatment failure among ARV-experienced HIV-positive populations, including the lack of routine virological monitoring and poor adherence to treatment (Brooks *et al.*, 2016; Oluoch *et al.*, 2016; Ochieng *et al.*, 2015; Rutherford *et al.*, 2014). What has not been determined by these and many other studies in this population, is to what extent this treatment failure is due to suboptimal exposures requisite therapeutic levels of the specific ARV drug components in the treatment regime.

1.3 Treatment Failure

In Kenya, treatment failure is monitored by routinely performing viral load test, 6 months and 12 months after the initiation of ART and performing CD4/CD8 counts; treatment failure is confirmed if the patient has persistently high viral load of $\geq 1,000$ copies/ml after being on ART for at least 6 months and also has a CD4 count of ≥ 200 cells/mm³ (NASCO, 2018).

preponderous of evidence shows that high rates of treatment failure in Kenya, which was recently shown greater than 35% is associated among other factors, with poor or none adherence to ART (Ochieng *et al.* 2015) and drug-resistant mutations (Kwobah *et al.*, 2012; Hassan *et al.*, 2014; Chohan *et al.*, 2013). The study reported in this thesis was conducted in the context of the larger study by Ochieng 2015 and colleagues in which 35.9% of the 546 patients failed treatment by virological definitions. Specifically, this study sought to address the variable gaps in virologic failure, by assessing from an independent subset of patients, the associations of virologic failure with pharmacological factors.

1.4 Therapeutic Drug Monitoring of Antiretrovirals

The knowledge about the relationship between the systemic exposure of Antiretroviral and the response to treatment is very crucial in the process of identifying the drug dosage (Back *et al.*, 2002). Additionally, this knowledge is very crucial in understanding why different

patients respond differently to the same drug and dose (Lee *et al.*, 2014). Therapeutic Drug Monitoring has, therefore, been used to understand these issues, and this has been done for other drugs including antimicrobial agents, antineoplastic, anticonvulsants and antiarrhythmic. For these drugs, TDM has been used to improve the desired outcome for the drug by optimizing the drug concentrations. This is often guided by exposure-response relationships and the desired therapeutic range relationships. The therapeutic range is often established by various clinical studies that are able to determine the perfect dose to achieve a therapeutic effect and one that is least likely to induce toxicities (Lee *et al.*, 2014).

Antiretroviral that have been able to meet the criteria explained above and therefore, they are suitable for TDM (Lee *et al.*, 2014). Additionally, studies have shown that ARVs have significant interpatient variability (Focà *et al.*, 2018; Calcagno *et al.*, 2017; Siccardi *et al.*, 2015). Moreover, studies have also shown that the concentration of the drug in blood has significant correlations with treatment responsiveness as well as toxicities (Mouton, Cohen and Maarteens, 2016). Most importantly, studies have also shown that conducting TDM in clinical settings has resulted in improved virological response especially for patients who were initially failing treatment (Back *et al.*, 2002).

Therapeutic Drug Monitoring is not performed in Kenya, other measures such as clinical, virological and immunological responses as used to guide treatment and also to evaluate the effectiveness of the treatment receiving ARVs (Hickey *et al.*, 2014). Therapeutic Drug Monitoring, however, is performed in the United States, the procedure is not routinely and is performed only in special circumstances (Günthard *et al.*, 2016). The circumstances include suspicion of drug-drug interactions, drug-food interaction, changes in the pathophysiological state of the patient for example if the patient has impaired hepatic, renal or gastrointestinal function which may affect drug absorption, metabolism or elimination (World Health Organization, 2016). Additionally, it is performed in pregnant women who are at very high risk of virological failure and consequent mother to child transfer of the virus (World Health Organization, 2016). Therapeutic Drug Monitoring is also performed in cases where patient has been heavily treated with ARVs yet he/she is not responding to

treatment, finally, when the physician suspects drug-associated toxicities. The study will look at the usefulness of TDM in assessing virological treatment response as one of the tools that can be applied in the Kenyan context to fight the increasing rates of treatment failure.

1.5 Statement of the Problem

By the end of the year 2015, over 1.5 million Kenyans were living with HIV and approximately 900, 000 patients were on antiretroviral therapy (NASCOP, 2016). The increase in coverage and access to ART resulted in substantial decline in HIV related incidence, morbidity and mortality (Hassan *et al.*, 2014). However, the continuing emergence of treatment failure threatens to reverse the gains made by the scale-up of ART. A study conducted by Lehman *et al.* (2012) reported that up to 50% of the patients selected for their study had virological failure that were not due to NVP resistant mutations, other factors including suboptimal exposure to ARVs could be the reason for treatment failure. In the United States, therapeutic drug monitoring (TDM) is used to evaluate treatment response and toxicity, additionally, TDM is used to confirm nonadherence to treatment especially in cases where there is virological failure without drug resistance (Günthard *et al.*, 2016). It is therefore important to consider suboptimal exposures to ARV as one of the factors that can contribute to treatment failure in Kenyan patients undergoing HIV management.

1.6 Justification

A study conducted by Ochieng *et al.* (2015), reported that 35.9% of the 514 Kenyan patients enrolled in the study failed first-line regimen and 36% failed second-line regimen, the study looked at adherence as one of the factors that led to treatment failure. However, it did not look at the development of drug resistance mutations and also suboptimal exposures to ARV as one of the causes of treatment failure. Most of the HIV-positive patients will fail to achieve adequate suppression of plasma HIV RNA (that is < 50 copies/ml) with any of the current ART regimens. Even when they achieve viral suppression, viral rebound may

occur following discontinuation of ART or poor adherence to treatment (Chun *et al.*, 2014). This shows that treatment failure is multifactorial. A number of such factors that might singularly or in consort lead to treatment failure include the development of resistant mutations, poor adherence and other pharmacokinetic variables. For pharmacokinetic variables, suboptimal concentrations of drugs in blood is likely to compromise efficacy and occasion failure (Kimulwo *et al.*, 2017). Therefore, Therapeutic Drug Monitoring (TDM) is used to establish whether suboptimal exposures to ARV is the cause of treatment failure.

1.7 Research Questions

- 1) What are the plasma Nevirapine levels of HIV-1 infected patients failing treatment?
- 2) What are the plasma Nevirapine levels of HIV-1 infected patients not failing treatment?
- 3) Is there an association between plasma Nevirapine levels and virological treatment outcome?

1.8 General Objectives

To determine the associations of plasma Nevirapine concentrations and virologic treatment outcome in patients from Homa Bay and Kisumu Counties who were receiving Nevirapine as part of long-term 3-drug HAART regimen

1.9 Specific Objectives

- 1) To determine the plasma Nevirapine levels of HIV-1 infected patients failing treatment.
- 2) To determine the plasma Nevirapine levels of HIV-1 infected patients not failing treatment.
- 3) To assess the association of plasma Nevirapine levels with virological treatment outcome.

CHAPTER TWO

LITERATURE REVIEW

2.1 HIV Epidemiology

The HIV epidemic is thought to have occurred after zoonotic infections with the Simian Immunodeficiency Viruses (SIV) (Maartens *et al.*, 2014). The HIV virus has two major types HIV-1 and HIV-2. HIV-1 is mostly prevalent worldwide while HIV-2 is found in some regions of western and central Africa (Fanales-Belasio *et al.*, 2010). The HIV belongs to the genus *Lentivirus* and the family *Retroviridae*. Because of the high mutation and recombination rates of HIV-1, the virus is further classified into three distantly related groups Main group (M), Outlier group (O) and non-M-non-O group (N) (Khoja *et al.*, 2008). The M group is then further subdivided into 9 subtypes (A, B, C, D, F, G, H, J, K); and up to 33 circulating recombinant forms (CRF) (Khoja *et al.*, 2008). The study by Kitawi *et al.* (2015) revealed that subtype A1 was predominantly circulating in Kenya representing almost 74.5% of all the isolates collected in the study.

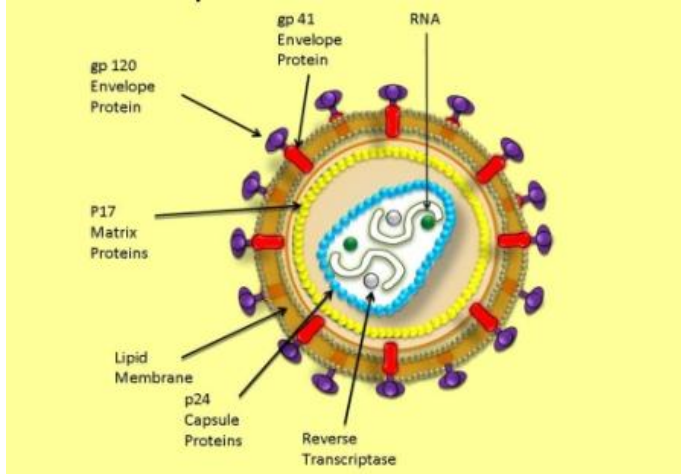
According to Hemelaar *et al.* (2011), subtype C is predominant in Africa and India and accounts for 48% of all HIV-1 cases in the world. However, according to a study conducted by Osman *et al.* (2013), there is a high prevalence of subtype A1 (89.7%) in Kenya. The results were similar to a study by Kitawi *et al.* (2015), who reported that subtype A1 is predominant in Kenya representing 74.5% of the species that were collected in their study HIV has been listed as the leading cause of disability and a major contributor to the global burden of disease. People living with HIV have increased risks of diseases like myocardial infarctions, liver disease, cancers and tuberculosis among many other infections (Smit, *et al.* 2015). This is common especially in low-income countries like Kenya. However, a lot has been done to reduce the burden of infections. This has been done through treatment and constant monitoring including the rolling out of programs such as the reduction of mother to child transmissions and improved access to treatment and health management which has helped to reduce the burden of infection (Baeten *et al.*, 2016). Despite all these attempts,

most of the patients are failing treatment which results in fast disease progression resulting in increased incidences of opportunistic infections.

2.2 The HIV Virion

The virus has two identical copies of the single-stranded RNA molecule that is characterized by the structural proteins genes *gag*, *pol*, *env* (Sundquist and Kräusslich, 2012). The *gag* gene encodes for the structural proteins found in the core of the virus these are the p24, p7, p6 and the matrix proteins; the *env* gene codes of the envelope proteins gp120 and gp41; the *pol* gene encodes for the enzymes that are required for the replication of the virus and these enzymes are; reverse transcriptase, integrase and protease (Fanales-Belasio *et al.*, 2010). In addition to the *gag*, *pol* and *env* gene, HIV-1 has six additional accessory genes *tat*, *rev*, *nef*, *vif*, *vpu*, and *vpr*, HIV-2 does not have the *vpu* but has *vpx* instead (Fanales-Belasio *et al.*, 2010).

The diameter of HIV is approximately 100nm and it is surrounded by a lipoprotein rich membrane that has a glycoprotein heterodimer complex composed of trimers of the gp120 on the external surface and gp41 transmembrane protein bound together (Sundquist and Kräusslich, 2012). In addition to the outer lipoprotein membrane, the virus also has a matrix inside the viral lipoprotein membrane (Fanales-Belasio *et al.*, 2010). The membrane and the capsid are composed of the polymers of the core antigen (p24), inside the capsid are the two copies of the single-stranded HIV RNA, the enzyme reverse transcriptase, protease and integrase (Fanales-Belasio *et al.*, 2010).



(Pattman *et al.*, 2010)

Figure 2.1: The Anatomy of the AIDS virus

The virion contains all the elements that are required for an infection to take place. These elements are; the two copies of the positive sense genomic RNA, cellular tRNA^{Lys}, 3 molecules to prime cDNA synthesis, the viral envelope (Env) protein, the Gag polyprotein, and the three viral enzymes: protease (PR), reverse transcriptase (RT), and integrase (IN) (Sundquist and Kräusslich, 2012). Viral enzymes are also present, they are packaged in the Gag-Pro-Pol polyprotein (Sundquist and Kräusslich, 2012).

2.3 HIV Pathogenesis

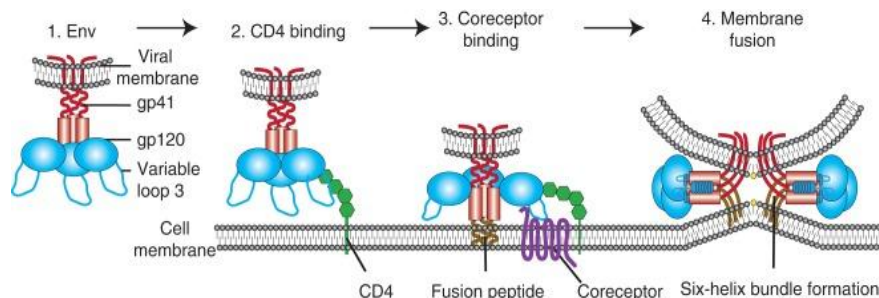
2.1.1 Viral Entry

The process of HIV infection is complex, and even though the process may not cause immediate lethal damage, the process will stimulate intracellular signal cascades that enable the virus to replicate itself in the host's cells (Sundquist and Kräusslich, 2012). The external glycoprotein (gp120) and the transmembrane protein (gp41) are crucial in the infection process (Wilén, Tikton and Doms, 2012). HIV entry into the cell is the first phase of replication and it begins when the virus attaches itself to the host cell. Viruses bind to the target cell mediated by the viral envelope, the envelope comes into close proximity with the viral receptor CD4 and receptors, thereby increasing the efficiency of infection (Coffin and

Swanstrom, 2013). The second step of virus entry and the first absolutely required for infection entails binding of Envelope to its primary receptor, the host protein CD4 as seen in figure 2.2. The viral envelope is heavily glycosylated with a trimer of gp120 and gp41 heterodimers (Wilén, Tikton and Doms, 2012).

The third step of the viral entry is the coreceptor binding, there are three types of HIV virus according to their receptor binding during the infection phase. There are viruses that bind to the CCR5 receptors and these are termed as R5 HIV (Sundquist and Kräusslich, 2012). The second category of the HIV use the CXCR4 and these viruses are termed as X4 HIV. The third category of HIV binds to both the CCR5 and the CXCR4 coreceptors and they are called the R5X4 HIV (Wilén, Tikton and Doms, 2012).

The fourth step of viral infection is the movement of the viral particles into the cell, this is also the step where membrane fusion occurs. The HIV usurps cellular transport pathways to enter into the host's cell after membrane fusion, additionally, the virus can enter the host's cell through the process of endocytosis (Sundquist and Kräusslich, 2012). The final step in the infection process is viral entry and membrane fusion mediated by the envelop proteins. The process of coreceptor binding exposes the hydrophobic gp41 fusion peptide. The process results in the folding of the peptides if the gp41 bringing an amino-terminal helical region and a carboxy-terminal helical region from each gp41 subunit together to form a six-helix bundle (Wilén, Tikton and Doms, 2012). Consequently, a fusion pore is formed that allows for the subsequent delivery of the viral contents into the host cell cytoplasm



(Wilén, Tikton and Doms, 2012)

Figure 2.2: Overview of the HIV infection process

2.1.2 Replication cycle

Once the viral contents are in the nucleus of the CD4 cell, the enzyme reverse transcriptase is used to synthesize a linear DNA strand from the viral RNA, this often occurs in cells that are newly infected. The RNA molecule act as mRNA that synthesizes the single-stranded DNA molecule with the help of tRNA. Consequently, the single-stranded DNA is used to synthesize the double-stranded DNA molecule, which is integrated into the host genome through the action of the integrase enzyme (Fanales-Belasio *et al.*, 2010).

The integrated DNA remains dormant in the host cell, however, in the latent stage of the infection, it is used to produce more viral copies utilizing the host's transcription factors (Fanales-Belasio *et al.*, 2010). During this stage the integrated viral DNA is transcribed into RNA then it undergoes splicing to produce copies of mRNA. These mRNAs are exported to the cytoplasm of the cell from the nucleus and then they are translated into *Tat* and *Rev* Proteins. These proteins are used to make the structural components of the virus (Coffin and Swanstrom, 2013). The *Rev* proteins bind to the viral RNA produced in the nucleus, the *Gag* and *Env* proteins are also produced. The *Gag* proteins are used to produce viral capsid proteins including the p24, and the matrix proteins such as the p17 (Coffin and Swanstrom, 2013). The *env* gene is transcribed to produce the envelope glycoproteins, finally, some of the full-length RNAs are used as the genome for the new virus. The next stage is recombination of the viral particles. The viral proteins and the viral DNA are assembled in the cytoplasm to produce a mature infectious virus that buds out of the cell to infect other new cells (Coffin and Swanstrom, 2013).

The early events of HIV infection take place rapidly. However, infection is permanently established thereafter, thus, prevention of infection events should focus on prevention or the interference of the early events (Campbell *et al.*, 2014). The transmission of HIV is mostly through sexual activities, this is because the dendritic cells that are near the mucosal surfaces play an important role in facilitating the binding of HIV envelope glycoprotein gp120 and the high-affinity cells (Levi, 2015).

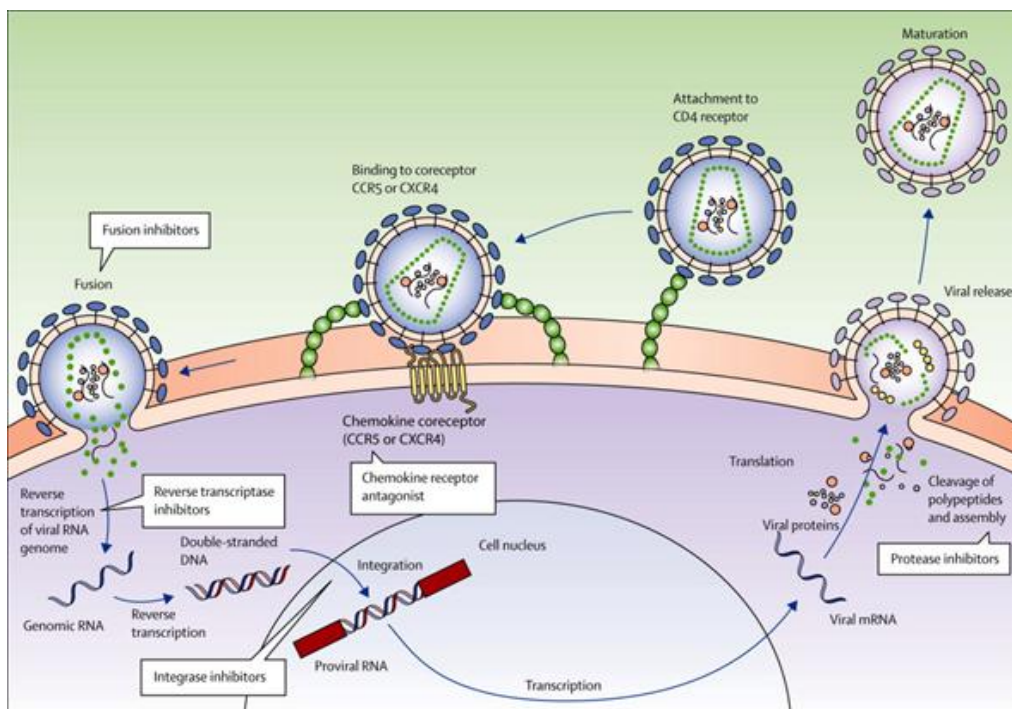
The HIV cells mainly target the activated CD4 T lymphocytes and the infection of the cells is *via* the interactions between the CD4 and the chemokine coreceptors, CCR5 or CXCR4 (Maartens *et al.*, 2014). Following infection of the cells is rapid replication that leads to increased viral load, this strikes and induction of inflammatory cytokines and chemokines (Fanales-Belasio *et al.*, 2010). The viral load then decreases to a setpoint because of the adaptive and innate immune response of the host (Insight Start Study Group, 2015). HIV-specific CD8 is also produced which kills cells infected by HIV, this is the first step before neutralizing antibodies are produced (Fanales-Belasio *et al.*, 2010).

Progression of the infection greatly depends on the host's immunity to contain viral replication and to reconstitute the pool of memory T-cells within the mucosa-associated lymphoid tissue or lymph nodes (Fanales-Belasio *et al.*, 2010). when the virus cannot be contained the destruction of the lymphoid system continues and the CD4+ T-cell levels continue to drop (Fanales-Belasio *et al.*, 2010). The drop in the levels of CD4+ T-cells places the patient at risk of opportunistic infections from viruses, fungi, bacteria, tumors and parasites. If the patient does not receive treatment the patient quickly progresses to AIDS and may succumb to AIDS-related complications (Fanales-Belasio *et al.*, 2010).

2.4 Management of HIV using Antiretroviral

The best option for the management of HIV to achieve viral suppression and reduction of morbidity and mortalities associated with HIV-1 infection is antiretroviral treatment (Coffin and Swanstrom, 2013). However, these drugs do not eradicate the infection and therefore, the patient would need to be on long life treatment to achieve viral suppression. The antiretroviral inhibit viral replication and therefore the host's immunity is given a chance to recover, therefore, the person will be able to fight off opportunistic infections (Baril *et al.*, 2016). However, due to the high rate of replication and the low fidelity of reverse transcription, there are diverse species and quasi-species of HIV, therefore there is a need to combine several potent antiretroviral agents to further suppress viral replication and reduce the incidences of drug-resistant HIV-1 mutants (Baril *et al.*, 2016). This is achieved through the use of highly active antiretroviral treatments (HAART). HAART is the combination of

at least three different classes of ARVs to suppress viral replication (Maartens *et al.*, 2014). The use of HAART was first developed in the late 1990s and its use transformed the once-fatal disease into a manageable chronic illness (Maartens, *et al.*, 2014). There are more than 25 licensed drugs that block, these drugs and are less toxic. They have a lower pill burden more effective are dosed less frequently than the initial regimens that were protease inhibitor-based (Maartens *et al.*, 2014). These drugs target various stages of the lifecycle of the virus as shown in Figure 2.3



(Maartens *et al.*, 2014)

Figure 2.3: Target of the various antiretroviral drugs

The standard regimen for the treatment of HIV includes the combination of two nucleoside reverse transcriptase inhibitors; emtricitabine or lamivudine together with one of abacavir, tenofovir, or zidovudine with a non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or integrase inhibitor (Maartens *et al.*, 2014). According to the Kenyan National AIDS and STI Control Programme (NAS COP) (2016), the aim of ARV is to improve the

patient’s quality of life, reduce morbidities and mortalities associated with HIV, restore and preserve the immunological function of the patient and suppress viral replication.

In Kenya, treatment of HIV is recommended for every individual regardless of the CD4 count, viral load or the stage of HIV infection (NASCOP, 2016). The recommended first-line ART regimen for infants, children, adolescents and adults according to NASCOP (2016) is shown in Table 2.1, Table 2.2 shows the recommended dosage forms for adults and adolescents

Table 2.1: First line regimen for infants, adolescents and adults as recommended by Kenya’s National AIDS and STI Control Program

Age	Preferred first-line ART
<2 weeks	AZT + 3TC + NVP
2 weeks- <3 years	ABC + 3TC + LPV/r
3 years-15 years (<35kg body weight)	ABC + 3TC + EFV
3 years-15 years (≥35kg body weight)	TDF + 3TC + EFV
>15 years	TDF + 3TC +EFV/NVP AZT + 3TC + EFV/NVP

(AZT- Zidovudine, 3TC-Lamivudine, ABC- Abacavir, LPV-Lopinavir , EFV- Efavirenz, TDF- Tenofovir)

Table 2.2: Administration of first-line ARVs in adults and adolescents

Name of the ARV	Dosage forms	Recommended adult dose
Zidovudine (AZT)	300 mg tablets	300 mg twice daily
Tenofovir (TDF)	300 mg tablets	300 mg once daily
Lamivudine (3TC)	150 and 300 mg tablets	150 mg twice daily or 300 mg once daily
Efavirenz (EFV)	200 mg(scored) and 600 mg tablets	600 mg once daily at bedtime
Nevirapine (NVP)	200 mg tablets	200 mg once daily for the first 2 weeks, thereafter, 200 mg twice daily
Abacavir	300 mg tablets	300 mg twice daily
Stavudine (D4T)	30 mg	30 mg twice daily

A table showing the dosage forms and dosage recommendations for adolescents and adults in Kenya (NASCOP, 2016).

Victoria *et al.* (2013) projected that by the year 2015, about 15 million people will be on ARVs, WHO (2016) reports that currently, about 14.9 million people are receiving ARV s accounting for approximately 40% coverage. In Africa, only 41% of the people have access to life-saving medication (WHO, 2016). In Kenya, the current prevalence of HIV is 5.9 %, this is approximately 1.5 million people, among those people only 59% are on ARVs (AVERT, 2016).

2.5 Nevirapine pharmacokinetics and Pharmacogenetics

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is combined with other drugs to treat HIV. After an oral administration, more than 90% of Nevirapine is readily absorbed and it is then distributed to all tissues including the brain due to its low protein binding capacity. Once Nevirapine is in the hepatic system, metabolism is induced by the action of the CYP enzymes 3A4 and 2B6 that leads to the transformation of the

Nevirapine to 2-, 3-, 8-, and 12-hydroxynevirapine (Fan-Harvard *et al.*, 2013). This process is then followed by the glucuronidation of these hydroxyl metabolites, that is the -, 3-, 8-, and 12-hydroxynevirapine through the action of the UGT enzymes and thereby extracted through the urinary system (Figure 2.4.) (Fan-Harvard *et al.*, 2013).

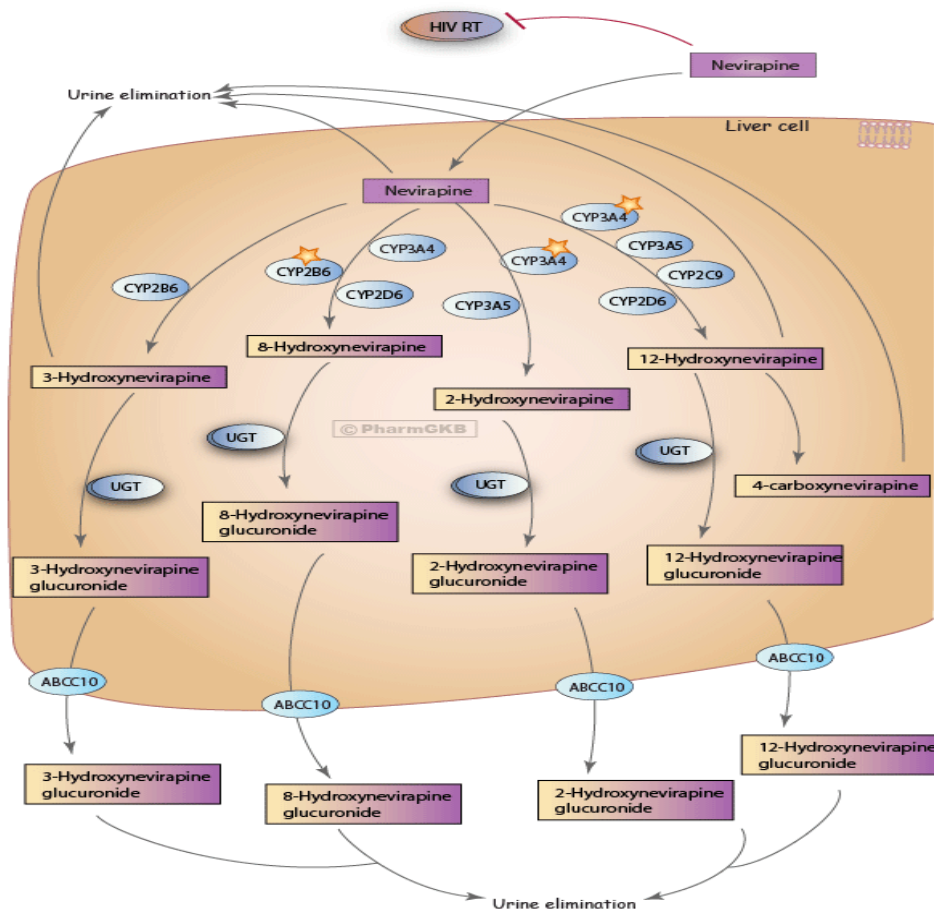


Figure 2.4: Pharmacokinetic Transformation of Nevirapine

Autoinduction of CYP 3A4 and CYP 2B6 isozymes leads to an approximately 1.5- to 2-fold increase in the apparent oral clearance of Nevirapine as treatment continues from a single dose to 2 to 4 weeks of 200 to 400 mg/day (Fan-Harvard *et al.*, 2013). Nevirapine has been associated with serious toxicities within the first 18 weeks of therapy. This is due to the impact of the CYP2B6 polymorphism, 516G>T, rs3745274, on NVP pharmacokinetics (Cammatt *et al.*, 2009). This has been associated with the development of

resistant mutations. Individuals who have CYP2B6 *6/*6 or *6/*18 haplotype have been significantly shown to have higher concentrations of Nevirapine in plasma (Cammett *et al.*, 2009).

The 12-hydroxynevirapine has been proposed as a factor in Nevirapine hepatocarcinogenicity, this is because of the impairment in the hepatic system because of reduced metabolic autoinduction. Therefore, in such patients, the difference between C_{max} and C_{min} would be lower because of the impaired hepatic clearance, this will increase the chances of the patient developing toxicities associated with Nevirapine (Cammett *et al.*, 2009).

2.6 Treatment Failure

The scale-up of ARV, as well as the availability of support for people living with HIV, has resulted in a substantial decline in HIV related incidences, morbidity and mortality (Günthard *et al.*, 2014). However, the emergence of drug-resistant mutants and subsequent treatment failure is threatening to reverse the gains made by ARV treatment especially where there is poor monitoring of treatment and poor adherence to ARV (Hassan *et al.*, 2014).

A systematic review by Barth *et al.* (2010) has shown that within 12 months of treatment 76% of individuals manage to maintain virological suppression, however, after 24 months, the percentage goes down to 67 %. Stadel and Richman (2013) conducted a systematic review that showed that 11% of the patients on ART for 12 - 23 months in resource-limited settings had HIV drug resistance. The number increased to 15% for patients on ARV for between 24-36 months and to 21% for patients on treatment for more than 36 months (Stadel and Richman, 2013).

In their study conducted in Kenya, Hassan *et al.* (2014) found out that 24.6% of their patients had treatment failure. A study by Ochieng *et al.* (2015) showed that 35.9% of their patients had treatment failure which was higher than the previously reported statistics, among the patients 81% were Nevirapine experienced.

Nevirapine (NVP) is a widely prescribed component of (HAART) and is used alongside other virus inhibitors to maximize viral suppression. Although a few studies have suggested that plasma NVP concentrations (cNVP) may affect the virologic outcome (Mudhune *et al.*, 2017; Tempestilli *et al.*, 2017; Baxi *et al.*, 2015), therapeutic drug level (TDL) measurements are excluded from HAART management of patients in many countries.

2.7 Therapeutic Drug Monitoring

Therapeutic Drug Monitoring (TDM) is the process whereby drug concentrations are measured in bodily fluids like saliva, serum, and plasma or blood (Tam *et al.*, 2016). The major aim of TDM is to individualize dosage so that the drug concentrations can be achieved within the recommended target range (Schoenenberger *et al.*, 2013). Drug levels cannot be measured at the specific site of action, the adverse effects correlates better with blood or plasma and that is why plasma, serum, saliva or blood is assessed for drug levels (Tam *et al.*, 2016). Therapeutic drug monitoring has been proposed by several studies as a means of optimizing the response to HAART (Duong *et al.*,2015; Wertheimer *et al.*, 2015).

Wertheimer *et al.* (2015) concluded from their study that TDM can effectively target the concentrations of ARVs and can be used in the management of HIV. It is important to monitor HIV infections in order to understand why the treatment fails despite the fact the patient is adhering to treatment (Back *et al.*, 2002). This study, therefore, examined whether the levels of nevirapine in plasma correlates with treatment responsiveness in terms of virological response to treatment. Additionally, this study examined whether adherence had an effect on drug levels of nevirapine in plasma.

Nevirapine is a widely prescribed ARV among Kenyan patients. Ochieng *et al.* (2015) reported that up to 81% of the patients they recruited in their study were on Nevirapine in combination with nucleoside reverse transcriptase inhibitors. Rutherford *et al.* (2014) indicated that adequate plasma concentrations of Nevirapine were required to achieve a successful response. Research has proven that subtherapeutic levels of < 3.0 mg/dl were associated with the development of mutations and consequently virological failure

(Gopalan *et al.*, 2017; Gopalan *et al.*, 2016; Lamorde *et al.*, 2014). Suboptimal concentrations may come as a result of poor adherence, however even in perfect adherence to regimen at standard dosages some patients will still have subtherapeutic levels of Nevirapine in plasma due to interpatient variability in nevirapine exposure, genetic factors, pregnancy, drug-drug interactions, drug-food interactions or other ethnic factors (Gopalan *et al.*, 2017; Gopalan *et al.*, 2016; Lamorde *et al.*, 2014;).

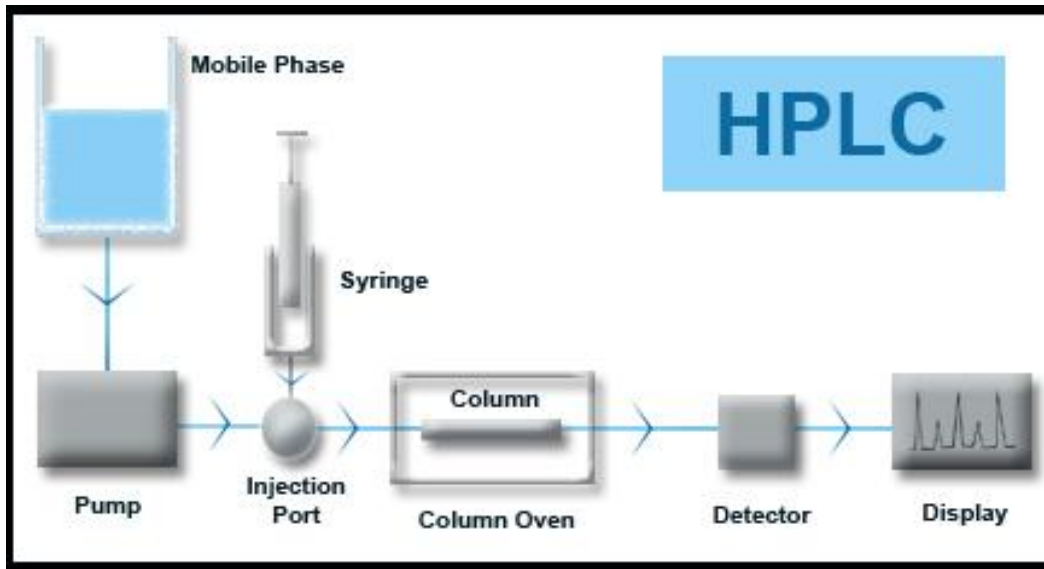
In the United States, TDM is a well-known tool that is used to optimize Nevirapine dosing (Rafaëlla *et al.*, 2008). However, in resource-limited settings, TDM is almost never performed due to the lack of simple and affordable methods to determine the levels in bodily fluids like saliva and plasma (Rafaëlla *et al.*, 2008). There are many protocols for determining the levels of nevirapine in plasma, the study used high-performance liquid chromatography (HPLC) because it is accurate, robust, available and affordable in the settings (Lamorde *et al.*, 2014)

2.8 High-Performance Liquid Chromatography (HPLC) for TDL Assays

High-performance liquid chromatography is a highly improved column chromatography, where a mixture in a solvent is separated into their basic components based on their molecular structure and composition (Snyder *et al.*, 2012). Unlike in column chromatography where the solvent is allowed to drip through the column under gravity, HPLC uses high pressure of up to 400 atmospheres to push the solvent through the column (Snyder *et al.*, 2012)

The mobile phase is held in the reservoir, the pump forces the mobile phase through the HPLC system at a set flow rate, typically the mobile phase is pumped at high pressures that can reach up to 400- to 600-bar (Snyder *et al.*, 2012) (See figure 2.5). The injection port serves to introduce the liquid sample into the HPLC system; approximately 5-10µl of the sample is introduced into the column (Snyder *et al.*, 2012). The column which is the stationary phase separates the components of the sample using chemical and physical

characteristics of the components. The individual components can then be picked up by the detector and then displayed on linked computers (Snyder *et al.*, 2012).



(Bhanot, 2011)

Figure 2.5: A figure showing the different parts of the HPLC system used for sample analysis

High-performance liquid chromatography is used in the TDM of ARVs in plasma or serum because it is an accurate and sensitive method (Back *et al.*, 2006). Therapeutic drug monitoring of Nevirapine is often performed using plasma samples however, many other studies have achieved successful results with saliva which is less invasive and more favorable especially when dealing with children (Lamorde *et al.*, 2014). However, clinical experience is limited and therapeutic cut-offs for saliva is not evaluated (Lamorde *et al.*, 2014). The advantages of using HPLC with ultraviolet (UV) detection for TDM of Nevirapine is that the process is efficient and accurate. Additionally, it is possible to quantify and identify different chemical components with a good resolution and the results are highly reproducible (Ana *et al.*, 2014). One major disadvantage of the process is that it

is time-consuming and arduous, the process of protocol optimization, sample preparation and data processing is time-consuming (Ana *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out in Kisumu County Referral Hospital in Kisumu County and Ndhiwa sub-County Hospital in Homa Bay County in Kenya. The reason for selecting this two counties is because of the high prevalence of HIV in the two counties. Homa Bay County has a HIV prevalence of 27.1% while Kisumu County has a prevalence of 18.7%, (Kenya AIDS Indicator Survey, 2014). The two hospitals, were included in the study because it had the most established clinics for HIV. Additionally, they offer free HIV treatment and management, therefore, many people go to these hospitals.

3.2 Study design

This was a cross-sectional study that collected both quantitative and qualitative data collection

3.3 Study population

The study population included both adults and children; recruitment from HIV positive patients receiving ART care from Kisumu County Referral Hospital in Kisumu County and Ndhiwa sub-County Hospital in Homa Bay County in Kenya. Patients above 18 years of age were required to sign the consent form willingly. However, for children below 18 years old, the consent was obtained from their parents or guardians.

3.3.1 Inclusion criteria

- HIV-1 positive patients who also voluntarily agreed to participate
- Patients between the ages of 16 and 65 years

- Patients receiving Nevirapine as one of the 3-drug ART regimen and registered at government treatment facilities
- Patients who had been taking ARV for more than fourteen days prior to the collection of samples

3.3.2 Exclusion criteria

Patients not meeting the inclusion criteria above

3.4 Ethical considerations

Clearance to carry out the study was obtained from the Kenya Medical Research Institute (KEMRI) Scientific Steering Committee and Ethical Review Committee (SSC/ERC Number-2477) (Appendix 4 and 5). Samples were collected by qualified personnel after the informed consent was signed.

3.5 Sample size estimations

The sample size was calculated on the basis of the country's national HIV-1 prevalence rate of 5.6%, using Fisher's test of proportions and applying respectively, a confidence level of 95% and a two-sided error of 0.05%.

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

d^2

Where

n: Desired sample size of the population valid only when the population is greater than 10,000

Z: The standard deviate, usually 1.96 that corresponds to 95% confidence level

P: The national prevalence of HIV 5.6%

d: Degree of accuracy (0.05)

D: Design effect, usually 1 where there is no replication or comparison.

n= 81.23 which is approximately 81 patients from both counties.

3.6 Sample collection, transport and storage

The study participants were sampled using convenience sampling method from patients attending the Kisumu County Referral Hospital and Ndhiwa sub-County Hospital comprehensive care center clinics (CCC) in their respective hospitals. All the patients who met the inclusion criteria were selected and upon consent, 4ml of venous blood was collected in EDTA tubes. Blood was collected in intervals of 1, 4 and 24 hours after the patient had taken a Nevirapine pill. Sample preparation and processing

3.7 Plasma preparation

Plasma separation was done within thirty minutes of sample collection. Whole blood was centrifuged at 2000 revolutions per minute for 10 minutes. The blood was then transferred into carefully labeled cryotubes in replicates of three. The plasma was transported in liquid nitrogen to the CREATES laboratory and stored in a -80°C freezer.

3.8 Viral load tests

Viral load assays were conducted within two months of sample collection in duplicate. The Analyses were conducted at the Early Infant Diagnosis Laboratory at the Kenya Medical Research Institute (KEMRI) in Nairobi using Abbott RealTime HIV-1 assay system (Abbott, Abbot Park, IL) and using an Abbott m2000sp platform. The assay uses reverse transcription Polymerase Chain Reaction (PCR) to generate an amplified product of HIV-1 that is in a clinical specimen, which was plasma in the study. The limits of detection for the *m2000sp* ranged between 40 and 10,000,000 HIV-1 RNA copies. Plasma samples were loaded into the m2000sp for extraction of RNA, the RNA was then mixed with the Abbot Real-time master mix reagents in a 96-well microtiter plate which was then sealed and

transferred into the Abbot m2000rt instrument for amplification and quantification. All the analysis was performed in duplicates (Ceffa *et al.*, 2016)

3.9 Analysis of Nevirapine in plasma using HPLC-UV

A modified version of the liquid-liquid extraction protocol for NVP reported by Notari *et al* (2006) was developed and used. The protocol was borrowed and modified according to the HPLC 120 machine that was used to analyze the samples. This included the flow rate used and the detection range that was used in sample analysis. Frozen plasma was analyzed within three months of extraction. Drug level analyses were performed using an Agilent 1260 High-Performance Liquid Chromatography (HPLC, 1260) with dual-wavelength UV–VIS spectrophotometric detector (Agilent, Santa Clara, CA).

All samples from the three time-points of 1, 4 and 24 hours post-dosage from any one patient were analyzed during a single batch. Nevirapine was extracted from plasma and analyzed in duplicate. The mobile phase was a 15mM potassium phosphate buffer/acetonitrile solution (70/30% v/v). The mobile phase was made by dissolving 1.428g of potassium phosphate monobasic in 700ml of deionized water and the pH adjusted to 3.0 using orthophosphoric acid. Acetonitrile (300ml) was then added to the solution followed by sonication for 15 minutes to degas the mobile phase.

All standards were derived from 200mg Nevirapine (NVP) tablets (Boehringer Ingelheim, Ridgefield, CT, U.S), and were provided by the National Drug Quality Control Laboratory in Nairobi. A single Nevirapine tablet was weighed, crushed and re-weighed then reconstituted in HPLC grade acetonitrile to make a stock standard solution (10mg/ml). The stock standard solution was used to make a diluted working standard (0.1mg/ml). Calibration curves were prepared daily in blank healthy human plasma and were linear over the range 0-10µg/ml (0, 1, 2, 4, 6, 8, 10 µg/ml). Three quality control standards were similarly prepared at 1.5, 5.5 and 8.5 µg/ml concentrations.

Patient samples (250µl), calibration standards (250µl) and quality control standards (250µl) were added to separate 15ml glass tubes that were labeled accordingly. Ethyl acetate (2ml)

was added to each tube to extract the drug from the plasma. The solution was vortexed vigorously for 5 minutes then centrifuged at 4000rpm for 5 minutes. The supernatant was extracted into clean 15 ml glass tubes that were labeled accordingly. The supernatant was evaporated under a gentle stream of nitrogen bath in a water bath at 45°C until the tube was dry. Afterward, 200µl of mobile phase was added to the tubes and the tubes were again vortexed for 2 minutes to reconstitute the drug in the solution.

A total of 20 µl samples were injected into the column, with the entire runtime lasting 5 minutes per sample. Chromatographic conditions consisted of a 1.5ml/minute elution flow rate on a 5µm 150x4.6mm C18 column at 254nm wavelength (Notari *et al.*, 2006).

3.9.1 Optimization of the HPLC protocol

The protocol was validated in terms of selectivity, specificity, linearity, recovery and accuracy. Specificity and selectivity were assessed by comparing blank plasma from six different individuals with blank plasma that had been spiked by the low-quality control standard (LQC) to ensure that there was no endogenous interfering peak at the retention time of Nevirapine. Linearity was assessed using the calibration standards the Nevirapine calibration curve was linear over a range of 0-10000 ng/ml, the mean correlation coefficient (r^2) of six results was greater than 0.98. Recovery was assessed by comparing the QC samples that had been spiked in plasma and extracted using the protocol with QC samples that were directly injected into the column to represent 100% extractions. The recovery of the LQC was 90.27 %, Medium quality control was (MQC) 91.74% and High-quality control (HQC) was 90.79% which were within the acceptable limits of 85-115% (Matta *et al.*, 2012). The mean recovery for all the QC's was 90.93%.

3.10 Adherence and treatment failure definition

The World Health Organization (WHO) provides three criteria for the definition of treatment failure that includes clinical failure, immunological failure, and virological failure (VF) (WHO, 2014). Virological criterion was used for its comparable sensitivity. Virological Failure was defined based on two consecutive viral load measurements (VL1

and VL2). Treatment failure was considered if patients had both VL1 and VL2 that were persistently above 1000 copies/ml or if their VL1 was below 1000 copies/ml and VL2 above 1000 copies/ml.

The two facilities also offered optional adherence and compliance programs that relied on community peer support (CPS) mechanisms. The CPS groups comprise HIV+ peer ‘counselors’, who provide counseling and adherence support, including patient home visits and focused group discussions relevant to treatment compliance. The CPS councilors also verify pill count at refill and pill burden. Participation in these groups was voluntary, and patients were asked if they were actively, partly, or never involved CPS. Adherence assessment was based on residual pill count and on self-reports, focusing on dose-compliance during the 30 days preceding the last refill. The number of dose pills at refill was counted and reconciled against the dose counts dispensed at last refill. Additional pill count data was extracted from patient cards for the four months preceding the study period. Non-adherence was determined as the percentage of overdue dose at refill, averaged over a four-month period and used to assign adherence as good ($\leq 5\%$ dose skipped), fair (6-15% dose skipped) or poor ($>15\%$ dose skipped) ART Adherence was assessed based on self-report and on pill burden, and defined as good ($\leq 5\%$ dose skipped), fair (6-15% dose skipped) or poor ($>15\%$ dose skipped).

3.11 Data analysis and storage

Data entry was done at CREATES and all data were stored in Microsoft Excel (2007) worksheets. The Data was cleaned and entered into Statistical Package for Social Sciences (SPSS®) version 22 for analysis. Patient demographic and treatment characteristics were described using means, median was used to describe the duration of ARV treatment. T-tests were used to compare the patients’ baseline characteristics with viral loads and Nevirapine drug level results (cNVP1, cNVP4, cNVP24). Pearson correlations were used to assess the associations between Nevirapine plasma levels (cNVP1, cNVP4, cNVP24) and VL2.

The chromatographs produced the patient's plasma Nevirapine levels as Area under the curve (AUC) which were then converted into concentrations (ng/ml) using an equation that was generated from the calibration curve. All analyses were performed using SPSS version 22. Significance was accorded to every p-value less than 0.05.

To ensure the integrity of data the calibration curve used to generate concentrations of unknown samples were prepared daily with the samples. All stock solutions and working solutions were prepared in triplicates and stored under -20°C to be used within a month. Validation of the HPLC protocol was performed prior to sample analysis to ensure the repeatability of the results, accuracy of the extraction and recovery. Data was then presented in tables of frequency and bar graphs. All data was stored in a secure computer with a backup located in the CREATES server.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics

A total of 82 patients were recruited for the study, all the patients were receiving Nevirapine as part of their ARV regimen. The median duration of treatment was 42 (range, 12-156) months. However, only 61 patients had their samples collected at 1, 4 and 24-hour time points. The rest of the patients (21 patients) who did not have all their samples collected were excluded from the study. Three patients were not receiving the optimal triple ART regimen; these patients had the highest baseline viral load (VL1) of 3.51 log₁₀ HIV RNA copies compared to the other regimen groups (See table 4.1). The patients who were in a non-standard therapy were also excluded from the study. There was no reason on record for this apparent non-standard therapy; therefore these patients were excluded from the study.

Table 4.1: Baseline demographic characteristics of the study participants

		Number (%), HAART duration†		
		Males	Females	Total
Age group in years	≤25	0 (0), 0	3 (100), 126	3, 126
	26-45	11 (31.4), 36	24 (65.6), 36	35, 36
	>45	8 (40), 46.5	12 (60), 38.5	20,45
Regimen Type¶	AZT	7 (36.8),35	12 (63.2),48	19,36
	D4T	7 (43.8),46	9 (56.3),43	16,45
	TDF	5 (25),57	15 (75),36	20,36
	Others*	0 (0),0	3 (100),54	3,54
Adherence	Good	8 (25.8),46	23 (74.2)38	31,43
	Fair	7 (50),46	7 (50),43	14,44.5
	Poor	4 (30.8),28	9 (69.2),39	13,32
CPS activity	Active (++)	8 (34.8),40	15 (68.2),38	23,38
	Occasional (+)	6 (38.3),43.5	11 (64.7),43,	17,43
	None (-)	5 (27.8),46	13 (72.2),39	18,41
Total		19(32.8), 44	39(67.2),39	58,42

*Others- is used to describe the 3 patients who were on non-standard therapy; NVP+TDF, 3TC+NVP and NVP only; ¶ Regimen backbone includes 3TC+NVP. CPS, community peer-to-peer support. † Median duration on HAART at enrolment

4.2 Virologic Treatment response

Virologic response was defined using two successive viral load (VL) measurements as either virologic failure or virologic success. The median duration between VL1 and VL2 was 12 months for this study subset. It was observed from the study that 25 (43.1%) of the patients failed treatment. Comparing virologic response across ART regimen, patients receiving D4T backbone had significantly higher VL (mean VL2 4.11 log₁₀ copies) than patients on other treatment arms (p=0.006). Virologic treatment outcome was next assessed

against adherence. Defining adherence as good, fair or poor; 31 patients (53.4%) had good adherence, 14 (24.1%) had fair adherence while 13 (22.4%) had poor adherence. Adherence was strongly associated with virologic outcome (p-value <0.001). Patients with good adherence also had the lowest VL (2.4log₁₀ copies/ml) compared to those with fair (3.31 copies/ml) or poor (4.8 copies/ml) adherence. These data are detailed in table 4. 2.

The associations between participation in CPS and VF were next assessed. A total of 22 subjects (40%) were active in CPS (CPS++), 15 (27.2%) were partly involved (CPS+) and 18 (32.7%) were never involved (CPS-). CPS activity was significantly associated with VF outcome (χ^2 p<0.001). Specifically, 90.9% CPS++ patients experienced virologic success (non-VF) compared to just 22.2% of CPS- patients experiencing virologic success. Similarly, CPS++ subjects had the lowest mean VL (2.40 log₁₀) compared to CPS+ (3.22 log₁₀ copies/ml) or CPS- (4.18 log₁₀ copies/ml) subjects, and these differences were significant in an ANOVA test (p<0.001). Moreover, adherence and CPS were positively correlated in a chi-square test (p<0.001), with more patients in CPS++ showing good adherence and more CPS- patients showing poor adherence. Age and gender were not significantly associated with VF outcome (Table 4.2.).

Table 4.2: Virologic treatment response of various categories of patients

		<u>VL2_‡, Number, (%)</u>		
		ART failure	ART responsive	Total
Gender	Males	4.44,6 (31.6)	2.59, 13 (68.4)	3.17,19 (100)
	Females	4.15, 17 (47.2)	2.20, 19 (52.8)	3.12,36 (100)
	p value _¶ (Significance levels)			<u>0.898</u>
Age group	≤25	0, 0 (0)	2.42,3 (100)	2.42,3 (100)
	26-45	4.32,15 (46.9)	2.59,17 (53.1)	3.40,32 (100)
	>45	4.05,8 (40)	2.02,12 (60)	2.82,20 (100)
	p value _¶ (Significance levels)			<u>0.219</u>
Regimen arm _‡	AZT	3.75,7 (36.8)	2.08,12 (63.2)	2.70,19 (100)
	D4T	4.86,10 (62.5)	2.87,6 (37.5)	4.11,16 (100)
	TDF	3.72,6 (30)	2.39,14 (70)	2.79,20 (100)
	p value _¶ (Significance levels)			<u>0.002</u>
Adherence	Good	2.92,5 (17.9)	2.13,23 (82.1)	2.28,28 (100)
	Fair	4.38,6 (42.9)	2.51,8 (57.1)	3.31,14 (100)
	Poor	4.68,12 (92.3)	6.27,1 (7.7)	4.80,13 (100)
	p value _¶ (Significance levels)			<u>0.000</u>
Community peer support network	Active (++)	3.30,2 (9.0)	2.24,20 (91.0)	2.40,22 (100)
	Occasional (+)	3.77,7 (46.7)	2.45,8 (53.3)	3.22,15 (100)
	None (-)	4.58,14 (77.8)	2.76,4 (22.2)	4.18,18 (100)
	p value _¶ (Significance levels)			<u>0.000</u>

Regimen backbone includes 3TC+NVP; ‡, VL2 represents viral load taken at the second study time-point and is presented as log₁₀ copies/ml; ¶, ANOVA p-value for comparison of mean VL2 between independent variables

4.3 Plasma nevirapine concentrations of subjects failing treatment and subjects responding to treatment

The trajectory of cNVP showed a steady rise from low levels at 1 hour and peaking at 4 hours before tapering off 24 hours after dosing. On average, and in a significant proportion of patients experiencing good and fair adherence, as well as those with virologic success, cNVP peaked at 4 hours post-ART dosing. These peak cNVP levels were either equal to or above a threshold of 3µg/ml concentrations. This pattern held largely true when comparing cNVP by adherence (Figure 4.1. A), gender or virological outcome, except for patients with poor adherence and those with virologic failure who experienced delayed peak cNVP. Patients experiencing virologic failure and those with poor adherence had lower cNVP that rose slowly but steadily before peaking at 24 hours. Those experiencing virologic success (non- virologic failure) had cNVP starting about 5-fold higher than virologic failure patients, but peaking early at 4 hours before tapering off at 24 hours (Fig 4.1. B). Overall, cNVP at 24 hours was comparable regardless of adherence, VR status or gender. All virologic failure patients, as well as those with poor adherence, appeared unable to sustain cNVP above 3ug/ml within the first 4 hours of ART dosing.

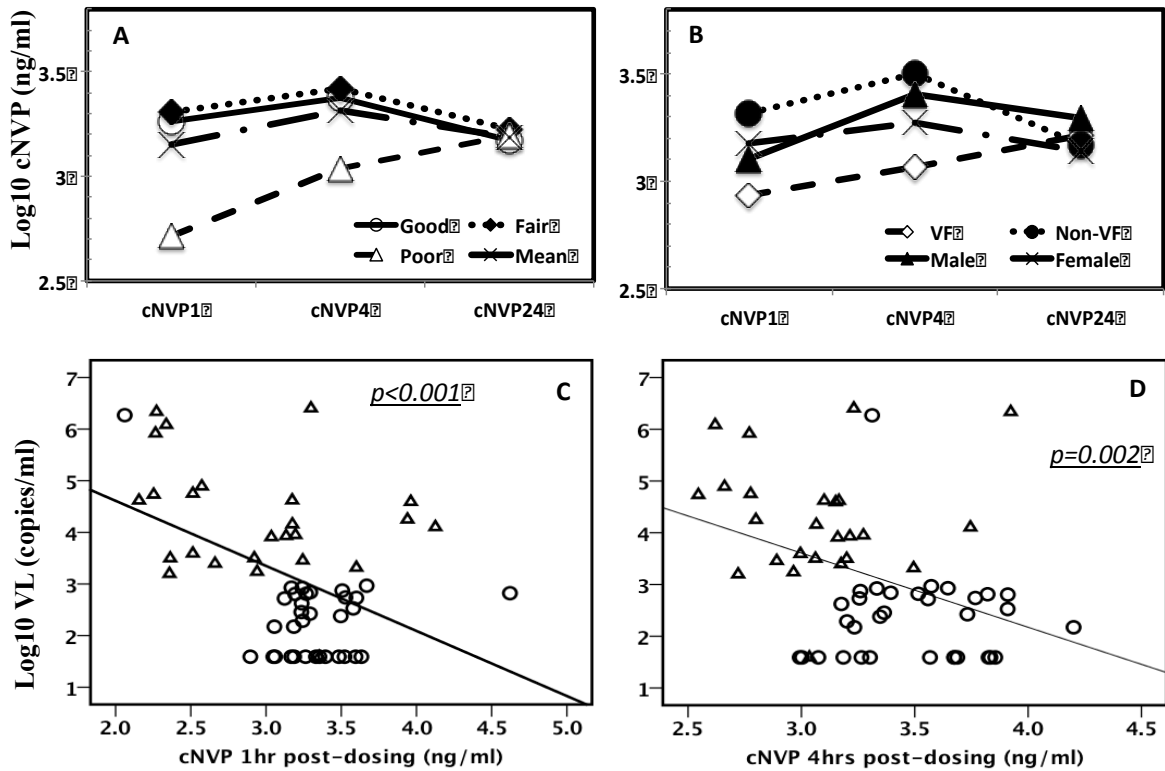


Figure 4.1: Trajectory plasma Nevirapine concentrations and association with viral load

Plasma Nevirapine concentration (cNVP) is compared for various groups over 24 hour period according to adherence (A:- open circle, good adherence; closed diamonds, fair adherence; open triangle, poor adherence; solid line, mean), and according to virologic response or gender (B:- open diamond, virologic failure; closed circle, virologic success or non-virologic failure; closed

Triangle, male; crosses, female). Patients with good and fair adherence and those with virologic success (non-virologic failure) had peak cNVP at 4 hours while cNVP for virologic failure patients started low at 1hr and peaked later at 24hrs. Significant inverse correlations are observed between same day viral load with cNVP at 1 hour (C) and at 4 hours (D). Circles, virologic success; triangles, virologic failure.

4.4 Plasma NVP concentrations are associated with adherence outcome

The relationship between cNVP with adherence outcome was analyzed. The mean cNVP was higher in patients with good (3,392-3344 ng/ml) and fair (3,368-3,433 ng/ml) adherence compared to those with poor adherence (1,278-1,638 ng/ml) at 1 hour and 4 hours (Table 4.4). It was observed that both cNVP1 and cNVP4 were significantly less for patients with poor compared to those with fair or good adherence ($p \leq 0.009$). Non-parametric tests were used to test the hypothesis that the distribution of cNVP was similar across adherence all the groups. The results also revealed that cNVP1 ($p=0.005$) and cNVP4 ($p=0.014$) were significantly different across adherence groups. At the time when majority of patients experienced peak cNVP levels at 4 hours post-ART dosing, achieving cNVP at or above $3\mu\text{g/ml}$ coincided with good adherence and virologic success. Using $3\mu\text{g/ml}$ as threshold for optimal or therapeutic NVP drug levels (TDL), the patients were grouped into either sub-therapeutic ($<3\mu\text{g/ml}$ cNVP) or therapeutic ($\geq 3\mu\text{g/ml}$ cNVP) categories and the association of TDL with adherence was investigated. The proportion of patients with poor adherence ($n=13$) and having sub-therapeutic cNVP (12/13, 92.3%) was higher than those achieving therapeutic cNVP under the same poor adherence category (1/13, 7.7%, $p=0.05$). This relationship between TDL and adherence was significant at peak cNVP at 4 hours but not at 1 or 24 hours post-ART dosing. No significant association was found between TDL and age group or gender, or between TDL24 and any grouping variables.

Based on the results, it was observed that cNVP at 4 hours and adherence had a significant effect on the longitudinal virological definition (p Values 0.003 and 0.000 respectively). However, changes in cNVP at 4 hours accounted for 15.6% variations in longitudinal VR keeping adherence constant while changes in adherence accounted for 35.7% variations keeping cNVP constant. Additionally, both adherence and cNVP at 4 hours predicts for up to 42.9% variation in virological treatment failure.

4.5 Associations of cNVP with virologic outcome

The relationship of cNVP with treatment outcome was also investigated. It was observed that cNVP1 and cNVP4 were significantly low for patients experiencing virologic failure compared to those experiencing virologic success ($p \leq 0.004$, Table 4.4). Virologic failure patients had significantly lower cNVP1 ($p=0.004$) and cNVP4 ($p<0.000$) but not of cNVP24 than did virologic success patients. Compared by regimen arm, patients receiving AZT and D4T had 2 to 3-fold less cNVP than those on TDF backbone, though these differences were only significant at 1 hour ($p=0.042$). The therapeutic drug level (TDL) threshold in which cNVP $<3\mu\text{g/ml}$ represented sub-TDL and $\geq 3\mu\text{g/ml}$ represented optimal TDL at any of the three pharmacokinetic time points (TDL1, TDL4 and TDL24) was applied. Majority of the patients had cNVP below $3\mu\text{g/ml}$ at 1 hour (41/55, 74.5%), at 4 hours (36/55, 65.5%) and at 24 hours (48/55, 87.3%). Applying this threshold to test for independent variable associations, TDL4 (χ^2 $p=0.002$) but not TDL1 or TDL24 was significantly associated with virologic response (defined as virologic failure or virologic success based on VL). Among the patients experiencing virologic failure, significantly more had sub-TDL4 (82.6%, $n=19/23$) compared to just 9.4% ($n=3/32$) who achieved optimal TDL yet still failing treatment. Using TDL threshold of $3\mu\text{g/ml}$, VL was significantly lower for patients who had cNVP4 at or above this threshold concentration ($n=20$, mean \log_{10} 2.59) compared to patients with cNVP4 below the threshold ($n=36$, mean \log_{10} 3.41 $3\mu\text{g/ml}$, $p=0.049$). None of these analyses conducted with cNVP taken at 1hr or at 24hrs post-dosing returned significant statistics except where otherwise mentioned. The cNVP1, cNVP4 and cNVP24 were fitted alternately into a bivariate correlation model with VL as the other scale variable. Using Pearson's statistics to test associations with VL measured at the time of determining cNVP (VL2), VL was significantly and inversely associated with cNVP1 ($p<0.001$, Fig 4.1. C) and with cNVP4 ($p=0.002$, Fig 4.1. D) but not with cNVP24.

Table 4.3: cNVP correlation with demographic, behavioral and treatment characteristics

Grouping variable		cNVP in ng/ml, post-dosing at		
		1 hour	4 hours	24 hours
Adherence	Good	3391.72	3343.86	1872.36
	Fair	3368.36	3433.21	2356.75
	Poor	1277.75	1637.68	2356.75
	p value¶	<u>0.002</u>	<u>0.009</u>	<u>0.788</u>
CPS	Active (++)	3983.01	3374.63	1234.62
	Occasional (+)	2163.47	2884.92	3028.17
	None (-)	2147.67	2526.00	6168.36
	p value¶	<u>0.078</u>	<u>0.384</u>	<u>0.123</u>
Virologic response	ART failure (25)	2164.90	1660.47	5056.10
	ART responsive (33)	3404.48	3899.75	2103.89
	p value¶	<u>0.004</u>	<u>0.000</u>	<u>0.972</u>
Gender	Males	2058.38	3259.43	6319.89
	Females	3322.97	2807.04	1764.91
	p value¶	<u>0.657</u>	<u>0.192</u>	<u>0.108</u>
Age group (years)	≤25	2604.80	3828.60	5315.15
	26-45	2424.83	3383.73	4431.59
	>45	3666.36	2160.87	1292.83
	p value¶	<u>0.642</u>	<u>0.495</u>	<u>0.107</u>
Regimen arm‡	AZT	1496.07	2878.39	5985.37
	D4T	2280.63	2042.53	2075.74
	TDF	4691.05	3780.65	1834.04
	p value¶	<u>0.042</u>	<u>0.56</u>	<u>0.910</u>

¶P value compares mean log 10 plasma nevirapine concentrations (cNVP) between respective grouping variables. CPS, community peer-to-peer support activity.

CHAPTER FIVE

DISCUSSION

5.1 Background of the study participants

The study was conducted to investigate associations of plasma Nevirapine concentrations (cNVP) with treatment outcome among Kenyans receiving NVP as part of a long-term ART regimen. More than half (53.4%) of these patients had good adherence, in this study adherence was described through pill count, Non-adherence was determined as the percentage of overdue dose at refill, averaged over a four-month period and used to assign adherence as good ($\leq 5\%$ dose skipped), fair (6-15% dose skipped) or poor ($>15\%$ dose skipped) ART Adherence was assessed based on self-report and on pill burden, and defined as good ($\leq 5\%$ dose skipped), fair (6-15% dose skipped) or poor ($>15\%$ dose skipped). Patients with virologic success achieved significantly high cNVP within one hour of dosing compared to delayed cNVP peak for those with virologic failure. Conversely, patients failing treatment showed poor adherence and significantly reduced cNVP. A study conducted by Ochieng *et al* (2015) reported high virologic failure (35.9%) in a larger population of Kenyans who received HAART for between 12 and 228 months. In this study, 25 patients (43.1%) failed treatment, which is higher than the results obtained by Ochieng *et al.* (2015)

Nevirapine is extensively used in Kenya as part of first-line therapy, and VL monitoring for ART management is accessible only to a limited extent. Moreover, neither drug level nor toxicity monitoring is included in clinical management of Kenyan HIV patients. The median duration between VL1 and VL2 for this study subset was 12 months, which was in line with the average duration of VL measurement intervals for clinical ART management in Kenya (Kimulwo *et al.*, 2017). This study however, expands virologic failure assessment to include therapeutic drug level (TDL) monitoring, and the longer duration between VL1 and VL2 would otherwise not change the virologic response status determination; under these conditions though, patients failing treatment may not benefit from timely clinical

decisions. The study shows that adherence significantly affects virologic outcomes and plasma cNVP: good adherence was associated significantly with suppressed VL and higher cNVP while poor adherence was associated with significantly high VL and lower (sub-therapeutic) cNVP levels. Moreover, TDL measured at peak cNVP (4 hours post dosing) was significantly predictive of both virologic response and adherence outcome.

In a study conducted in Malawi to examine factors that affect treatment outcomes in patients receiving ART, up to 47% of the patients were non-adherent to treatment (Van Oosterhout *et al.*, 2005). That study also showed adherence to be significantly associated with subtherapeutic NVP plasma levels and with virologic failure. Adherence in this study was measured by a combination of pill burden and self-report, with a total of 77.5% being labeled as either good or fair adherent. Although this study was not powered to define the effective therapeutic NVP levels, mean cNVP was at least two-fold higher for patients with either good or fair adherence compared to poor adherence.

Accumulating evidence shows that community support networks (CPS) enhance social relationships that demystify HIV-associated stigma (Campbell *et al.*, 2007; Zachariah *et al.*, 2007). The larger study showed that participation in CPS networks significantly improved adherence and treatment outcome (Ochieng *et al.*, 2015). In this current subset of patients, those actively involved in CPS tended to reach peak cNVP early at 4 hours post-dosing, and this cNVP also were substantially higher than seen in patients not actively involved in CPS. It is believed that CPS affected cNVP outcome by influencing adherence, a secondary relationship observed in the earlier larger patient population.

5.2 Plasma nevirapine concentrations in patients failing treatment

In the study 43.1% of the patients failed treatment, this group of patients had significantly lower levels of plasma Nevirapine concentrations. As observed from the study, cNVP concentrations were predictive for virological treatment outcome at 4 and 24 hours. The patients who were failing treatment also had poor adherence to treatment.

Maintaining an optimal plasma concentration of Nevirapine is important, a study was conducted to establish the associations between plasma drug levels of Nevirapine and the development of genetic mutations in HIV. According to the study, having trough concentrations of Nevirapine that are below 3µg/ml is associated with a high risk of developing drug-resistant mutants especially in the pol gene. Other studies, however have described that the rates of developing drug-resistant mutants are higher for trough concentrations of lower than 4.5 µg/ml. The results produced in this study were similar to the studies by (Gopalan *et al.*, 2017). Patients who were failing treatment had trough concentrations (4 hours) of 1.6 µg/ml

A study conducted in the Chinese population also mentioned that trough concentrations of more than 3.9 µg/ml were required to maintain a good response to treatment (Wang *et al.*, 2011)). This results were different from those of study conducted Requena *et al.* 2005)who found out that in their population, there was need to achieve a trough level of more than 4.3 µg/ml to achieve a suppression of all polymorphic natural variants of HIV. Many other studies also suggested trough concentrations of more than 3 µg/ml to ensure adequate viral suppression (Zoufaly *et al.* 2013; Nikanjam *et al.*, 2012; Corbet *et al.*, 2010; and Vanprapar *et al.*, 2010; Ellis *et al.*, 2007). It is clear from evidence that the most common cause of treatment failure is the development of drug-resistant mutants, this is especially true for patients who have good adherence to treatment (Wensing *et al.*, 2015). Studies have also shown that subtherapeutic levels of Nevirapine in plasma is associated with the development of drug-resistant mutations (Oluka *et al.*, 2015)

5.3 Plasma nevirapine concentrations in patients not failing treatment

The study indicated direct correlation between plasma Nevirapine levels and ART responsiveness. In the study, it was observed that patients who had good response to treatment had significantly higher levels of Nevirapine at 1 and 4 hours. These levels were higher than 3 µg/ml which were the recommended trough levels for Nevirapine according to the study conducted by Vanprapar *et al.* (2010). In this category of patients who were

responding to treatment the plasma concentrations at 1 hour and 4 hours were 3.4 µg/ml respectively.

The steady-state plasma pharmacokinetics of Nevirapine after the administration of 200mg of Nevirapine reveals a trough concentration of greater than 3 µg/ml which is sufficient for suppression of viral replication (Cooper and Van Heeswijk, 2007). In this group of patients, the plasma concentrations peaked at 4 hours, similar to the results obtained in this study for patients who were responding to treatment. Additionally, the lowest concentrations were observed at the 24 hour period, similar to the results obtained in this study. In the study, the mean concentration of Nevirapine was highest at 4 hours (3.4 µg/ml) for patients responding to treatment and lowest at 24 hours (2.1 µg/ml). Having a low concentration of Nevirapine at 24 hours was a good indication that this group of patients was metabolizing the drugs well with sufficient elimination half- life, clearance and volume of distribution (Bertrand *et al.*, 2012). This prevents toxicities that result in poor adherence to treatment due to the associated adverse reactions that result from them (Yogev, 2015; Ena *et al.*, 2003). As evidenced in this study, patients who had good adherence to treatment also had high concentrations of Nevirapine and were responding well to treatment.

5.4 Association of between plasma Nevirapine levels and treatment outcomes

According to the results of this study, plasma Nevirapine levels of Nevirapine was predictive for treatment outcomes, patients who had subtherapeutic levels at 1 hour and 4 hours significantly failed treatment while those with therapeutic levels were responsive to treatment at 1 and 4 hours. A number of studies have also explored the relationships between the pharmacokinetics of Nevirapine and the virological response to therapy. This studies have been able to show that a steady- state Nevirapine trough concentrations of about 3 µg/ml were sufficient to inhibit viral replication *in vitro* (Cooper and Van Heeswijk, 2007). Additionally, it has been demonstrated that higher concentrations of more than 3.4 µg/ml of Nevirapine in plasma are associated with a more rapid viral load decline and a long-term efficacy for patients who were initially treatment naïve. De Vries- Sluijs *et al.* (2003). also showed that there is more than fivefold higher risk of treatment failure if

Nevirapine levels are less than 3 µg/ml. In this study, a significant majority of patients who experienced virologic success and those who had good adherence also achieved cNVP of at least 3µg/ml four hours after initial ART dosing and significantly suppressed their plasma viral load. These data provide evidence to support the recommendation that therapeutic NVP level monitoring should be included as part of ART management, especially in settings where this drug is extensively used in suppressive HAART (Leth *et al.*, 2006; Kouanfack *et al.*, 2008).

5.5 Limitations of the study

The results should be interpreted within the limits of the study design and context. The study was not designed for intensive PK analyses, which is otherwise not practical in a clinical setting as a routine TDL monitoring practice. Under intensive PK studies, the V_{Max} and trough levels for cNVP might deviate from what was observed, but these are unlikely to affect overall conclusions. However, the design allows for the findings to remain appropriate for patients in similar setting where resources and drug options are limited. One of the limitations of the study was that adherence was measured using pill count and self-report which is not a very reliable test for adherence. Additionally, it was hard to predict whether the suboptimal levels of plasma Nevirapine were due to adherence, liver metabolism or due interactions with other drugs since the cNVP was not able to predict the circulating levels of other drugs including other ARV's. Additionally, treatment failure is caused by multiple factors including presence of drug-resistant mutants which was not examined in this study. Finally, drug-drug interactions, drug-food interactions and differences in drug metabolism across age, gender and ethnicity may affect plasma concentrations of Nevirapine. However, the study design looked at concentration levels and adherence which has shown to be predictive for treatment response.

5.6 Conclusion

The study showed that patients who were failing treatment had subtherapeutic plasma Nevirapine levels which were below the recommended levels of < 3µg/ml. These patients

had a peak concentration at 24 hours rather than at 4 hours which is the required trough levels. Additionally, these patients had poor adherence to treatment which is also a contributing factor to treatment failure and sub-therapeutic levels

Patients who were not failing treatment had higher plasma Nevirapine concentrations above the recommended trough levels of 3µg/ml. additionally, these patients had their peak plasma concentrations at 4 hours and the lowest (C_{min}) concentrations at 24 hours. Furthermore, the patients had good adherence to treatment.

The study proves that plasma Nevirapine concentration has a significant association with treatment failure. Patients with higher concentrations at 1 and 4 hours according to the study were responding well to treatment and these patients had lower HIV viral load as compared to patients who had lower concentrations. Patients with lower concentrations of Nevirapine in plasma had higher viral load.

5.7 Recommendations

Plasma Nevirapine concentrations should be monitored in clinical settings particular in patients who are failing treatment. Such patients can benefit from informed decision on drug selections to improve therapeutic efficacy

Plasma Nevirapine concentrations are not necessary in resources limited settings for patients who are not failing treatment because of the cost and intensity required for the procedure.

While detailed pharmacokinetics (PK) investigations in clinical settings are valuable treatment monitoring tool, the results of this study warrants recommending expanding investigations to emphasize on the relationship between pharmacogenomics and treatment outcome in settings of extensive NVP and other ART exposure so as to shed more light on other host factors that might affect optimal TDL.

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APPENDICES

Appendix 1: Questionnaire

(Administer in after the general study questionnaire and consent has been administered)

Unique study No: _____

Personal details

Patient's number: _____

Age: _____ Sex: _____

Occupation: _____ Residence: _____

Weight: _____

Medication details (From the patients' file)

Medication taken: _____

Number of times the medicine is taken: _____

Approximate time the medicine taken: _____

Last CD4 count: _____ Last Viral Load: _____

Pill count _____

Questions

What are the specific times that you take the medicine in a day? Do you have something to remind you to take the medicine at the specific times?

Has the doctor ever told you to change the medicine because it stopped working or it affected you in a bad way?

How many times have you skipped taking your medicine in the past seven days? (One can carry out a pill count to check adherence)

Do you participate in community peer support programmes?

Appendix 2: Informed Consent

Consent information

Therapeutic drug levels and treatment responsiveness in patients infected with human immunodeficiency virus type 1 (HIV-1) from Kisumu county and Homa bay county

Investigator: **KIMULWO M. JEBICHII.**

Affiliations: 1. Centre for Research in Therapeutic Sciences, Strathmore University, Nairobi, Kenya,

2. Institute of tropical medicine and infectious disease.

Information to the study participant

HIV is an infection that is treated with antiretrovirals. Sometimes this treatment fails due to the following reasons; not taking drugs as the doctor prescribed, viruses that do not respond to treatment and suboptimal drug levels in the blood.

After you take a drug, the blood is distributed with the blood to various parts of the body where it is expected act. One hour after taking the blood and four hours after taking the drug there is a certain amount of drug that should be present in your body. Again just before you take the next drug, there should be an amount of drug present in your blood, that is why we will take the blood three times.

Purpose of the Study:

The purpose of this study is to examine whether the suboptimal exposure to nevirapine affects treatment outcomes

Procedure to be followed

If you agree to participate in this study, we will give your unique study number that will help us identify you; this is to maintain your confidentiality. Afterwards you will answer a

few questions and we will draw about 4ml of blood. We will draw your blood approximately one hour after you took your first tablet. Meanwhile, we will give you some refreshments as you wait for three hours when we will draw blood again from you the same amount. Tomorrow you will come again just before you take your drug so that we can take the last sample.

We will compensate you for your transport since we are requesting you to come outside your normal clinic visits.

Benefits of the study

The study offers only indirect benefits to you, which in the long-term will help your physicians manage your conditions better. We will test to see if you are metabolizing the drugs as it is required through what is called ‘therapeutic drug monitoring’. This study will also help us find out whether suboptimal drug exposures can cause treatment failure. This will help us develop policies to control treatment failure.

Confidentiality of your identity

The information you give us about you will not be disclosed to any other parties, not even your medical records. We will give you a unique study number which will help to return the results of the tests to you, otherwise, your identity will not be made public in any forum.

Medical risks and problems.

We don’t expect that this investigation will cause you any medical problems other than the reasons for which you normally visit this health facility. However, you will experience a small pain when blood is being drawn.

Storing your blood for future studies

Part of the blood that you will give us will be stored for future studies to analyze or control for the present factor analyses. You will not be required to give extra blood for this purpose

and you may still refuse to have your blood stored or used in the future. You will be given and guided through a questionnaire form to make your choice.

Obtaining additional information

You are encouraged to ask any questions that occur to you at this time or ask questions at any time in the course of your contact with the investigators. You will also be given a copy of this agreement for your continued information. If you desire more information at a later date, you may call the following people at any time:

Maureen Kimulwo

Mobile phone number; 0725175505

Email address; maureenkimulwo@gmail.com

Washington Ochieng

Mobile number; 0715425583

Email address; wochieng@gmail.com

Basis of participation

You are free to refuse to participate in this study. If you choose to do so, your rights to be attended to in this or other hospitals now or in future will not be affected.

Consent signing:

I have read the information stated above and have had the opportunity to ask questions all of which were answered satisfactorily. I also understand that I can still refuse to consent and withdraw from the study at any time of my choosing. I, (check and sign/thumbprint below):

Adults

I agree declineto participate in this study.

Blood storage for future analysis:

I further agreerefuseto have my samples stored for future analyses.

Project Personnel to fill this section before the participant:

Unique study

No.....Nationality/origin.....

Age..... Sex

Occupation..... Locality

Patient Recruited..... Not recruited to the study

Appendix 3: Approval letter from Scientific Steering Committee/KEMRI



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54849-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0723-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website: www.kemri.org

ESACIPAC/SSC/101264

13th December, 2012

Bernhards Ogutu

Thro'
Director, CCR
NAIROBI


*Forwarded
17/12/2012*

REF: SSC No. 2477 (Revised) – Genetic characterization of HIV-1 strains circulating in Kenya and determination of known mutations conferring resistance to antiretroviral drugs

Thank you for your letter received on 7th December, 2012 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval


Sammy Njenga, PhD
SECRETARY, SSC

Appendix 4: Approval letter from Ethical Review Committee/KEMRI



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-208901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website www.kemri.org

KEMRI/RES/7/3/1

April 15, 2013

TO: WASHINGTON OCHIENG (PRINCIPAL INVESTIGATOR)

THROUGH : DR. JUMA RASHID;
DIRECTOR, CCR

Forwarded 15/04/2013

RE: **SSC PROTOCOL NO. 2477 – REVISED (RE-SUBMISSION): GENETIC CHARACTERIZATION OF HIV-1 STRAINS CIRCULATING IN KENYA AND DETERMINATION OF KNOWN MUTATIONS CONFERRING RESISTANCE TO ANTIRETROVIRAL DRUGS**

Make reference to your letter dated 4th April, 2013, Received on April 4, 2013.

We acknowledge receipt of the Revised Study Protocol.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document listed above and is satisfied that the issues raised at the 211th meeting held on 26th November, 2012 have been adequately addressed.

The study is granted approval for implementation effective this **15th day of April 2013**. Please note that authorization to conduct this study will automatically expire on **April 14, 2014**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **March 4, 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Sincerely,

**Dr. Elizabeth A. Bukusi,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE**

Appendix 5: Approval letter from the Principal Investigator

CREATES/RCDG/2012/CCRF/02

4th July 2013

Dr Michael Kiptoo,
ITROMID Coordinator,

Subject: Graduate Students completing thesis projects curved out of and appended to an Approved Protocol, SSC#2477

SSC protocol #2477 was approved by both the Scientific Steering Committee and the Ethical Review committees and is currently under implementation.

The following four students are completing their Masters Thesis projects under the main umbrella of the approved protocol but with independent projects answering various components of the main protocol. Kindly facilitate the consideration of these students' projects within the context of the approved protocol

Student	Project's topical area
Rose Kitawi	HIV type 1 genetic diversity and transmission patterns in Machakos, Kajado and Nakuru counties
Geoffrey Masankwa	Associations of Host Genetic polymorphisms with treatment and virological outcomes among HIV-1 infected individuals in Kenya
Maureen Kimulwo	Therapeutic drug levels and treatment responsiveness in Kenyan subjects infected with human immunodeficiency virus type 1
Timothy Nzomo	Molecular genetics of antiretroviral drug resistance among HIV-1 infected Kenyan subjects

Yours Sincerely



Washington Ochieng', PhD

Principal Investigators, KEMRI/CREATES/ACCT
Assistant Professor of Biology