PHARMACOGENETIC AND PHARMACOECOLOGICAL DETERMINANTS OF THERAPEUTIC RESPONSE TO NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AMONG HIV PATIENTS IN KENYA

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AUGUST, 2022

DECLARATION

I, the undersigned, hereby declare that this thesis is my original work has not been previously presented in its entirety or in part, for the award of any other degree or to any other university

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DEDICATION

My immeasurable and unending gratefulness are to my father, my maker, the Almighty God for the everlasting mercies, strength, will and capabilities to finalize this degree. It has been longer than expected. I dedicate this work to my wife and number one supporter, Evelyn Awino for her relentless love, prayers, support, patience and belief in me and to my five adorable children: George Otieno Tyris, Nadra Ngayo, Naji Muganda, Naila Ogoma and Nalani Musa for their strong love for me and outpouring encouragement. Thank you all, big.

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GLOSSARY

Allele: A variant form of a gene; in humans an allele is a member of a pair of different forms of a gene.

Association: A statistical finding that shows connection or cooperative link between two variables

Confidence interval: A range of values so defined that there is a specified probability that the value of a parameter lies within it

Enzyme: A protein molecule produced by living organisms that catalyzes the chemical reaction of substances (including drugs).

Etiology: the study of the causes, origins, or reasons behind the way that things are, or the way they function, or it can refer to the causes themselves.

Evaluation research: is the systematic assessment of the worth or merit of time, money, effort and resources spent in order to achieve a goal.

Gene: The basic biological unit of heredity – a segment of DNA that contributes to phenotype/function genes, up to the whole genome, and variable drug effects.

Genotype: The genetic constitution of an individual organism; that is the specific allelic makeup of an individual.

Haplotype: A set of genetic variants that are inherited together.

Heterozygote: A person who has two copies of an allele that are different.

Holistic: Dealing with or treating the whole of something or someone and not just a part

Homozygote: A person who has two copies of an allele that are the same.

Locus: A specific position on the genome, such as where a particular nucleotide is located.

Matrix effect: In chemical analysis, matrix refers to the components of a sample other than the analyte of interest. The matrix can have a considerable effect on the way the analysis is conducted and the quality of the results are obtained; such effects are called matrix effects.

Metabolism: Metabolism is the set of life-sustaining chemical reactions in organisms. Conversion to another chemical species

Mutation: A permanent alteration in the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA. Viral genomes can be of either DNA or RNA

Normal distribution/ the Gaussian distribution: The probability distribution that is symmetric about the mean, showing that data near the mean are more frequent in occurrence than data far from the mean

Nucleotide: Small molecules that are the basic constituents of DNA

Pharmacodynamics: The study of the relationship between drug concentrations and drug effects.

Pharmacoecologic factors: Non-biological factors those that influence the day-to-day concentration of drugs (adherence and drug interactions)

Pharmacogenetic factors: Are more fixed aspect of the host factors that potentially affect drug efficacy and toxicity. These include genes that may affect the overall disposition and activity of a drug, such as those related to absorption, distribution, metabolism, and excretion; drug transport; or the drug target itself

Pharmacogenetics: A term used to define inherited variability in response to drug treatment

Pharmacogenomics: The study of how all of the genes (the genome) can influence responses to drugs.

Pharmacokinetics: The study of the relationship between drug dose and drug concentrations (often as a function of time) in plasma or tissue.

Phenotype: The observable physical or behavioral traits of an organism largely determined by the organism's genotype but also influenced by environmental factors.

Polymorphism: The occurrence in the same population of multiple discrete allelic states of which at least two have high frequency of not less than 1%

Quality control standards: Are details of requirements, specifications, guidelines and characteristics that products, services and processes should consistently meet in order to ensure: their quality matches expectations. they are fit for purpose. they meet the needs of their users.

Resistance mutation: is a mutation in a virus gene that allows the virus to become resistant to treatment with a particular antiviral drug

Response: Reaction to drug

Sensitivity: The proportion of true-positive cases that are correctly identified by a test.

Selectivity: The discrimination shown by a reagent in competitive attack on two or more substrates or on two or more positions in the same substrate

Single-nucleotide polymorphism (SNP): The most common type of genetic variation in humans, which occurs when a single nucleotide [adenosine (A), guanine (G), cytosine (C) or thymine (T)] in the genome sequence is changed.

Statistical significance: The likelihood that a relationship between two or more variables is caused by something other than chance

Sub-therapeutic/ Sub-optimal: A dose or concentration of a drug) lower than that usually prescribed to treat a disease effectively

Substrate: Substance that is acted upon by an enzyme.

Therapeutic: concerned with the beneficial response to treatment of disease and the action of remedial agents.

Therapeutic drug monitoring (**TDM**): A branch of clinical chemistry and clinical pharmacology that specializes in the measurement of medication levels in blood. Its main focus is on drugs with a narrow therapeutic range, that is drugs that can easily be under- or overdosed.

Treatment outcome: In research infers to as a situation whereby the "emphasis is upon measuring significant aspects of personality before and after treatment and noting the nature and extent of the resulting changes

Xenobiotics: Chemical substances that are foreign to the biological system such as drugs.

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LIST OF ABBREVIATIONS AND ACRONYMS

3TC	Lamivudine
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
AZT	Zidovudine
CD4	Subgroup of T lymphocytes carrying CD4 antigens
CNS	Central Nervous System
CRDR	Center for Respiratory Disease Research
CYP2B6	Cytochrome P450 2B6
D4T	Stavudine
DNA	Deoxy-ribonucleic Acid
EFV	Efavirenz
FACES	Family AIDS Care and Education Services
FDA	Food and Drug Administration
h	Hour
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HQC	High quality control
KEMRI	Kenya Medical Research Institute
LLOQ	Lower limit of quantification
LMICs	Low middle-income countries
LOD	Limit of detection
LOQ	Limit of quantification
LQC	Low quality control
KNBS	Kenya national bureau of statistics
ME	Matrix effect
ml	Milliliter
mm	millimeter

MQC	Middle quality control
NNRTIs	Non-nucleoside/Nucleotide Reverse Transcriptase Inhibitors
NRTIs	Nucleoside/Nucleotide Reverse Transcriptase Inhibitors
NVP	Nevirapine
PI	Protease Inhibitor
PK	Protein Kinase
PMTCT	Prevention of Mother to Child Transmission
QC	Quality Control
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
S/N	Signal Noise
SID	Subject Identification Number
SNP	Single Nucleotide Polymorphism
SSC	Scientific Steering committee
TB	Tuberculosis

ABSTRACT

Despite the efficacy of antiretroviral therapy (ART) and the advancement in the prognosis of persons living with HIV/AIDS, a considerable proportion of persons on ART do not realize or retain adequate virologic suppression. Currently, 68% of adults and 73% of children living with HIV in Kenya are receiving ART treatment following the implementation of the 2015 World Health Organization (WHO) test and treat guidelines. During this study, the first-line ART treatment recommendations in Kenya typically comprised of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI): either nevirapine (NVP) or efavirenz (EFV). High interpersonal variability in the pharmacokinetics of ARV drugs have been reported, which may jeopardize ART treatment gains if not appropriately managed. Therapeutic drug monitoring (TDM) of antiretrovirals (ARVs) purposes to identify elevated or sub-therapeutic ARV concentrations which allows for prompt dosage adjustments preventing patients' exposure to toxic or subtherapeutic concentrations. Optimal ART outcomes inevitably require an understanding of the individual variation in response to ART. This study determined the pharmacogenetic and pharmacoecological determinant of sub-therapeutic responses to NVP and EFV among HIV patients in Nairobi Kenya.

In this non-comparative cross-sectional study, a total of 599 HIV patients were recruited who provided 5ml blood samples 8 hours post ART medication as well as participated in a face to face structured interview. The CD4 cell, viral load, full blood hematology and blood chemistry measurements were determined according to manufactures' instruction. HIV drug resistant mutations were identified using an in-house sequencing method while cytochrome P450 (CYP26B) and constitutive androstane receptor (CAR) single nucleotide polymorphisms (SNPs) were identified using Real Time Polymerase chain reaction (RT-PCR). The NVP and EFV plasma levels were measured using ultra-high-performance liquid chromatography with a tandem quadruple mass spectrometer (LC/MS/MS). All data were subjected to descriptive statistical analysis. The NVP and EFV plasma levels were first tested for normality and presented as the median and interquartile range (IQR). Inferential data analysis of drug plasma levels were

evaluated using the student's t-test and one-way ANOVA. Categorical variables were summarized as frequencies and percentages while the Pearson's chi square and fisher-exact tests used for inferential analysis of categorical variables. Host factors relate to NVP and EFV plasma level either directly or indirectly by affecting ART adherence. The relationship between pharmacoecological factors with ART adherence was first evaluated using fisher-exact or chi-square and only significant variables were evaluated for association with NVP or EFV plasma level using quantile linear regression. Data analysis was done using STATA v 13 software (StataCorp LP, Texas, USA). The allele, genotype and haplotype frequency and deviation from Hardy-Weinberg of CYP26B and CAR single nucleotide polymorphism (SNP) was analyzed using an online SNPStats software (https://www.snpstats.net/start.htm). The level of significant was set at $p<0.05$.

All the 599 patients enrolled (100% responses rate) had data for objective one, while 566 (94.5% responses rate) had data for objectives two, three and four. The median age of the 599 patients was 41 years [IQR 35-47 years] with the majority (60.3%) being female and 56.1% were receiving EFV based regimen. The CD4 cell count (mean ± SD) significantly increased at the12-month post ART initiation (301.7 ± 199.4 cell/ml to 329.4 ± 305.8 cell/ml; $P<0.05$). Hepatotoxicity and renal abnormalities occurred more frequently at month 12 compared to baseline; ALT (2.5% versus 10.5%), AST (5.3% vs 23.4%) and creatinine (63.4 vs 68.84%). Fewer patients at month 12 had anemia (29.4% vs 56.4%), leucopenia (42.4% vs. 46.9%) and thrombocytopenia (6.5% vs. 84.1%) compared to baseline. The median [interquartile range – IQR] NVP plasma (n = 254) concentration was 6237.5 [4518 – 8964 ng/ml] and 2739.5 [1878 - 4891.5 ng/m] for EFV (n = 312). Majority 54.3% of patients had supra-therapeutic NVP plasma levels followed by 31.5% and 14.2% with sub-therapeutic and therapeutic plasma concentrations respectively ($p < 0.001$). Patients on EFV (63.8%, 31.7% and 4.5%) had therapeutic, supra-therapeutic and sub-therapeutic plasma concentrations respectively ($p < 0.001$).

Thirteen CYP2B6 (329G>T, 341T>C, 444 G>T/C, 15582C>T, 516G>T, 548T>G, 637T>C, 785A>G, 18492C>T, 835G>C, 1459C>T and 21563C>T) and one CAR (540C>T) SNPs were detected among study patients. Hardy-Weinberg equilibrium could not be tested for CYP2B6 329G>T, 341T>C, 444 G>T/, 637T>C, 835G>C, and 548T>G SNPs due to lack of heterozygous and/or homozygous mutants. Linkage disequilibrium (LD) was observed among CYP2B6 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T, resulting in 8 haplotypes among which CTGCTTCC and CGATTCCT had the highest and the lowest frequency. The most frequently occurring mutations with a prevalence of more than 30% were CYP2B6 516G>T, 785A>G and 21563C>T followed by CYP2B6 18492C>T and 15582C>T at a frequency of between 10 to 20%. The CAR (540C>T) and CYP2B6 983T>C occurred at a rate of 5 to 10% with CYP2B6 1459C>T, 329G>T, 341T>C, 444 G>T/C, 637T>C and 835G>C rarely occurred (>0.05%). The genotype and allele frequencies of SNPs were similar, regardless of the ART regimen. The following SNPs: CYP2B6 329 G>T; 15582C>T, 516G>T, 785A>G and 21563C>T, were associated with reduced NVP metabolism; while CYP2B6 18492C>T and 983T>C with increased NVP metabolism. The SNPs CYP2B6 15582C>T, 516G>T, 785A>G, and 21563C>T were associated with reduced metabolism of EFV and CYP2B6 18492C>T with increased EFV metabolism. The CYP2B6 and CAR inferred phenotypes associated with a reduced metabolism were found in 171/566 (30.2%) of all study patients; and 91/312 (29.2%) on EFV and 80/254 (31.5%) on NVP. Phenotypes associated with increased metabolism were found in 39.9% (n = 226/566) of the patients, 122/312 (39.1%) patients on EFV and 104/254 (40.9%) patients on NVP.

On multivariate quantile regression analysis, factors that remained associated with high NVP plasma concentration included; feeling guilty for being HIV positive (adjusted β 954, 95% CI 192.7 to 2156.6; p =0.014) or feeling worthless for being HIV positive (adjusted β 852, 95% CI 64.3 to 1639.7; p =0.034), disclosing patient's HIV status to neighbors (adjusted β 1731, 95% CI 376 to 3086; p = 0.012), regular uptake of porridge (adjusted β 1780, 95% CI 121.4 to 3438.6; p =0.036), missing taking current ARVs for the whole day or more (adjusted β 4287.9, 95% CI,

826.2 to 7749.6; p = 0.015), current white blood cell concentration (adjusted β 4012.4, 95% CI 1032.2 to 6992.6; p < 0.001). The SNPs associated with NVP plasma levels on multivariate analysis included; CYP2B6 329G>T (adjusted β 11343.2, 95% CI 5738.8 to 16947.6; p <0.001), CYP2B6 341T>C (adjusted β -8614.2, 95% CI -9621.7 to -7606.7; p < 0.001), CYP2B6 516G>T (adjusted β 3603.4, 95% CI 589.6 to 6617.2; p = 0.019), CYP2B6 18492 C >T (adjusted β -1301.4, 95% CI -2195.3 to -407.5; p = 0.004), CYP2B6 983T>C (adjusted β 2948, 95% CI 313.2 to 5582.8; p =0.028), CYP2B6 CAR 540 C>T (adjusted β -1543.2, 95% CI -2683.7 to -402.7; p =0.008) and number of SNPs per patients (adjusted β 1129.6, 95% CI 729.9 to 1529.3; p <0.001).

On multivariate quantile regression analysis factors that remained significantly associated with high EFV plasma levels included; disclosing HIV positive status (adjusted β 363, 95% CI, 97.9 to 628.1; p = 0.007), obtaining ARV pill uptake information from other sources (adjusted β 18421.3, 95% CI, 16291.5 to 20551.2; p <0.001), stopping taking current ARVs due nausea (adjusted β 713, 95% CI 425.5 to 1000.5; p <0.001) and stopped taking current medication due to skin problems such as rash (adjusted β 1847, 95% CI 137.9 to 3556.1; p =0.035) and the presence of body pain in the past 30 days (adjusted β 475, 95% CI, 117.2 to 832.8; p = 0.009). The SNPs CYP2B6 516G>T (adjusted β 2414, 95% CI 1876.6 to 2951.4; *p <0.001*), CYP2B6 835 G >C (adjusted β 2877, 95% CI 1498 to 4256; *p <0.001*) and number of SNPs per patient (adjusted β 570, 95% CI 362 to 778; *p <0.001*).

Some of the important limitations worth mentioning in this study included. First, the use of NVP-based ART regimens in Kenya and other countries, especially developed countries, has been considerably reduced in the recent past, meaning that this study could be relevant to a restricted number of patients. Second, standardized tools for measuring pharmacoecological variables are not available, limiting the generalizability of this study outcomes. Third, this was a cross-sectional study, which only permitted the description of the relationship between pharmacogenetic and pharmacoecological factors and NVP/EFV plasma concentrations and not a causal conclusion. Such outcomes can be confirmed in a longitudinal study. These limitations notwithstanding, the

following conclusions can be drawn. These data establish prolonged immunologic/virologic response to ART among patients continuing on therapy. The prevalence of anemia, thrombocytopenia and leucopenia was low with minimal renal and hepatotoxicity impairment observed. Wide interindividual variability were observed in NVP and EFV plasma concentrations with a large proportion of patients falling outside of therapeutic window, proposing potentially an increased risk of treatment failure or toxicity. This study demonstrates the importance of therapeutic drug monitoring in determining the ARV treatment outcome. Six SNPs of the *CYP2B6* gene (CYP2B6 329G>T, CYP2B6 637T>C, CYP2B6 785A>G, CYP2B6 18492C>T, CYP2B6 21563C>T and 15582C>T) and one CAR (540T>C) could potentially act as additional independent predictors of NVP and EFV plasma concentrations beyond that provided by the *CYP2B6* c.516G>T and CYP2B6 983T>C polymorphism. Host pharmacoecological factors influence ART drug adherence impacting on the NVP and EFV plasma concentrations.

PREFACE

Chapter one

This chapter provide an introduction of the subject matter. The chapter highlights the current HIV situation in Kenya touching on the prevalence, treatment options, treatment limitations, assessment of sub-optimal drug concentrations and an overview of the etiology of sub-therapeutic ART treatment outcome. The chapter also provides the study justifications and objectives.

Chapter two

This is the literature review section. The chapter gives an exposition of ART therapy with regards to type, efficacy and biotransformation. It further describes the etiology of sub-therapeutic ART treatment outcomes. This is segregated to include factors attributed to the virus, co-morbidity, drugs, and host pharmacoecologic and pharmacogenetic characteristics. Under host pharmacogenetic factors, this section provides an overview of cytochrome P450 enzyme system as well as the constitutive androstane receptor (CAR). The role of therapeutic drug monitoring to individualize ARV treatment is also highlighted.

Chapter three

This is the experimental chapter of the study. The chapter describes all the materials, equipment, methodological and procedural set up applied to achieve the objectives of this study. The chapter also provides a detailed description of data management and analysis.

Chapter four

This chapter is organized into specific objectives; which are handled separately. Each objective is accompanied by (i) an introductory preview (ii) results, (iii) discussion of the results in comparison to published findings (iv) limitations, conclusions and recommendations of each objectives

Chapter five

This chapter discusses in summary significant findings of each objectives.

Chapter six

This chapter is highlights key conclusions and recommendations of each objectives

PUBLICATIONS

The following papers and abstracts/ presentations are either published or in different stages of publication

Published papers

1. Ngayo MO, Okalebo FA, Bulimo WD, Mwachari C, Guantai AN, et al. (2016) Impact of First Line Antiretroviral Therapy on Clinical Outcomes Among HIV-1 Infected Adults Attending One of the Largest HIV Care and Treatment Program in Nairobi Kenya. J AIDS Clin Res 7: 615. doi: 10.4172/2155-6113.1000615
2. Ngayo MO, Oluka M, Bulimo WD, Okalebo FA. Association between social psychological status and efavirenz and nevirapine plasma concentration among HIV patients in Kenya. Sci Rep. 2021 Nov 11;11(1):22071. doi: 10.1038/s41598-021-01345-9. PMID: 34764325.
3. Musa Otieno Ngayo, Margaret Oluka, Zachari Arochi, Wallace Dimbuson Bulimo, Faith Apolot Okalebo. Effects of cytochrome P450 2B6 and constitutive androstane receptor genetic variation on Efavirenz plasma concentrations among HIV patients in Kenya. PlosOne. 2021 doi: 10.1371/journal.pone.0260872

Proposed papers

1. Variability of efavirenz and nevirapine plasma concentrations among HIV patients receiving first line antiretroviral therapy in Nairobi, Kenya. Target journal AIDS Research and Therapy

2. Adherence to antiretroviral therapy among HIV infected adult measured by self and medical report, pill count drug level in Nairobi Kenya. Target journal AIDS Research and Therapy
3. Association between EFV and NVP plasma concentrations and clinical outcome: a focus of pathological, hematological, side effects and medical adverse outcomes among HIV patients in Nairobi, Kenya. Target journal: BMC Pharmacology and Toxicology

4. Human CYP2B6 and CAR genetic variability across Kenyan ethnic community: a case of haplotype diversity and EFV and NVP plasma concentrations. Target journal Pharmacogenomics

CHAPTER ONE

INTRODUCTION

1.1. Background information

Treatment of HIV is complex and many factors must be considered for a successful clinical outcome. To help reduce the burden of HIV, the utilization antiretroviral therapy (ART) has played a fundamental role. Worldwide, significant achievements have been attained in the HIV testing and treatment cascade. Globally, by 2020, 73% (about 27.4 million) of 37.6 million HIV infected persons were receiving ART treatment (UNAIDS, 2020). Notable concerted effort to increased ART uptake has positioned Kenya on the pathway to attain the target on AIDS-related deaths. Kenya, by 2015 initiated the implementation the test and treat World Health Organization (WHO) guidelines which has spiraled further, the utilization and access of ART treatment (UNAIDS, 2016). By the end of 2020, approximately 74% adults and 73% children in Kenya requiring ART were truly utilizing it (UNAIDS, 2020). By 2020, an outstanding percentage of HIV patients on ART (68%) were virally suppressed (UNAIDS, 2020). During the current study, the first-line ART used according to the Kenyan guidelines comprised a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs; stavudine [d4T], tenofovir [TDF], zidovudine [AZT], or lamivudine [3TC]), plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) either efavirenz (EFV) or nevirapine (NVP) (NASCOP, 2018).

Optimal ART treatment outcomes inevitably require appreciation of patient's variation in response to ART which includes both efficiency and toxicity. ART treatment outcomes are influenced by the viral factors (resistance pattern) (Parra et al., 2011; Swenson et al., 2010) and drug factors (including toxicity profile, convenience, pill burden, and penetration into the central nervous system (CNS). Lastly, host factors are equally important in the individualization of ART and comprise both pharmacoecologic and pharmacogenetic factors (Parra et al., 2011). Pharmacoecologic factors; are those that influence the day-to-day drug exposure (adherence and drug interactions) (Phillips & Mallal, 2009). An immutable factor includes pharmacogenetic factors that possibly affect drug efficacy and toxicity. These

comprise genes that may affect the entire disposition and action of a drug, including those associated to drug target and transportation, drug absorption, distribution, metabolism and excretion (Phillips & Mallal, 2009). Understanding genetic variations that predict drug response is beneficial in guiding selection of the starter drug regimen, promoting effectiveness and reducing adverse reactions (ADRs) (Chantratita et al., 2011). Currently, ART treatment in Kenya is not individualized and majority of patients are affected by sub-optimal clinical outcomes. Hence the need to adopt more beneficial and innovative approaches including the use of therapeutic drug monitoring (TDM).

The vital aspect of ART administration is the optimal exposure to drug. The extended usage of NVP and EFV or suboptimal exposure to these drugs, is associated with significant risks that might jeopardize ART treatment success especially to NNRTIs (Muro et al., 2005). Sub-therapeutic plasma levels especially for NVP and EFV are prognostic of virologic failure and emergence of HIV drug resistant strains (Fabbiani et al., 2011; Khoo et al., 2006). Reports in Africa have shown wider interpersonal dissimilarity in the ART plasma levels in HIV infected persons with treatment failure, and a huge proportion dropping below the ART therapeutic window (Gunda et al., 2013; Kimulwo et al., 2017). Although several reports have linked plasma NVP and EFV concentrations with selected treatment outcomes (Baxi et al., 2015), in many developing nation, Kenya included therapeutic drug monitoring has not been integrated in HIV management programs (Kimulwo et al., 2017). This study investigated the pharmacogenetic and pharmacoecological etiology of therapeutic responses to efavirenz and nevirapine among HIV patients on first-line ARV management in Nairobi Kenya with a view together information that might help guide individualization of ART management treatment in Kenya.

1.2. Study problem

A sizeable number of HIV infected persons live in the developing countries. In most of these countries the health budgets allocation are meager, and are further faced with huge shortage in the number of doctors, poor laboratory monitoring, a significant weight of HIV infection, and high rates of tuberculosis co-infection (UNAIDS, 2020). Consequently, the degree to which

any new ARVs will aid in the management of HIV infection will be guided by a number of factors including: toxicities, cost, usability, various formulations, and co-admissibility with other commonly used drugs (Clumeck et al., 2008). Efavirenz and Nevirapine had gained huge importance in treatment of HIV infection for decades in several countries including Kenya (Clumeck et al., 2008; Gazzard et al., 2008). Efavirenz retains a role after failure of a first PI - based regimen. However, some EFV recipients experience virological failure (Gulick et al., 2006) and/or central nervous system (CNS) symptoms (Clifford et al., 2009). Nevirapine was the primary NNRTI used to treat women because EFV was contraindicated for women of child-bearing potential. Although many reports have associated plasma NVP and EFV concentrations with ART treatment outcomes (Baxi et al., 2015), unfortunately, measurement of plasma drug concentration is not routinely done as part of HIV treatment guidelines in Kenya (Kimulwo et al., 2017).

Successful HIV treatment involves many complex and interrelated factors working in synergy to achieve optimum therapeutic outcomes. These treatment outcomes are influenced by several factors which must be controlled for. These factors are often multi-factorial involving the nature of HIV virus itself such as the presence of ART resistant mutation (Parra et al., 2011) and ART drug related factors affecting toxicity. The host related factors are equally important in shaping the outcome of any treatment. These factors are pharmacoecological and pharmacogenetic in nature (Parra et al., 2011). Pharmacogenetic factors are fixed affecting drug efficacy and toxicity. Generally, NVP and EFV are metabolized by majorly the hepatic enzyme CYP2B6, CYP3A4 and the influence of the constitutive androstane receptor (CAR) (Ward et al., 2003). Although CYP2B6 785A>G, 983T>C, 516G>T and 1459C>T are well documented to influence NVP and EFV plasma levels (Ngaimisi et al., 2013), single SNP analysis of CYP2B6 516G>T only might be inadequate in generating satisfactory data to predicting intra and inter personal differences of EFV and NVP plasma concentrations. Multifactorial approach to establish association of battery of factors working in synergy to affect ART treatment outcomes is preferred to help guide ARV individualization.

An important feature of these hepatic enzymes as well as other human genetic driven functions, is that they are ethnic or race dependent. Kenya just like as in many Sub-Saharan African

populations, have over time acquired enormous genetic diversity than any other race in the world (Prugnolle et al., 2005). In a race against time to optimize ART treatment outcomes having in mind the 2015 WHO recommendations of immediate ART treatment to people diagnosed with HIV (UNAIDS, 2016), evaluating the role of ethnic diversity was important in contributing to the individualization of ART treatment in Kenya.

1.3. Study Justification

At the time of this study, NVP was predominantly prescribed NNRTI back-born of first line ART regimen in Kenya primarily due to its application in PMTCT, effectiveness, availability and affordability (WHO, 2016). For decades, EFV has been widely used in the management of HIV infection and has majorly contributed to the development of highly active antiretroviral therapy (HAART) in many countries (Clumeck et al., 2008; Gazzard et al., 2008). However, these two drugs are associated with greater occurrence of severe and life threatening skin rashes; hepatotoxicity; teratogenicity; and central nervous system (CNS) or neuropsychiatric disturbances; and greater chance of development of HIV drug resistant mutations due to low genetic barrier (Maggiolo, 2009; Aizire et al., 2012; Clutter et al., 2016). This limits their usefulness. Understanding inter-individual variation in the response to ART treatment is key to achieving individualized therapeutic outcomes. Both sub-therapeutic and supratherapeutic ART responses, more so for NVP and EFV or any ARV drug with a low genetic barrier, offers innumerable risks for the success of treatment (Muro et al., 2005). Factors that affects optimal drug levels are two-fold; those that affect day to day drug concentration generally termed as pharmacoecological including (adherence, comorbidities, nutrition, social psychological attributes, and stigma) and host genetic factors referred as pharmacogenetic characteristics. These include patients genetic makeup affecting drug disposition and activity (Phillips & Mallal, 2009).

The liver enzyme CYP2B6 contributes majorly in the hydroxylation of both EFV and NVP with NVP also being metabolized by CYP3A4 (Ward et al., 2003). The constitutive androstane receptor (CAR) are involved in basal and inducible regulation of EFV metabolizing enzymes (Wyen et al., 2011). The focus of diverse studies have been on several SNPs of CYP2B6, such

983T>C, 785A>G, 516G>T, 1459C>T among others which are involved in the metabolism of both EFV and NVP (Maimbo et al., 2012; Ngaimisi et al., 2013). Unfortunately, data gathered from individual SNP may not be adequate in predicting the variations in EFV or NVP plasma levels. Substantial research has been geared towards understanding the association of numerous SNPs that reduce the metabolic function of CYP2B6 in order to promote their prediction accuracy (Carr et al., 2010). An important feature of these hepatic enzymes as well as other human genetic driven functions, is that they are ethnic or race dependent. Kenya just like many Sub-Saharan African populations, have over time acquired enormous genetic variety than any other race globally (Prugnolle et al., 2005). Ethnic diversity is shown to influence the distribution of variant alleles between populations leading to population genetic diversity which has been associated with intra and inter drug plasma exposure (Ngaimisi et al., 2013). Although these ethnic groups reside in close proximity to each other, their cultural and environmental variation is wide (KNBS, 2019). Subsequently, the Kenyan population is marked by vast individual genetical and environmental diversity that could alter the effectiveness and adverse events or treatment outcomes among individuals and ethnic blocks receiving similar ART regimen (Ngaimisi et al., 2013). Individual pharmacoecological parameters including age, sex, body mass index, CD4 and HIV viral load nadir play a key role on the NVP and EFV plasma levels (Bennett et al., 2008; Wang et al., 2014). This study therefore aimed at determining the level of sub-therapeutic outcomes and the role of pharmacogenetic and pharmacoecological factors in therapeutic responses among HIV patients receiving NVP and EFV first-line ARV treatment in Nairobi, Kenya.

1.4. Research questions

1. Are there significant differences in immunological, virologic, hematological and biochemical outcomes among HIV patients receiving nevirapine and efavirenz containing first line antiretroviral therapy between baseline and at 12 months into treatment?
2. Are there significant differences in the prevalence of sub-therapeutic and toxic plasma levels of NVP and EFV in HIV patients at 12 months into antiretroviral therapy?

3. What are the pharmacoecological factors affecting plasma concentrations of NVP and EFV among HIV patients at 12 months into antiretroviral therapy?
4. What are the pharmacogenetic factors (CYP2B6 and CAR (540C>T) genetic polymorphisms) affecting NVP and EFV plasma concentrations among HIV patients at 12 months into antiretroviral therapy?

1.5. General objective

To evaluate the pharmacogenetic and pharmacoecological determinants of therapeutic response to nevirapine and efavirenz among HIV patients receiving treatment in Kenya

1.5.1. Specific objectives

1. To determine the immunological, virological, hematological and biochemical outcomes among HIV patients receiving nevirapine and efavirenz containing first line antiretroviral therapy at 12 months into treatment
2. To establish the prevalence of sub-therapeutic and toxic NVP and EFV plasma concentrations among HIV patients at 12 months into antiretroviral therapy
3. To describe pharmacoecological factors affecting NVP and EFV plasma concentrations among HIV patients at 12 months into antiretroviral therapy
4. To determine pharmacogenetic factors affecting NVP and EFV plasma concentrations in HIV patients at 12 months into antiretroviral therapy

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of HIV in Kenya

Kenya together with Tanzania are ranked third-largest countries in terms of HIV epidemic globally with 1.4 million adults and children living with HIV in 2020 (UNAIDS, 2020). The review further shows that in 2020, 19,000 adults and children died due to AIDS-associated complications. Although these statistics are still abnormally high, the mortality due to HIV/AIDS has been marked by a progressive decline from approximately 65,000 deaths reported in 2010 (UNAIDS, 2020). The HIV infection was reported in Kenya around 1983-4 and in the 1990s, HIV infection was responsible for significant diseases and deaths in Kenya. The prevalence of HIV in Kenya has since reduced by almost half from 10.6% in the 1900s to around 4.6% currently. This significant mortality morbidity decline has been attributed to the upscale of utilization of ART for HIV treatment and management (MoH-NACC, 2018). Reports in Kenya shows that HIV epidemic is majorly due to sexual transmission with women bearing the largest prevalence of HIV infection (MoH-NACC, 2018). Further, geographical variation in HIV prevalence has been reported in Kenya with counties in western and Nyanza region reporting the highest prevalence of about 23% in Siaya to about below 1% in the Northern counties of Kenya such as Wajir, Mandera and Isiolo (Table 2.1).

Table 2.1. Estimated new HIV infections among adult population by county in Kenya (adopted from MoH-NACC, 2018)

Counties	New HIV Infections	Counties	New HIV Infections	Counties	New HIV Infections	Counties	New HIV Infections	Counties	New HIV Infections
Homa Bay	9,629.2	Muranga	1,640.4	Kericho	318.3	West Pokot	93.1	Bungoma	1,145.0
Kisumu	8,790.2	Uasin Gishu	520.2	Makueni	1,570.9	Embu	595.9	Marsabit	151.9
Siaya	7,700.3	Bomet	216.6	Meru	1,391.6	Samburu	57.6	Busia	1,466.9
Migori	5,092.7	Trans Nzoia	508.4	Kitui	1,546.7	Tharaka	486.3	Lamu	103.6
Kisii	2,071.9	Narok	308.8	Nyandarua	767.9	Elgeyo Marakwet	85.1	Tana River	124.7
Nakuru	800.9	Mombasa	2,426	Kilifi	1,413.0	Taita Taveta	526.6	Vihiga	735.5
Nairobi	4,719	Kajiado	393.8	Kirinyaga	741.9	Kakamega	1,934.7	Wajir	27.5
Turkana	437.7	Machakos	1,744.2	Baringo	108.6	Isiolo	193.4	Kenya	71,034
Kiambu	4,273.1	Nyeri	1,123.6	Laikipia	151.0	Mandera	72.9		
Nyamira	425.1	Nandi	217.9	Kwale	1,067.8	Garissa	54.6		

2.2. HIV treatment

The advancement and improvement in the treatment of HIV for the past two decades has been instrumental in prolonging the survival of people living with HIV/AIDS (Bhaskaran et al., 2008). The currently used ARVs are associated with better virologic suppression and limiting the rates of ARV discontinuation as a results improved ease of use and tolerability (Gazzard et al., 2008; Willig et al., 2008). The current ART recommendations are that the first line should contain two nucleoside reverse transcriptase inhibitors (NRTIs) and either one non-nucleoside reverse transcriptase inhibitor (NNRTI) or a boosted protease inhibitor (PI) (Clumeck et al., 2008; Gazzard et al., 2008; Hammer et al., 2008; NASCOP, 2018). In this study, the first-line ART in use comprised two of the following NRTIs (zidovudine – AZT, tenofovir - TDF, stavudine - d4T, or lamivudine -3TC), and one NNRTI either NVP or EFV (NASCOP, 2018). Following the 2015 WHO approvals, dolutegravir -DTG is nowadays the chosen first line NNRTI in a fixed-dose combination with TDF and 3TC used in many developing countries including Kenya (UNAIDS, 2020). The use of DTG in developed countries has been marked by limited side effects, and in its formulations of one small tablet taken on a daily basis makes

it is easy to use and it is currently associated with few if not none the development of HIV drug resistant mutations (NASCOP, 2018).

Antiretroviral therapy use is a key component used in lowering or eliminating the scourge of HIV. Fundamental heights has been achieved regarding HIV infection detection testing and management (UNAIDS, 2020). Extraordinary scale up of ART has positioned Kenya on pathway to attaining the target in reducing HIV/AIDS-related mortalities. As of 2015, Kenya implemented the WHO 2015 suggestions that necessitated countries to test and treat people infected with HIV irrespective of their CD4 or viral load status (UNAIDS, 2016). By 2020 about 76% adults and 72% children in Kenya requiring ART were essentially put on this life saving treatment (UNAIDS, 2020). An astonishing percentage of these patients about 68% reported significant virologic and immunologic gains (UNAIDS, 2020).

2.3. Efficacy of efavirenz versus nevirapine

During the period of this study, NVP was a predominantly prescribed ARV in many developing nations, Kenya included. This drugs' effectiveness, convenience, cheapness and its application in the PMTCT were the main reasons for its wide use (WHO, 2016). Unfortunately, NVP is associated with higher occurrence of skin rashes such as Stevens-Johnson syndrome which life-threatening disease (Van Leth et al., 2004), life-threatening risk of hepatotoxicity (Aizire et al., 2012) and a greater risk for development of drug resistant mutations are some of the factors that restricted NVP continued use (Ochieng et al., 2015; Clutter et al., 2016).

For over two decades, efavirenz has been an essential component of first line ART treatment of HIV infection and has added great value in the development of HAART in many countries (Clumeck et al., 2008; Gazzard et al., 2008). The effectiveness of EFV has been recognized in abundant reports in HAART-naive patients, including those with advanced infection. The one-pill, once-daily formulation of EFV plus two other NNRTIs had been preferred formulations (Clumeck et al., 2008). Efavirenz also maintains a fundamental function in the advent of treatment failure of a first protease inhibitor (PI) based regimen. This drug is essentially well endured; rash, teratogenicity and central nervous system (CNS)are the common experienced adverse reactions (Maggiolo, 2009). Just like NVP, EFV is also prong to development of HIV

drug resistant mutations limiting its use (Clutter et al., 2016). Efavirenz is contra-indicated for patients presenting with severe psychiatric disease and in pregnant women during the first trimester of pregnancy. Efavirenz has fewer effects on plasma lipid profiles than some boosted PIs. Lipodystrophy can occur during treatment with EFV but it may be reduced if concurrent use of thymidine analogues is avoided (Maggiolo, 2009).

In contrast to the findings of the non-randomized observational cohorts that compared NVP and EFV, two direct, prospective, randomized, head-to-head clinical trials between EFV and NVP failed to find a significant difference between the efficacies of the two NNRTIs (Van Leth et al., 2004). Other studies have shown different pictures; the Antiretroviral Therapy Cohort Collaboration (ART-CC), reported that patients initiated on NVP-based regimens were twofold more prone to have virologic failure or to die six months into treatment compared to patients initiated on EFV (Casabona et al., 2008). Another earlier study among patients from the Veterans Affairs also testified poorer virologic and immunologic outcomes with NVP than with EFV (Braithwaite et al., 2007). The study by Núñez et al., (2002) reported no meaningful variance in the percentage of patients attaining virologic suppression at 48 weeks post initiating either NVP or EFV (Núñez et al., 2002). In the larger 2NN trial (Van Leth et al., 2004), NVP was associated with more serious toxicities. In addition, among South African subjects in 2NN, rates of failure of NVP was higher than EFV-based regimens (50% versus 38.3%) (Van Leth et al., 2004). Similar results were observed in the study by Nachega *et al.,* (2008) which showed EFV was linked with greater virologic and clinical outcomes than NVP, proposing that EFV might be the desired NNRTI in resource-limited settings.

2.4. Immunological, hematological and biochemical outcomes during ART

Disease progression in HIV-infected patients is generally delayed by treatment with ART. The ARVs reduces viral replication and increases the number of CD4+ cells (Carbonneil et al., 2004). The treatment of HIV infection is a life-long undertaking, and therapeutic benefit can be limited by the evolution of drug-resistant virus and long-term toxicity resulting in treatment failure. However, the infection remains currently incurable and lifetime treatment is required due to the viral persistence in latent reservoirs that are not accessible to ART and not detectable

by the immune system (Murray et al., 2016). More so, the patients receiving ART will experience significant side effects. ART is known to be toxic to liver and bone marrow, in several studies the relationship of HIV viruses and anti-retroviral drug effect on several parameters has been reported (Baynes et al., 2016; Kayode et al., 2020). Hematological and metabolic abnormalities are frequent among HIV+ infected patients and may be directly attributed to the virus. The abnormalities may be caused by opportunistic infections, neoplasms that cause bone marrow suppression or hemolysis though that can be corrected, prevented and improved by treatment with ART (Enawgaw et al., 2014). Anemia is a common hematological abnormality in HIV+ patients and has been shown to predict disease progression and mortality (Kyeyune et al., 2014). But until now, the exact mechanism by which HIV in vivo alters the microenvironment in the bone marrow to inhibit hematopoiesis and directly lead to decreased blood cells remains unclear. Studies have shown an increase in hemoglobin, RBC, total leukocyte count and T CD4 lymphocytes in patients on ART (Tesfaye et al., 2014; Ebony et al., 2017). Liver dysfunction is the most common cause of death unrelated to AIDS, accounting for 14% - 18% of all deaths (Palella et al., 2006). Nevirapine has been reported as eosinophilia, granulopenia, jaundice and increased enzymes associated to liver function abnormality (Chamroonkul and Bansal, 2019). Some factors that can explain abnormalities of liver enzyme in HIV patients include opportunistic infections, AIDS related neoplasms, concomitant infection with chronic hepatitis C virus, chronic hepatitis B virus, alcohol abuse, and nonalcoholic fatty liver disease (Chamroonkul and Bansal, 2019).

2.5. Pharmacogenetics

Pharmacogenetics is the study of the inter-individual variability of the body's responses to drugs due to genetic factors. In combination with clinical (age, sex, weight, type of disease) and environmental (nutrition and co-medication) factors, genetic factors influence the effectiveness of a treatment and can cause the onset of side effects (Pirmohamed, 2011; Rotger et al., 2006). Following clinical observations of treated patients who had sub- or supra-optimal plasma or urine concentrations, the first inter-individual variations were identified in the genes responsible for drug metabolism. This phenotypic approach to visualize differences in the metabolism of a drug within a population was used until the late 1980s (Meyer, 2004). It was based on determining the metabolic ratio between a drug and its metabolite. This method made

it possible to differentiate between individuals based on their metabolic capacity. For example, those who have a high metabolic ratio corresponding to a slow rate of metabolism (poor metabolizer) having two non-functional alleles. On the other hand, individuals who have an intermediate metabolic ratio (intermediate metabolizer) having either one non-functional allele and one deficient allele, or two deficient alleles. Individuals who have a normal metabolic ratio (extensive metabolize having two functional alleles) and finally individuals who have a low metabolic ratio corresponding to a high rate of metabolism (“ ultra-rapid metabolizer ”having at least one gene duplication) (Figure 2.1) (Zanger, 2008). Pharmacogenetics play a key role in antiretroviral therapy because it helps determine the genetic factors that make antiretroviral drugs toxic or that vary the response to treatment in treated patients (Rodríguez-Nóvoa et al., 2006).

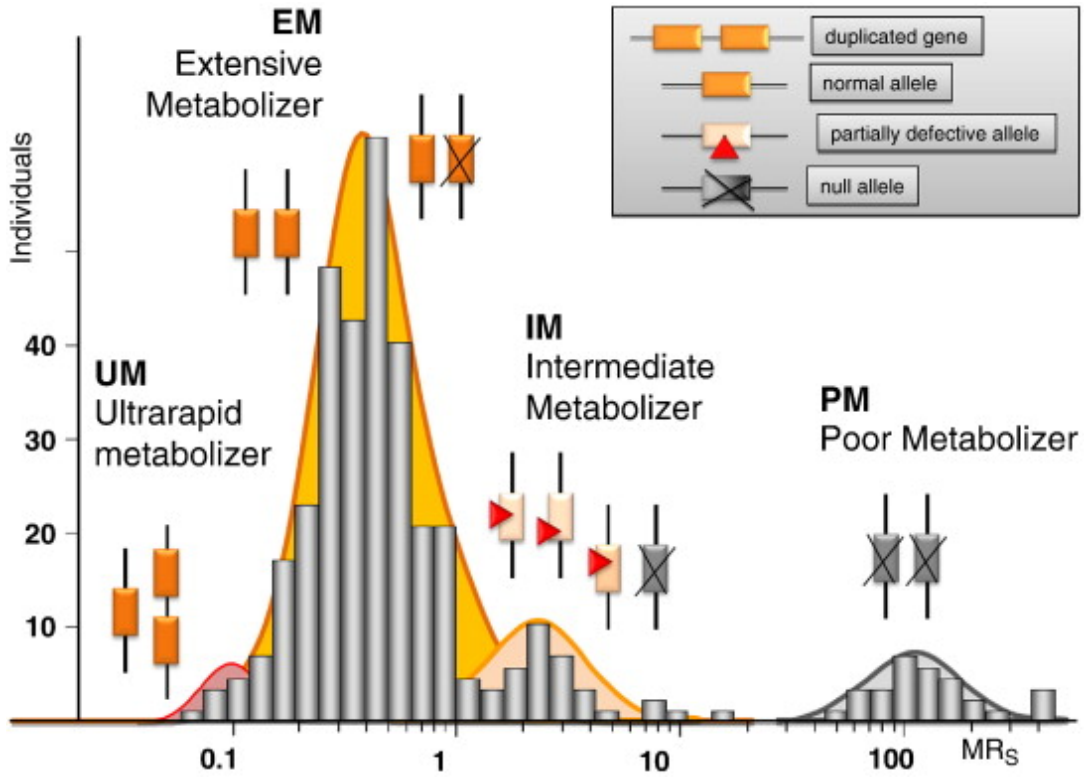


Figure 2.1. Phenotypic and genotypic distribution of the oxidation of spartein by cytochrome P450 CYP2D6: MR, metabolic ratio (adopted from Zanger 2008).

2.6. Therapeutic Drug Monitoring

Therapeutic Drug Monitoring (TDM) allows prompt dosage adjustments based on individual plasma levels to optimize the therapeutic effect of the medication and prevent exposure to toxic or subtherapeutic concentrations of the drug (Rakhmanina et al., 2004). The criteria for successful TDM include: a well-defined therapeutic range, strong correlation between drug concentration and therapeutic effect, high interpatient and low intra-patient variability in plasma concentrations and failure of the long-term out comes with inadequate target levels (Acosta et al., 2002). A reliable cost-effective method should be available for the determination of drug concentrations. While several features of antiretroviral therapy (ART) fit the criteria for TDM, a number of questions need to be answered before large- scale introduction of TDM can be justified. For the majority of antiretroviral drugs, the therapeutic window is narrow, with toxicities ranging from nausea and vomiting to pancreatitis, nephrolithiasis, and neurologic effects (Soldin et al., 2003). Some of these toxicities might be prevented with the appropriate use of TDM (Van Heeswijk et al., 1998). Concentrations of ARV agents vary widely among patients treated with the same dose, often more than 10-fold (Rakhmanina et al., 2004). The cause of such significant interpatient pharmacokinetic variability is multifactorial and included differences in metabolism, bioavailability and complex drug-drug and food drug interactions (Rakhmanina et al., 2004).

2.7. Etiology of sub-therapeutic nevirapine and efavirenz concentration

Constructed on the reference ARVs therapeutic ranges, defined as 1000–4000 ng/ml for EFV and 3400–8000 ng/ml for NVP (Duong et al., 2004), as: sub-therapeutic (below the lower therapeutic range limit), therapeutic (within the therapeutic range), and supra- therapeutic (above the higher therapeutic ranged limit) (Duong et al., 2004). The causes of variability in NVP and EFV plasma concentrations can be divided broadly into two factors including the human host related factors which could either be pharmacoecological or pharmacogenetical in nature (Parra et al., 2011; Gopalan et al., 2017) and indirectly due to virus related factors mainly due to the occurrence of drug resistant mutations (Parra et al., 2011).

2.7.1. HIV viral factors - HIV drug resistance

Due to the various side effects that can appear during antiretroviral therapy, treated patients may stop or not take their treatment regularly to reduce the side effects. Poor adherence generally results in suboptimal drug doses and causes therapy failure which results in an increase in viral load (virologic failure) and consequently a decrease in CD4 + T cells (immunologic failure). An inadequate plasma concentration of one or more antiretroviral drugs is usually accompanied by the development of HIV drug resistance mutations in the viral genome. The majority of resistance mutations are substitutions, but duplications, insertions and recombination have also been described. The rapid adaptation of HIV to therapeutic pressure results from several intrinsic properties of retroviruses such as their high mutation rate and high replication rate. Indeed, reverse transcriptase introduces an error every 1000 to 10,000 nucleotides per replication cycle, which means that the viral genome with its size of around 10kb can contain up to 10 mutations. The number of resistance mutations can thus be very high because approximately 1010 viral particles are produced per day in an untreated individual (Perelson et al., 1996). It is therefore likely that viruses already exist at the start of antiviral therapy in the viral quasi-species that are less susceptible or even resistant to one or two antiretrovirals (Coffin, 1995). More than 70% of patients treated contain viruses resistant to at least one antiretroviral drug (Scott et al., 2004). Transmission of viruses carrying one or more mutations seems inevitable and has become a significant public health problem (Turner & Wainberg, 2006).

2.7.1.1. HIV Drug Resistance to non-nucleoside reverse transcriptase inhibitors

Decreased adherence to non-nucleoside reverse transcriptase inhibitors (NNRTIs) to reduce, for example, neuropsychiatric side effects can lead to resistant viral strains. Although these groups of drugs are very effective in inhibiting HIV replication, they have a low genetic barrier, which means that only one mutation in reverse transcriptase is sufficient to prevent the activity of the drug (Gardner et al., 2010). Amino acid substitutions of the reverse transcriptase peptide chain (L100, K101, K103, E148, V179, Y181, Y188) which render resistance have been described (Figure 2.2) (Johnson et al., 2011). The most frequent mutations are K103N and Y181C which induce resistance against EFV and NVP (De Clercq, 2004). These mutations are

all found in the binding region of reverse transcriptase inhibitors. Substitution of lysine for asparagine at position 103, for example, creates a network of H-bridges with neighboring amino acids, which seems to prevent the inhibitor from interacting with the binding zone (Hsiou et al., 2001). Most resistance mutations furthermore cause cross-resistance so that NVP treatment cannot be replaced after failure by EFV (Antinori et al., 2002). Second generation NNRTIs (such as etravirine) have a better genetic barrier (two mutations are needed to inhibit the action of the drug) and are less affected by cross-resistance between NNRTIs (Croxtall, 2012). Resistant viral strains do not only appear due to poor drug adherence but can also develop in certain areas of the body where the concentration of antiretroviral drugs is lower compared to the plasma concentration. The central nervous system is such an example. EFV is one of the only ARVs that can traverse the blood-brain barrier and reduce HIV replication in the central nervous system (Von Giesen et al., 2002). In some treated individuals who have undetectable plasma viremia, levels of the virus could still be determined in the cervix, sperm and respiratory system indicating that the antiretroviral drugs were failing to inhibit the proliferation of the virus in these sanctuary sites (Politch et al., 2012). The ineffectiveness of some antiretroviral drugs in these compartments may be related to pharmacokinetic interactions which influence the absorption, transport, distribution, metabolism or excretion of the drugs (De Maat et al., 2003).

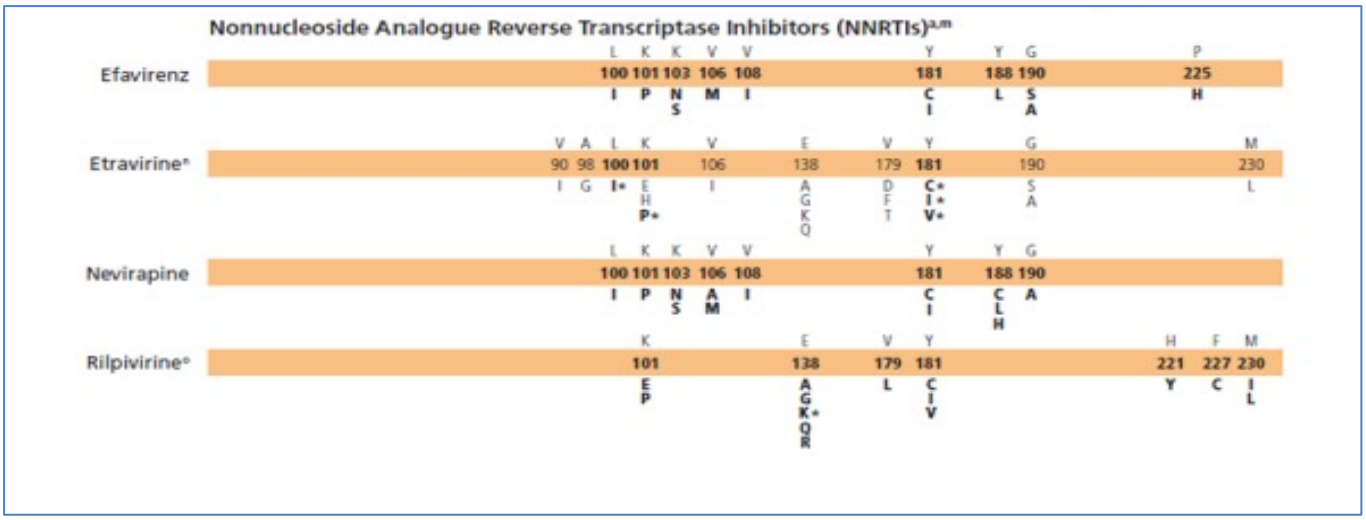


Figure 2.2. HIV-1 reverse transcriptase mutations conferring resistant to Non-nucleoside Reversed Transcriptase inhibitors (adopted from Johnson et al., 2011).

2.7.2. Host pharmacoecological factors associated with drug therapeutic response

Patients factors are equally important determinants of ARV drug plasma concentrations and are also key in the individualization of ART (Parra et al., 2011). The pharmacoecologic factors include factors relating to adherence, lifestyle of the patient, co-medications and drug interactions, co-morbidities, pre-existing organ dysfunction including liver or kidney disease, or pregnancy (Pavlos and Phillips, 2012). Patients demographic factors such as age, gender (Swaminathan et al., 2011; Wyen et al., 2008), nutritional status such as body weight, growth rate (Schipani et al., 2011) have been shown to influence the treatment outcomes credited to the difference in physique mass and drug clearance between genders (female and male) (Gopalan et al., 2017). Studies have shown NVP and EFV metabolism in young children is more faster compared to adults meaning that children would require substantially higher NVP doses to attain therapeutic concentrations (Gopalan et al., 2017; Swaminathan et al., 2011). Furthermore, females generally have elevated median NVP and EFV plasma concentrations than males. This variance in ARV drug plasma levels by gender has been accredited to the differences in body structure and size across gender (Gopalan et al., 2017; Swaminathan et al., 2011).

2.7.2.1. Adherence and NNRTI treatment outcomes

Adherence rate to ARV uptake is a key cause of virologic failure which could guide and predict the development of HIV drug resistant mutation among people infected with HIV. However, the connections between occurrence of resistance, virologic response and ART adherence vary among the type of ARV drug in use (Bangsberg et al., 2007). A study surveying the rates of virologic rebound, occurrence of HIV drug resistance mutations and rate of ART adherence in patients with undetectable viral load at baseline showed the rate of rebound was >10% among patients receiving PIs (14.7%) or boosted PIs (11.7%) at an adherence rate of about 76% to 95%, and about 30% to 50% at the lowest adherence rates (<55%). For NNRTIs, adherence of < 55% was required to attain a comparable virologic rebound rate (17.6%) (Maggiolo et al., 2007). This study further showed that, occurrence of HIV drug resistant mutation decreased as the level of adherence to NNRTIs increased, while the relationship inverse for patients on unboosted PIs. Studies show patients initiating HAART with adherence rate lower than 95% to NNRTIs are significantly associated with accumulated HIV drug resistance mutations

compared to patients with more than 95% adherence rates (Tam et al., 2008). The number of pills taken per day has also been shown to affect the level of adherence. Currently, most HAART are dispensed once-daily regimens (Stone et al., 2004).

2.7.2.2. The effect of Hepatitis B and Hepatitis C co-infection on therapeutic outcomes

HIV patients co-infected with either hepatitis B or C viruses (HBV or HCV) are faced with worse prognosis compared to patients with HIV only and treatment guidelines commend early treatment of both infections (Gazzard et al., 2008). All the ARVs potentially can cause hepatotoxicity, which is worsened in patients with HBV/HCV co-infection. Elevated liver injuries marked by increased liver enzymes, are common among patients co-infected with HBV/HCV and are on EFV-based treatment (Brück et al., 2008). Studies have shown HBV/HCV co-infection leads to elevated exposure to EFV hence liver related side-effects. It should be noted that not all HIV patients with and without HBV/HCV co-infection results to significant differences in EFV plasma levels (Pereira et al., 2008). The foundation for the higher danger for liver toxicity is still unclear. In generally, studies report that NNRTIs plays a vital role in the management of HBV/HCV co-infected patients, but the status of liver enzymes must be consistently monitoring.

2.7.3. Host pharmacogenetic factors influencing HAART therapeutic response

The important interpersonal variation in drug exposure to the NNRTIs and protease inhibitors, are mainly caused by genetic polymorphisms in drug transporters, nuclear receptors, and drug-metabolizing enzymes (Parra et al., 2011). Although research is increasing on the pharmacokinetic/pharmacogenetic of these drugs, studies directly associating treatment efficacy and response of these drugs and genetic polymorphism in absorption, distribution, metabolism, and excretion and drug transporter genes are significantly varied (Phillips & Mallal, 2009). Data linking the use of pharmacogenetics to guide antiretroviral dose/ dosing / dosage are skewed in developing countries. Although opinion varies, others advocates the usage of therapeutic drug monitoring in combination with pharmacogenetics which is currently not available in many clinical practice aimed at improving treatment efficacy (Figueroa et al., 2010).

2.7.3.1. Cytochromes P450

Discovered in 1955 in the hepatic cells of rats, cytochromes P450 are present in all eukaryotic organisms (animals, plants and fungi) and in certain prokaryotes (Meunier et al., 2004). There are about sixty human cytochrome P450 (CYP) genes and sixty pseudogenes, which are inactivated cytochrome genes, have been described in humans and classified according to the similarity of their amino acid sequence into 18 families (indicated by a number: 1-5, 7, 8, 11, 17, 19-21, 24, 26, 27, 39, 46 and 51) and 43 sub-families (indicated by a letter: AG, J, RU, VX, Z) (http: // drnelson.uthsc.edu/human.P450.table.html). The majority of isozymes belong to the CYP1 to CYP4 families. About 15 isozymes of the CYP1, CYP2, CYP3 and CYP4 families are involved in the phase I biotransformation of exogenous substances such as drugs and other xenobiotics. Some of these isozymes also play a role in the metabolism of endogenous substances such as steroids (estradiol, testosterone, steroids, prostaglandins, fatty acids, eicosanoids and vitamin D3) mainly by CYP4 and CYP51 (Lewis, 2004). The CYP isozymes are expressed in several tissues, but the majority are localized in the liver. They are the main players in the hepatic elimination of protease inhibitors and NNRTIs. Their role in drug metabolism is to inactivate substances which are generally lipophilic in nature by transforming them into hydrophilic metabolites, which facilitates their elimination by the kidneys (Weinshilboum, 2003).

In addition to the induction of CYP by various exogenous substances, their enzymatic activity also depends on the genetic modifications of the genes encoding the isozymes. Indeed, CYP are highly polymorphic. More than 660 alleles of 29 cytochromes P450 have been described by the "Human CYP allele nomenclature committee" of the Karolinska Institute (www.cypalleles.ki.se). The mutations which modify the function of the cytochromes can be either deletions or duplications of genes. Deleterious mutations such as, insertions or deletions cause a change of the reading frame and consequently production of inactive proteins. Moreover, point mutations can also modify the specific activity of the enzyme by substitution of a nucleotide base in an exonic region which can cause the change of an amino acid in the peptide sequence. Polymorphisms of coding regions are not, however, the only ones to have an effect on the activity of cytochromes. Mutations in regulatory regions flanking genes and those

in introns can, for example, increase transcription and alter or create splicing sites resulting in truncated proteins (Ingelman-Sundberg et al., 2007). Point substitutions (either substitutions of one purine by another purine (A G) or of a pyrimidine by another pyrimidine (C T) are the most common genetic variations (Ingelman-Sundberg et al., 2007).

2.7.3.2. Metabolism of EFV and NVP

The EFV and NVP NNRTIs are two substrates of CYP2B6. Two pathways of EFV metabolism have been described in vivo and in vitro using microsomal preparations of human liver. The first is the main pathway for the oxidation of EFV and converts between 77% and 92% of it into the 8-hydroxyefavirenz metabolite (Figure 2.3). This step of EFV metabolism is mainly carried out by the action of CYP2B6 with a low participation of CYP1A2, CYP2A6 and CYP3A4 / 5. The 8-hydroxyefavirenz metabolite is subjected to a second oxidation step which adds a hydroxyl group at position 14 resulting in the 8,14-dihydroxyefavirenz metabolite (Ward et al., 2003). However, the latter can also be formed during the hydroxylation at position 14 of the 8-hydroxyefavirenz glucuronide metabolite which results from the rapid glucuronidation of 8-hydroxyefavirenz in vivo (Ogburn et al., 2010). The second pathway of metabolism which converts less than 8 to 23% of EFV to 7-hydroxyefavirenz is mainly due to the action of CYP2A6 and, to a lesser extent, the enzymatic activity of CYP2B6. Direct conjugation of EFV with glucuronic acid initiated by UDP-glucuronosyltransferase specifically UGT2B7, and results in the metabolite N-glucuronide-efavirenz which is the most common metabolite in urine after the first dose of EFV. After multiple doses of EFV, 8-hydroxyefavirenz glucuronide becomes the most predominant metabolite in urine. The 7-hydroxyefavirenz and 8,14-dihydroxyefavirenz metabolites are themselves glucuronidated, inter alia, by UGT2B7 and eliminated in the urine (Bae et al., 2011; Mutlib et al., 1999).

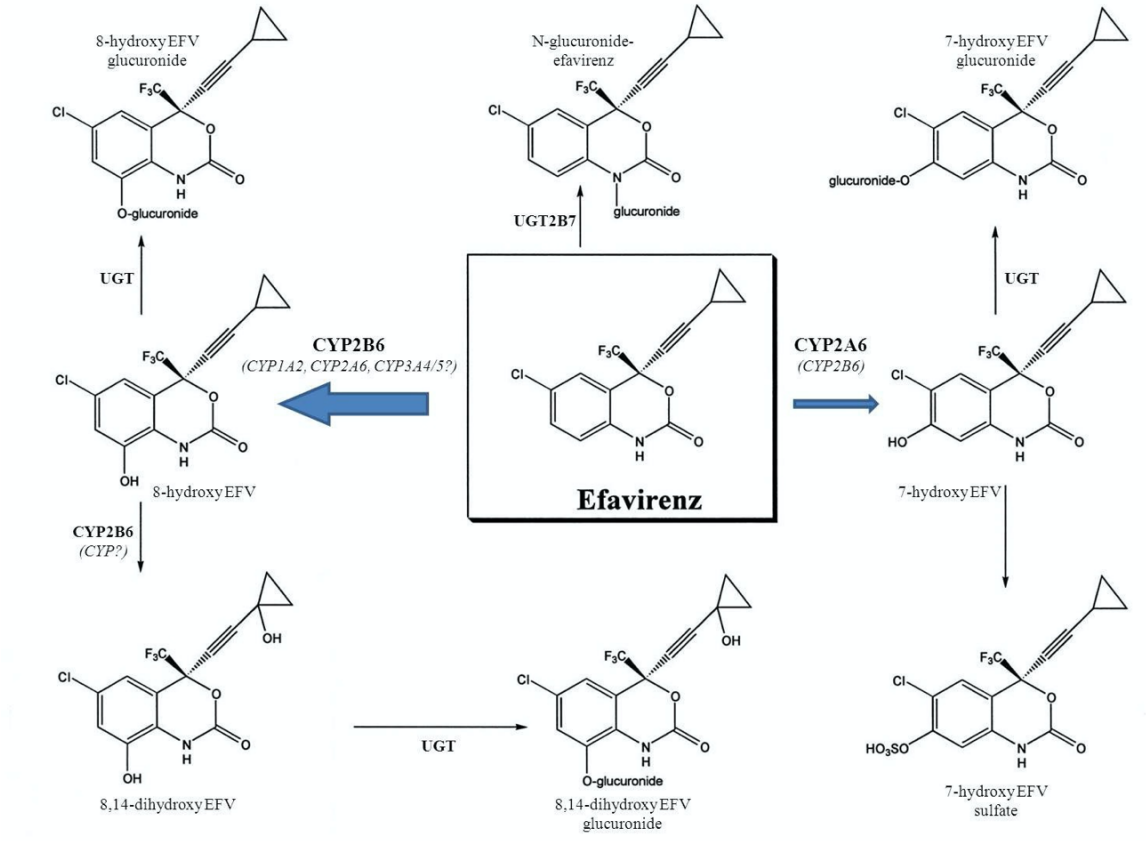


Figure 2.3. Proposed metabolic pathways for the efavirenz (modified from Mutlib et al., 1999).

Biotransformation of NVP by hydroxylation creates the metabolites 2-, 3-, 8- and 12-hydroxynevirapine which are subsequently glucuronidated and largely eliminated by the kidneys (Figure 2.4). Cytochromes CYP3A4 / 5 and CYP2B6 produce the 2- and 3-hydroxynevirapine metabolites, respectively. 8-Hydroxynevirapine results from the enzymatic action of the cytochromes CYP2B6, CYP2D6 and CYP3A4. 12-Hydroxynevirapine is formed by CYP3A4 / 5, CYP2D6 and CYP2C9. However, the CYP2D6 and CYP2C9 cytochromes play only a minor role in the catabolism of NVP (Kwara et al., 2010; Riska et al., 1999).

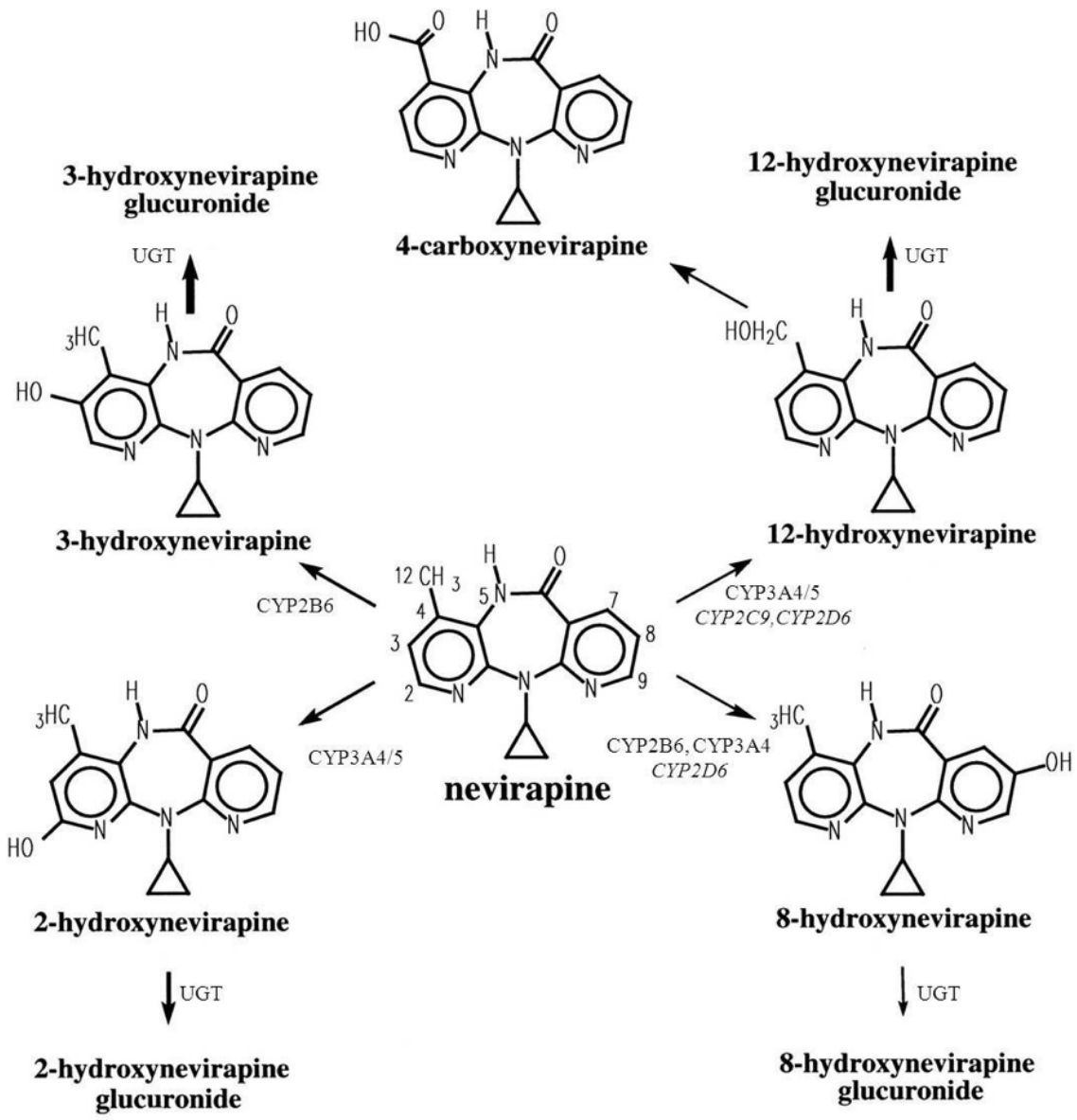


Figure 2.4. Biotransformation of nevirapine (adopted from Riska et al., 1999).

2.7.3.3. The CYP2B6 enzyme

Human CYP2B cytochrome P450 consists of a functional CYP2B6 gene and a CYP2B7P pseudogene that results from duplication of CYP2B6 genes. CYP2B is localized on the long strand of chromosome 19 (19q13.2) and is part of the CYP2ABFGST group of 350kb which contains the genes and pseudogenes of the cytochromes CYP2A, 2B, 2F, 2G, 2S and 2T (Hoffman et al., 2001). Hepatic expression of CYP2B6 is highly variable and protein levels

can differ more than 100 times between individuals. This variability is not only due to drugs and chemicals and environmental substances that have an activating effect on CYP2B6 but also depends on genetic variations. After the discovery in 2001 of the first SNPs which were associated with a decrease in the expression or the enzymatic activity (Lang et al., 2001), a hundred polymorphisms have been described so far and assigned to 37 different alleles (www.cypalleles.ki.se/cyp2b6.htm). Sequence variations can be missense, nonsense or silent SNPs and can modify the promoter or intronic regions. Most of these polymorphisms are in linkage disequilibrium forming haplotypes which have important effects on the function of the isozyme CYP2B6. Some SNPs, on the other hand, are more common than others with frequencies of 5% to more than 50% depending on the different ethnic groups (Zanger et al., 2007).

2.7.3.3.1. Influence of CYP2B6 genetic variation on the metabolism of nevirapine and efavirenz

While genetic polymorphism of cytochrome CYP2B6 has not been extensively studied compared to other cytochrome P450 isozymes, a large genetic variability has been demonstrated which influences the metabolism of xenobiotics including efavirenz and nevirapine (Table 2.2). The CYP2B6 metabolic pathway is also able to adapt to changes in enzyme activity caused by different SNPs (Haas et al., 2009). The 516G> T polymorphism of CYP2B6 exon 4 is one of the most important SNPs in the biotransformation of efavirenz and nevirapine. The 516G> T SNP is associated with greater interpersonal variability of plasma and intracellular concentrations of EFV and NVP (Manosuthi et al., 2013). The CYP2B6 516G> T polymorphism induces aberrant splicing that cleaves the region from exon 4 to exon 6 of CYP2B6 messenger RNAs which cumulatively reduces the amount of protein and the enzymatic activity (Hofmann *et al*., 2008).

The CYP2B6 c.21563C> T SNP of intron 8 has been associated with elevated plasma NVP/EFV levels (Carr et al., 2010; Manosuthi et al., 2013). On the contrary, CYP2B6 SNP c.18492T> C of intron 5 is associated with decreased NVP/EFV plasma concentrations. In addition, this polymorphism exerts an opposite effect on the enzymatic activity compared to other SNPs; whereas the homozygous mutated SNP c.21563TT induces an increase in the

plasma level of EFV or NVP; the highest concentrations of the two NNRTIs occurred in individuals with CYP2B6 c.18492TT SNPs (Carr et al., 2010; Sukasem et al., 2012). Both polymorphisms are located in activating regions of introns, but they are unlikely to have a direct effect on gene function. Their functional contribution is indirect through linkage disequilibrium with other functional SNPs of the CYP2B6 gene (Carr et al., 2010; Sukasem et al., 2012). Patients with heterozygous and homozygous mutant of CYP2B6 18492T>C hence lower plasma NVP/EFV concentrations (Manosuthi et al., 2014; Soeria-Atmadja et al., 2017). Studies have shown the presence of this CYP2B6 18492T>C SNP combined with co-administration of a strong CYP inducer could rise the probability of subtherapeutic plasma efavirenz concentrations (Manosuthi et al., 2014).

Table 2.2. CYP2B6 genetic variations associated with intracellular concentrations of efavirenz and nevirapine (adapted from Zanger et al., 2007)

CYP2B6 Allele	SNP	Effect on the NVP and EFV plasma levels
**6*, **26, *29*	c.516 G>T	Interindividual variability of plasma and intracellular concentrations (Vandercam et al. 2010) Association with elevated plasma EFV concentrations and increased risk of developing neurotoxic side effects (Rakhmanina and van den Anker 2010)
*27, *29	c.593 T>C	High plasma EFV level (Rotger et al. 2007)
*4	c.785 A.G	Increased hydroxylase activity and 8-hydroxyefavirenz level (Ariyoshi et al. 2011).
*16, *18	c.983 T>C	High plasma concentrations (Wyen et al. 2008)
*28	c.18492 T>C	Decreased plasma concentrations (Sukasem et al. 2012).
	c.21563 C>T	Increased plasma level (Cressey et al. 2012)
CAR	540C>T	Significant association of the CAR-CC genotype with discontinuation of EFV treatment (Wyen et al. 2011).

The CYP2B6 * 16 and the CYP2B6 * 18 allele consist of the 785A> G and 983T> C polymorphisms, respectively. Both of these two SNPs appears to be more common in African populations because it could not be demonstrated in Asians or Caucasians (Oluka et al., 2015; Wang et al., 2006). These two SNPs are associated with elevated NNRTI levels with the rate being higher for the EFV based ARV regimen (Manosuthi *et al*., 2013).

The C> T polymorphism at position 1459 of exon 9 of the CYP2B6 gene also exhibits significant interethnic variability. While this SNP has not been detected in Korean and Taiwanese populations and is only weakly represented in Japanese (2.9%) and Ghanaians

(2.9%), the frequency among the Caucasians and African Americans reaches 10 % (Klein et al., 2005). The * 5 (1459C> T) and * 7 (516G> T, 785A> G, 1459C> T) alleles have been assigned to the CYP2B6 R487C variant. The SNP at position 1459 is however tri-alleli. The frequency of this polymorphism is 0.7% (Jamshidi *et al*. 2010). SNP 1459C> T reduces expression of the isozyme CYP2B6. Although the CYP2B6 1459C> T variant is only weakly expressed, no significant effect could be demonstrated on the metabolism or clearance of EFV and NVP (Wyen et al., 2008).

Five SNPs c.329G> T, c.341T> C, c.444G>T, c.548T> G and c.637T> C first identified among the Rwandese population by Radloff *et al*., (2013) were shown to code for CYP2B6 proteins with reduced function or completely inactive proteins. These findings are consistent with those of Marth *et al*., (2011). The role of these SNPs on NVP and EFV concentrations both in-vivo and in vitro is an opening for future investigations.

The nuclear receptors constitutive androstane receptor (CAR), the preganane X receptor (PXR) and the Ah receptor ("aryl hydrocarbon receptor", AhR), are responsible for the activation of isozymes by increasing the rate of transcription of cytochrome P450 genes. AhR regulates cytochromes of the CYP1 family. PXR induces the cytochromes CYP2A, CYP2B, CYP2C and CYP3A. CAR influences mainly CYP2B cytochromes but can also induce CYP2C cytochromes and CYP3A. If the xenobiotic concentrations are high, the CAR, PXR and AhR receptors also promote the transcription of enzymes of the metabolic pathway II such as that of UDP-glucuronosyl-transferases (UGT) to increase the expression of enzymes involved in the catabolism of foreign substances (Wortham et al., 2007; Wyen et al., 2011). Specifically, CAR appears to play a role in basal and inducible regulation of the enzymes involved in efavirenz metabolism. Genetic variability in CYP2B6 and CAR contributes to early treatment discontinuation for efavirenz-based antiretroviral regimens due to high EFV plasma concentration (Wyen et al., 2011). In South Africa, CAR 540C>T (rs3003596T> C) polymorphism has been found in intron 3 of the CAR gene (Swart et al., 2013). The study showed homozygous CC and heterozygous TC forms were significantly associated with a reduction in plasma EFV concentration (Swart et al., 2013).

2.7.4. Ethnic background and CYP2B6 polymorphisms

Ethnic background has been linked to the variation in the frequency of variant alleles between different populations. These ethnic driven variant alleles has been associated with variation in plasma levels (Ngaimisi et al., 2013). In Kenya, three broad linguistic groups: Bantu, Nilotic and Cushites have been recognized. Although these ethnic groups reside in close proximity to each other, they have widespread environmental and cultural diversity between them (KNBS, 2019). Subsequently, huge host genetic and environmental diversity are evident which could impact the treatment outcome, drug efficacy and drug adverse event between different these ethnic groups managed using identical ART regimen (Ngaimisi et al., 2013).

The human CYP2B6 contributes a vital function in the metabolism of diverse ART drugs used for HIV life-long therapy. CYP2B6 is a largely polymorphic enzyme influencing individual therapeutic response (Hedrich *et al*., 2016). Importantly, African ethnic groups show a wide polymorphism in the *CYP2B6* gene (Čolić *et al*., 2015). The constitutive androstane receptor (CAR) contributes both in basal and inducible regulation of the enzymes responsible for the metabolism of efavirenz. Data currently associated genetic polymorphisms in CYP2B6 and CAR with early treatment discontinuation among patients receiving EFV -based ART regimens due to high EFV plasma concentrations (Wyen et al., 2011). Data are skewed correlating the relationship between ethnic grouping and NVP/EFV in Kenya evaluating the impact of these polymorphisms on efavirenz and nevirapine plasma concentrations.

2.8. Summary of determinants of ARV plasma concentration

Several factors work in synergy to causes of variability in NVP and EFV plasma concentrations can be divided broadly into three factors including; viral factors mainly the presence of ART resistant mutations (Parra et al., 2011). Antiretroviral therapy drug related factors affecting plasma concentration and toxicity. The host related factors which could either be pharmacoecological or pharmacogenetical in nature summarized in Figure 2.5 (Parra et al., 2011).

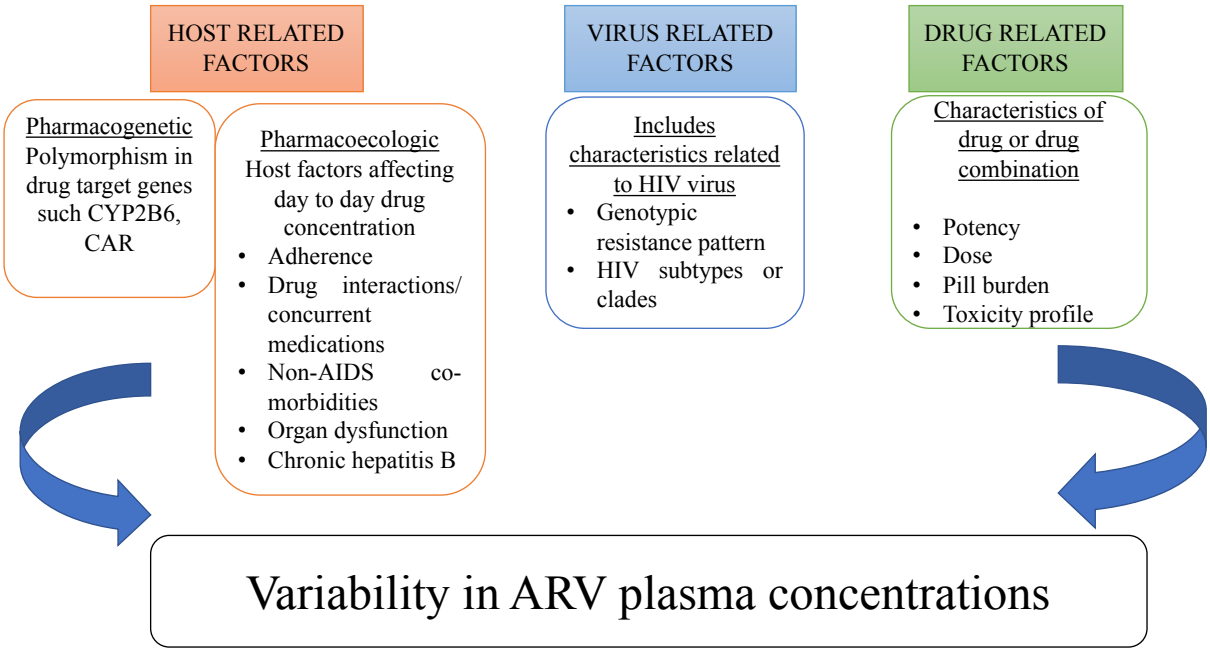


Figure 2.5. Factors affecting therapeutic response to Antiretroviral therapy useful in personalization of antiretroviral prescription (adopted from Phillips & Mallal, 2009)

CHAPTER THREE

METHODOLOGY

3.1. Study Site

This study recruited patients receiving HIV treatment at the Family AIDS Care and Education Services program (FACES), located at Kenya Medical Research Institute (KEMRI), Center for Respiratory Disease Research (CRDR) KEMRI, Nairobi. The collection of data on clinical and biochemical parameters and adherence were done at FACES-KEMRI. FACES program generally offers HIV/AIDS management and care and is located in Kisumu and Nairobi. This program is a collaboration between KEMRI and mainly University of California, San Francisco (UCSF) and the better part of operational grants comes the US President's Emergency Plan for AIDS Relief (PEPFAR). At the time of the current study, FACES was supporting 82 HIV sites, 80 in Nyanza Province and 2 in Nairobi. Some of the program activities included; HIV Testing and Counseling, Prevention of Parent-to-Child Transmission, Voluntary Medical Male Circumcision. TB diagnosis and treatment, reproductive health care, nutritional support and laboratory services. During the current study period, the FACES program in Nairobi had two sites one in CRDR and the other in Mimosa Clinics. Overall, the Nairobi FACES program had enrolled 5,288 HIV patients of whom 4,861 were adults and 427 pediatrics patients. Out of these patients in this program, 2,635 (54.2%) of the patients were receiving ART treatment,

The EFV/NVP the plasma levels, HIV drug resistance mutation detection and the pharmacogenetic analysis were done at the Retrovirology laboratory, Public Research Centre for Health, Luxembourg. Shipment of samples to Luxemburg was approved by KEMRI ERC which the patients consented during recruitment (SSC Protocol No. 2539).

3.2. Study Population

This study enrolled participants who were HIV-1 sero-positive and receiving first-line ART at the FACES Nairobi treatment center. At the time of this study, first line ART comprised of zidovudine (AZT) or abacavir (ABC) or tenofovir (TDF) or stavudine (d4T), lamivudine (3TC), and efavirenz (EFV) or nevirapine (NVP) for at least 12 months (NASCOP, 2018).

3.3. Study Design

This was non-comparative cross-sectional study conducted among HIV-1 infected patients receiving care and treatment at KEMRI Nairobi. This is a type of research design in which research collect data from many different individuals at a single point in time. In cross-sectional research, you observe variables without influencing them. Some of the reasons for choosing this design is that cross-sectional studies are relatively cheap and less time-consuming. Cross-sectional studies allow for data collection from a large pool of subjects and compare differences between groups. Because cross-sectional studies capture a specific moment in time, it provides a snapshot of conditions in that country at that time.

3.4. Inclusion and exclusion criteria

This study enrolled patients if they were consenting HIV/AID infected adult (18 years and above) and had been receiving first-line ART with either nevirapine or efavirenz NNRTI backbone. The patients should have been attending FACES HIV treatment program for in the past 12 months (a duration deemed sufficient to have attained steady state drug plasma levels). Lastly, the study recruited patients who were able to provide 10 ml blood samples 5 to 8 hours post medication as well as participated in a face to face structured interview. On the other hand, the study excluded patients from enrollment if they were minors and unable to give consent for participation. The study also excluded patients who were pregnant, had a history of any mental illness and other disorder that could impair their capability to provide written informed consent. Patients on either efavirenz or nevirapine first line ART regimen for less than less than 8 weeks and those whose past 12 months health and laboratory records were unavailable were also excluded from the study.

3.5. Sample size

This study applied the population proportion estimation with a specified relative precision formula described by Lemeshow et al., (1990) to calculate the study sample size. The formulation is presented in equation one. The alpha (α) was set at 0.05, relative precision (ε) set at 0.20 and percentage of HIV-1 seropositive with sub-optimal NVP/EFV plasma levels during a 12-month ARV treatment at 15% (Gunda et al., 2013; Oluka et al., 2015). This set up yielded a total of 599 HIV patients recruited to attain 0.95 power.

Equation one

The Lemeshow formula for computation of sample size for descriptive studies

Equation 3.1. The formula for estimating population proportion with a specified relative precision

$$n = Z^2_{1-\alpha/2}\,(1-P)/\,\varepsilon^2 P$$

Where;

- $Z1-\alpha/2$: represents 95% confidence intervals = 1.96
- ε – is the test precision set at = 0.2
- α - Significance level set at = 0.05
- P – The prevalence of HIV-1 infected patients with sub-optimal NVP/EFV plasma levels 12-month post ARV initiation at 15% (Gunda et al., 2013; Oluka et al., 2015)

Substituting the variables in the equation one formula gave n =544. Therefore, minimum sample size screened in order to obtain the desired sample types was n = 544. The calculated sample was increased by 10% or 54 patients was added to cover for non-response rate or lost to follow-up or missing information giving an overall total of 599.

To calculate the number of patients on NVP and EFV recruited was done based on proportionate to size equation two.

Equation two

Formula for computation of patients on NVP and EFV

Equation 3.2. The formula for probability proportional to size

$$n = n/N\ (b)$$

Where;

- n – Actual number of patients per treatment arm (1183 on NVP and 1452 on EFV)
- N – Total number of patients for all treatment arms (1452+1183 = 2635)
- b – Calculated sample size = 599

The calculated sample size for patients on NVP was 269 and those on EFV was 330

3.6. Sampling Procedures

The head of FACES program was briefed about the study and gave permission to conduct this study within the program. The program head thereafter provided the names of HIV patients eligible to participate in this study. The patients' calendar was generated and used to schedule the consenting and recruitment of patients into the study. The patients meeting the eligibility criteria and consenting (Appendix 1) were consecutively sampled until the target sample size achieved in each treatment arm. Each patient provided 10 ml of blood sample 5 hours post medication. These patients also underwent a face to face structured interview to gather study related information.

3.7. Data collection

All the enrolled patients were clinically examined to gather relevant clinical data followed by blood sample collection. The patients then underwent structured interviews to gather study

related information. Secondly the study collected retrospective data form patient records. This was mainly to gather data on adverse drug reactions and treatment failures if any.

3.7.1. Patients' interviews

A detailed structured face to face interview was conducted among study patients collect attributes related to patient's socio-demographic variables, healthcare utilization, ART regimens, duration on ART, ART change or switch, dietetic profiles, concurrent prescriptions, ART adherence, social psychological status, co-morbidities, clinical outcomes, sexual behavior, disclosure and substance use (Appendix 3)

3.7.2. Review of patients' previous records

The second part of data collection involved retrospective review of patient medical registers. The patients' medical records were retrieved and data abstracted to cover additional demographic data, liver enzymes tests, baseline CD4 count, and medical history, history of skin reaction, hepatitis B infection, any adverse drug reaction, CD4 decrease, ART change and viral load rebound, co-morbidity, comedications, change of ART regimens, adherence to clinic visits (Appendix 2).

3.7.2.1 ART drug adherence assessment

The ART adherence screening in this study was conducted by reviewing the pharmacy refill data as well as medical records as described by Ochieng et al. (2015) (Appendix 3). Briefly the adherence was measured based on dose compliance during the 30 days preceding the latest refill. The quantity of dose pills at refill was counted and reconciled against the dose counts dispensed at last refill. Furthermore, pill count data were obtained from patient cards for the four months preceding the study period. Nonadherence was determined as the percentage of overdue dose at refill, averaged over a four-month period and used to assign adherence as good (<= 5% dose skipped), fair (6-15% dose skipped) or poor (>15% dose skipped).

3.7.3. Laboratory Investigations

3.7.3.1. Sample collection

From each of the 599 subjects enrolled about 10 mL a single draw of whole blood sample was collected into EDTA tubes (Becton, Dickinson and Company, USA) by veno-puncture at 5 – 8 hours after the last dose. About 2 mL of the blood was used for CD4 determination and CYP2B6 polymorphism studies while the remaining 8 mL whole blood was centrifuged using Neofuge 18R (Heal Force Bio-meditech Holdings Limited, China) at 10,000g for 10 minutes to collect plasma which was used for virological, immunological, biochemical and drug resistance studies. If not used immediately the plasma samples at -80 °C pending analysis. Sample (whole blood and plasma) aliquot were sent to Laboratory of Retrovirology, Public Research Centre for Health, Luxembourg for validation, viral load testing, HIV drug resistance testing, determination of plasma drug concentration and for genotyping the following CYP2B6 (329G>T, 341T>C, 444 G>T/C, 15582C>T, 516G>T, 548T>G, 637T>C, 785A>G, 983C>TA, 18492C>T, 835G>C, 1459C>T and 21563C>T) and CAR (540C>T) single nucleotide polymorphisms (SNP)

3.7.3.2. Immunological and Biochemical assessment

The CD4 counts was measured as absolute numbers and percentages using a FACSCount TM flow cytometer (Becton Dickinson, BD Biosciences, San Jose, USA). Full hemogram was measured by the SYSMEX KX21N haematology mechanism (Sysmex Corporation, Kobe, Japan). Blood clinical chemistry (AST, ALT, Creatinine) were measured using the Lisa 300 Plus analyser (Hycel Diagnostics, Massy, France).

3.7.3.3. Determination of plasma HIV-1 RNA concentration

To measure the HIV-1 RNA concentration (viral load) involved, first the extraction of viral RNA from 1ml plasma samples of study patients using QIAmp viral RNA mini kit (Cat. No. 52906, Qiagen Inc., USA) in line with the manufacturer guidelines. A total volume of 10µL of HIV RNA was quantified using the Generic HIV Viral Load assay (Biocentric, Bandol-France). The amplification of HIV RNA was achieved using the ABI Prism 7300 Sequence Detection System (Applied Biosystems). The amplification condition was set at 50°C for 10

minutes and 95°C for 5 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. The test has detection limit of 40 HIV-1 RNA copies/ml.

3.7.3.4. Determination of efavirenz and nevirapine plasma concentration

The EFV and NVP plasma concentration was determined at Laboratory of Toxicology, Public Research Centre for Health, Luxembourg using methods modified from by Reddy *et al.,* (2016). The plasma levels were quantified using ultra-high performance Xevo TQ-S tandem quadrupole mass spectrometer (Waters Corporation, U.S.A).

3.7.3.4.1 Preparation of solutions

The stock solution of nevirapine and efavirenz (200 µg/ml) was purchased from Vivan Life (Sciences, Mumbai, India). The stock solutions were prepared in methanol. These stock solutions (200 µg/ml) were then diluted with 50 % methanol in water to achieve concentrations ranging from 752.27 and 121,333.33 ng/ml for nevirapine and 523.56 and 62000.00 ng/ml for efavirenz.

3.7.3.4.2 The method calibration curves

Weighted linear regression was used to analyze the calibration curves of the area ratio of EFV and NVP to their corresponding internal standard against nominal drug levels. These calibration standards were analyzed in independently in five batches. To acceptable linearity limits non-zero calibration standards required that the mean deviations of concentrations to be within ±15% while the lower limit of quantification be within ±20% from nominal concentrations. The linear regression equation ($1/X^2$) was used to determine the concentrations of the peak area ratio versus the concentration of EFV and NVP.

3.7.3.4.3 Inactivation of HIV samples for drug quantification

An elaborate in-house method was used to first inactive the HIV virus in a biosafety level three laboratory. Briefly, 50µl patient's plasma samples and 5µl internal standard (2µg/ml nevirapine, purity: 100 %, from Vivan Life Sciences, Mumbai, India in methanol) were added into a 1.5ml Eppendorf tube. The mixture then heated at 65°C for 10 minutes and cooled at

room temperature for 10 minutes. In each of the samples, 100µl of cold methanol (-20^{O}C) was dispensed and let to stand for 10 minutes at -20°C. The supernatant was collected by centrifuging the sample mixture at 20,000g and set 20°C for 8 minutes. In a 1.5ml clean tube containing the supernatant, 850µl of ammonium acetate buffer (pH = 3.00) with a brisk centrifugation. At this state, the HIV virus was inactivated and the ARV quantification was done in biosafety level two or lower laboratory.

3.7.3.4.4. Extraction and quantification of efavirenz and nevirapine

The bond Elut C18 cartridges was used for solid phase extraction using the Visiprep Vacuum Manifold with a standard lid (Merck, Germany). First, to condition the Bond Elute C18 150×4.6mm, 5µm column, 1ml methanol was passed and washed by 1ml ultrapure water. Secondly, 150µl of samples prepared above were passed/charged through the columns and washed twice using 1ml ultrapure water. The first wash was collected into clean sterile tubes. The second wash discarded. The columns were vacuum dried (5–10 kPa). The NVP elution was done twice each using 500µl methanol at a flow rate of 1ml/min and dried using vacuum between the two elution. Using Thermo ScientificTM Reacti-VapTM Evaporators (Thermo Fisher Scientific Inc, USA) the eluents were totally evaporated for 30 minutes at 37°C.

Into 50ml capped vials 100µl 1:1 acetonitrile and water (equal ration 1:1) was used to reconstitute the eluents by short vortexing. The vials were used for the NVP and EFV quantification using the Xevo TQ-S platform. The platform automatically injects 1µl of the samples and the NVP and EFV quantities obtained in five minutes.

3.7.3.4.5 Preparation of standard and quality control solution

The NVP and EFV standard stock solutions were each diluted in 50% methanol giving working solution comprising all drugs at 400, 800, 1600, 3200, 6400, 12,800, 25,600, 51,200 and 64,000µg/L for EFV and NVP. The 100 ng/mL working solution for internal standard (nevirapine D4 for NVP and $^{13}C_6$-efavirennz for EFV) was achieved by diluting internal standard in 50%. Six plasma calibrators were prepared by mixing 20µL of working standard solution and 20µL internal standard solution by dilution in 200µL plasma from ART naïve

patients. Quality controls samples were made in blank human plasma at 80, 640 and 5120μg/L for EFV and NVP

3.7.3.4.6 The method specificity

Examination of the methodical interference from endogenous elements were achieved by analyzing six plasma samples from ARV naïve individuals'

3.7.3.4.6.1 Matrix effects

The role of matrix on the analytical ion suppression or enhancement was tested using the method described by Reddy et al., (2016). Batches of five different blank plasma were extracted using methods described in section 3.7.3.4.4. The high-quality control (HQC), middle quality control (MQC) and low-quality control (LQC) of the analytes was used to induce or spike the plasma extracts. Comparison was done between the area of the spiked plasma extract (A) the aqueous standards in 50% methanol (B) at corresponding level. The matrix effect (ME) is the ratio of (A/B×100). The 100% ratio matrix effect shows that the analytes in mobile phase and in the plasma are similar. The ME greater than 100% represents enhanced ionization while ME less than 100% shows suppressed ionization. In this study the ratio of limit of detection to signal/noise (S/N) was programmed at greater than 3.0 in chromatogram while the limit of quantization at more than 10 of signal/noise.

3.7.3.4.6.2 Method precision, accuracy and recovery

The intra- day method precision was determine using three QCs (HQC, MQC and LQC). The study prepared and analyzed five independent QC samples in the same standard curve. Fifteen independent samples were analyzed in triplicate days in order to determine the inter-day calibration curve. The method accuracy was determined using the following formula; ((the observed mean concentration) divided by (spiked mean concentration)) multiplied by 100%. The assess the absolute recovery rate was achieved was by comparing the area of the peak of plasma extract against the standards injected directly.

3.7.3.5. CYP2B6 and CAR genotyping

3.7.3.5.1. DNA Preparation

The human DNA was extracted from EDTA collected blood samples using automated NucliSENS® easyMAG® system (BioMérieux - Boston, USA) using to the guidelines of the manufacturer. Briefly the 2000µl lysis buffer was automatically dispensed into the sample extracting vessels. About 200µl whole blood was gently mixed and loaded into the lysis buffer. A 740µl silica beads dissolved in lysis buffer was then added to each vessel containing the samples and pipetted up and down before launching the automated DNA extraction which lasted 61 min for a run of 24 samples. The ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) instrument was used to quantify genomic DNA extracted.

3.7.3.5.2. Real Time PCR genotyping

Determination of SNPs was done using ABI 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA) platform. Genetic variation for the following SNPs *CYP2B6* 516G>T (rs3745274), CYP2B6 983T>C (rs28399499), CYP2B6 15582C>T (rs2279345), CYP2B6 18492 C>T (rs2279345), CYP2B6 21563 C>T (rs8192719) and CAR 540C>T (rs2307424) was achieved using Applied Biosystem pre-validated Taqman assays. The assay IDs were C___7817765_60, C_60732328_20, C__26823975_10, C__26823975_10, C__22275631_10 and C__25746794_20 respectively. Using the manufacturer's guidelines, in a final volume of 20ul for each reaction, the following reagents were added; 2X TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 20X Drug Metabolism Genotyping Assay and 10ng DNA. The amplification was achieved by an initial stage of 95°C for 10 minutes and 50 cycles at 92°C for 15 seconds and 90°C for 60 seconds.

The primers for CYP2B6 329 G>T (rs186335453), CYP2B6 341T>C (rs139801276), CYP2B6 444 G>T (rs1053569), CYP2B6 548 T>C (ss539003292), CYP2B6 637 T>C (ss539003292), CYP2B6 785A>G (rs2279343), CYP2B6 835 G>C(rs139029625) and CYP2B6 1459 C>A (ss539003296) are listed in Table 3.1. The amplification was achieved using a 20ul master mix containing 2X TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA),

primers forward (10um) and reverse (10 uM), wildtype and mutant probes (10 uM each), H_2O and 10ng sample DNA. The PCR amplification was achieved by initial phase of 50°C for 2 minutes, followed by second stage of 95°C for 10 minutes and 45 cycles at 95°C for 15 seconds and the last stage of 60°C for 60 seconds. The allelic discrimination setting was used to determine the SNPs genetic variations using SDS, version 1.3.0 software. This SDS software was also used for the post-assay SNP analysis defining the SNPs results as either homozygous wild type, heterozygous and homozygous mutant.

Table 3.1. List of CYP2B6 SNPs primers used for genotyping in this study (Radloff *et al*., 2013)

SNP	Region	Variant (rs number)	Direction	Sequence (5' – 3') c
329 G>T	Exon 2	rs186335453	F	CGACCCATTCTTCCGGGTATATGGTGTGATCTTTG
			R	CAAAGATCACACCATATACCCGGAAGAATGGGTCG
341 T>C	Exon 3	rs139801276	F	CCGGGGATATGGTGTGACCTTTGCCAATGGAAACC
			R	GGTTTCCATTGGCAAAGGTCACACCATATCCCCGG
444 G>T	Exon 3	rs1053569	F	GGAGCGGATTCAGGATGAGGCTCAGTGTCTG
			R	CAGACACTGAGCCTCATCCTGAATCCGCTCC
548 T>C	Exon 4	ss539003292	F	CATCATCTGCTCCATCGGCTTTGGAAAACGATTCC
			R	GGAATCGTTTTCCAAAGCCGATGGAGCAGATGATG
637 T>C	Exon 4	ss539003294	F	CTTTTTCACTCATCAGCTCTGTACTCGGCCAGCTGT
			R	ACAGCTGGCCGAGTACAGAGCTGATGAGTGAAAAAG
785 A>G	Exon 5	rs2279343	F	AGGCAAGTTTACAAAAACCTG
			R	CCCTCCCTAGTCTTTCTTCTTCC
835 G>C	Exon 6	rs139029625	F	GAAAAAGAGAAATCCAACCCACACAGTGAATTCAGCC
			R	GGCTGAATTCACTGTGTGGGTTGGATTTCTCTTTTTC
1459 C>A	Exon 9	ss539003296	F	CAACATACCAGATCAGCTTCCTGCCCCGC
			R	GCGGGGCAGGAAGCTGATCTGGTATGTTG

3.7.3.6 Drug Resistance Testing

3.7.3.6.1. RNA extraction

The HIV viral RNA was extracted from plasma samples using QIAamp viral RNA extraction kit, (Qiagen Inc., USA) using the guidelines of the manufacturer.

3.7.3.6.2. Nested PCR amplification and visualization for HIV RNA

Genotypic resistance testing was conducted using an in-house method. Briefly, the AmpliTaq Gold (Roche Molecular Systems, Branchburg, NJ) was used for the nested PCR. The first round PCR amplification of the pol gene was achieved using the following primers

(RT18:5′GGAAACCAAAAATGATAGGGGGAATTGGAGG3′ and RT21 5′ CTGTATTTCTGCTATTAAGTCTTTTGATGGG 3′). The PCR cycling condition was as follows 1 cycle of 45°C for 1 min and 94°C for 2 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, and final extension of 72°C for 2 min. The following primers were used in the second round of nested PCR (RT1: 5′CCAAAAGTTAAACAATGGCCATTGACAGA 3′ and RT4: 5′ AGTTCATAACCCATCCAAAG 3′). The PCR amplification condition was set as follows 1 cycle of 94°C for 2 min and 30 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, and final extension of 72°C for 10 min as described by (Overbaugh *et al.,* 2009). The PCR products were visualized using agarose gel stained with ethidium bromide. The positive sample was visualized at 645 base pairs

3.7.3.6.3. Bigdye sequencing reactions

The nested PCR positive samples were first cleaned using ExoSAP-IT™ PCR technology in line with manufacturer's guidelines. The Exosap removes excess nucleotides and primers. This single step PCR clean up involved adding 5ul of positive PCR product and 2ul of ExoSAP-IT reagent into a 200ul PCR tubes. Using the AmpliTaq Gold (Roche Molecular Systems, Branchburg, NJ) machine these tubes are held at 37ºC for 15 minutes and heated up to 80ºC for 15 min. This cleaned PCR products were sequenced directly using Bigdye sequencing reaction.

The HIV sequencing was done using the Big Dye terminator kit (Applied Biosystems). The sequencing was done using the ABI Prism 3300 equipment (Applied Biosystems, Foster City, US). The final volume of 10 µl for sequencing reaction consisted of 5.5 µl DNA free ultra-pure water, 2 µl 5x Big Dye buffer, 1 ul Big Dye, 0.5µl each of the primers (RT 1: 5' CCAAAAGTTAAACAATGGCCATTGACAGA 3' and RT4: 5' AGTTCATAACCCATCCAAAG3'), and 1ul of ExoSap IT cleaned PCR product. The Bigdye PCR amplification was achieved follows 1 cycle of 94°C for 10 sec and 25 cycles of 94°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min (Overbaugh *et al.,* 2009).

Before sequencing, the 10 ul bigdye PCR products were first cleaned using Sephadex® G-50 (Sigma, US) impregnated spin columns centrifuged at 20,000g and set -20°C for 5 minutes. The cleaned products were denatured using 10 ul Hi-Di™ Formamide heated at 95°C for 2 minutes using the AmpliTaq Gold (Roche Molecular Systems, Branchburg, NJ) machine. The 20ul of the denatured bigdye product was then loaded into 96 sequencing well plate and directly sequenced using Big Dye technology on ABI 310 (Applied Biosystems, Foster City, CA) (Overbaugh *et al.,* 2009).

3.7.3.6.3.1. HIV Drug resistance interpretation

The sequences were then aligned and cleaned using Sequencer - sequence analysis software version 5.4.9 (Gene code Cooperation, MI, US). To determine the HIV subtypes and presence of HIV drug resistant mutations, the sequences were blasted in the Stanford University and International AIDS society-USA web site (http://hivdb.stanford.edu) as described by (Johnson et al., 2008). Using the Stanford Genotypic Resistance Interpretation Algorithm, HIV drug resistance was defined as the occurrence of resistance mutations associated with impaired drug susceptibility.

3.7.4. Quality assurance

All laboratory personnel received training on Good Laboratory Practice (GLP) and Good Clinical Practice (GCP). Standard operating procedures (SOPs) of KEMRI and those of Laboratory of Retrovirology, Public Research Centre for Health in Luxembourg were adhered to; especially those pertaining to labeling of specimen containers, specimen collection, transportation, analysis and posting of results. All reagents were prepared in accordance with SOPs used at the two laboratories. Equipment operation were done according to manufacturer's instructions.

3.7.5. Data Management

Patients in this study were identified using a unique subject identification number (SID). Data and patient's information gathered and generated in this study both in the data collection forms and databases were only associated with the unique SID. All the database and study related files were password protected using a double entry system. All other hard copy records such

as questionnaires, consent forms and laboratory results were in a lockable filling cabinet in access restrict office at KEMRI. The biological samples were stored in -80 ^{o}C freezers with restricted access.

3.7.6. Statistical Analysis

The descriptive and regression statistical analyses were performed using Stata version 13 (StataCorp. LP, College Station, USA). Linkage disequilibrium, allele, genotype and haplotype frequency analyses were done using the SNPStats software (Online SNP analytical tool accessible at https://www.snpstats.net/start.htm. The significance level was set at $P \leq 0.05$.

All data were subjected to descriptive statistical analysis. Continuous variables especially NVP and EFV plasma levels were first tested for normality, using the Shapiro Wilk test. Categorical variables were presented as frequencies and percentages. Chi-square or Fishers' exact test were used to describe associations between variables. Means ± standard deviation (SD) was used to present normally distributed outcomes. The inferential data analysis was carried out using one-way ANOVA the Student's t-test. Linear regression models were used to determine the relationship between dependable variables and laboratory outcomes following ART initiation.

Steady-state nevirapine and efavirenz plasma concentrations was tested for normality using the Shapiro- Wilk test, and subsequently $\log_{10}$-transformed due to lack of normality. The relationship between NVP and EFV plasma concentration were tested using quantile regression analysis. NVP plasma level was classified as <3100 ng/mL (below therapeutic range), 3100–4300 ng/mL (therapeutic range) and >4300 ng/mL (above therapeutic range). For EFV, the concentrations of <1000 ng/ml considered below therapeutic range, 1000 to 4000ng/ml considered therapeutic range and >4000 ng/ml considered supratherapeutic level (Duong et al., 2004; Gopalan et al., 2017).

Evaluation of Hardy-Weinberg equilibrium (the calculation of the p value was achieved using the Markov chain method) for the 13 CYP2B6 SNPs and 1 CAR genetic variation, Linkage disequilibrium, allele, genotype and haplotype frequency and differences in the SNP/allele frequencies between groups/ populations was analyzed using the SNPStats software

(https://www.snpstats.net/start.htm). The expected heterozygosity was determined using the Wright's F statistics. Variation in log_{10}-transformed NVP and EFV plasma levels were evaluated using the Kruskal–Wallis test by ranks while factors associated with NVP and EFV plasma levels was determined using the quantile regression statistics. Only the CYP2B6 and CAR SNPs conforming to Hardy-Weinberg equilibrium were evaluated.

The expected metabolic scores attribution was made according to: 15582 TT = −2; 15582 CT = −1; 15582 CC = 0; 516 TT = −2; 516 GT = −1; 516 GG = 0; 785 GG = +2; 785 AG = +1; 785 AA = 0; 18492 TT = −2; 18492 CT = −1; 18492 CC = 0; 983 CC = −2; 983 TC = −1; 983 TT = 0; 21563 TT = +2; 21563 CT = +1; 21563 CC = 0; 1459 TT = −2; 1459 TC = −1; 1459 TT = 0; 540 TT = +2; 540 CT = +1; 540 CC = 0. The summation for each genotype (15582, 516, 785, 983, 18492, 21563, 1459, and 540) per sample was done to obtain the "metabolic score". PM: poor metabolizers; I: intermediate metabolizers; EM: extensive metabolizers; UR: ultra-rapid metabolizers. The divergence from normal distribution for the metabolic scores of NVP and EFV were analyzed using Kolmogorov-Smirnov test.

3.7.7. Ethical Considerations

Guidance for Good Clinical Practice and the principles articulated in the pronouncement of Helsinki (Edinburg, October 2000) (WHO, 1995) were applied in this study. This procedure and the resulting informed consent forms used in this study were reviewed and permission obtained from, the KEMRI research and ethics committee granted permission on 21st May 2013 (SSC No. 2539 on 21st May, 2013) (Appendix 1). Written informed consent was acquired from all the study patients before recruitment into the study.

The potential participants were engaged on the study related activities, addressing the issues of benefits and risks for participation. The study also explained to the patients their role in the study. The patients were given the right to choose to take part in the study or not. Eligible patients were expected to comprehend and sign a consent form summarizing the discussion prior to enrollment (Appendix 1). The consent form was filled in duplicate and one copy retained in the study and the other issued to the patients (Appendix 1).

All data gather from the patients were kept in confidence and exclusively used for achieving the study objectives. Unique subject identification number (SID) was. Used to identify all the recruited patients.

The study made it clear that participation was absolutely voluntary and patients could opt out at any moment at will. For those who opted out, their reasons were documented.

It was explained to the patients that the investigators and or institutional ethical review committee reserved the right to stop the study at any time should need a rise. Reasons for termination (if any) was provided to the participants. The researcher reserved the right to terminate the study for safety reasons at any time. Study samples and records have been stored at the KEMRI laboratory for a period of ten years.

The patient's laboratory results obtained during screening was issued to them in line with the laid down clinical protocol. Referral for promote relevant treatment were initiated in cases of those found to have abnormal immunological, virological, drug resistance and pharmacogenetic profiles. Data presentation and publications were devoid of personal identification information.

CHAPTER FOUR

RESULTS

4.1. PREVALENCE OF IMMUNOLOGICAL, VIROLOGICAL, HEMATOLOGICAL AND BIOCHEMICAL OUTCOMES

4.1.1. Introduction

The use of highly active antiretroviral therapy (HAART) has slackened HIV/AIDS disease progress, reduced mortality, and enhanced the quality of life for huge number of people infected with HIV (Lahuerta *et al.*, 2014; Mutimura *et al*., 2015). During the study period, measurement of both CD4 cell count and HIV RNA viral load were vital indicates of the effectiveness ART treatment. Robust increases in CD4 cell counts have been reported due to ART uptake (Mutimura *et al*., 2015). The initial CD4 cell count prior to ART medication, significantly influences the degree of patient's immunologic and virologic ART treatment outcomes and the consequent morbidity and mortality (Honda *et al*., 2012; Ingole *et al*., 2013; Nakanjako *et al*., 2016). Gradually, toxicities and adverse effects have emerged as a significant threat jeopardizing the clinical benefits of these ART treatment. Liver induced injuries and hematologic abnormalities, are commonly associated with some of the ART affecting HIV/AIDS disease progression and reduced survival rate (Enawgaw *et al*., 2014; Kovari *et al*., 2011).

In the course of ART treatment, the incidence of severe increased liver enzymes (hepatotoxicity) usually ranges between 5 and 10 per 100 person-years (Peters *et al*., 2010). All ARV in use are associated with increased liver enzyme level elevation, with NNRTI-based regimens causing severe hepatic outcomes compared to either NRTI- or PI-based regimes (Peters *et al*., 2012). Blood abnormalities including thrombocytopenia, anemia, neutropenia and cytopenia are common during ART treatment (Ibeh *et al*., 2013).

Data presented in this objective were aimed at generating information regarding the association between treatment with NVP and EFV based regimen and virologic, immunological and pathological outcomes which are still skewed in sub-Saharan African (Lahuerta *et al*., 2014).

4.1.2. Methods

The methods used in this section are presented in chapter three sections 3.7.3.1, 3.7.3.2 and 3.7.3.3 while data management and statistical analysis are presented in section 3.7.5 and 3.7.6

4.1.2.1 Case Definition

Elevated liver enzymes were categorized as ALT more than 56U/L or AST more than 40U/L. Creatinine abnormality was defined as more than 0.8mg/dL. Hemoglobin blood count less than 13 g/dl for men and less than 12 g/dl (women) was considered anemic. The total WBC count lower than 4.3 x 10^9 cells per liter was considered leucopenia. Thrombocytopenia was defined as total platelet counts lower than 150 × 10^3/µl. Lymphocytosis, was defined as increased level absolute lymphocyte count of more than 4000 cells/µl.

The HIV RNA as count above 1000 copies/ml 6 months into treatment under ART adherence support was defined as virologic failure. Immunological failure was described as decrease in the CD4 count below baseline count or a constant CD4 count lower than 100 cells/mm.

4.1.3. Results

4.1.3.1. Comparison of the socio-demographic, hematological, renal and liver characteristics of study patients

Table 4.1 summarizes the characteristics of study participants. All the five hundred ninety-nine (599) enrolled patients (100% responses rate) had all the immunological, virological, hematological and biochemical data and were evaluated. Based on both CD4 and viral load; 357 (59.6%) patients were ART treatment responders while 242 (40.4%) patients as treatment failures. For the 242 patients failing ART treatment 21/242 (8.7%) was due to virologic failure, 207/242 (85.5%) due to immunologic failure while 14/242 (5.8%) were filing both in viral load and CD4 counts (Figure 4.1).

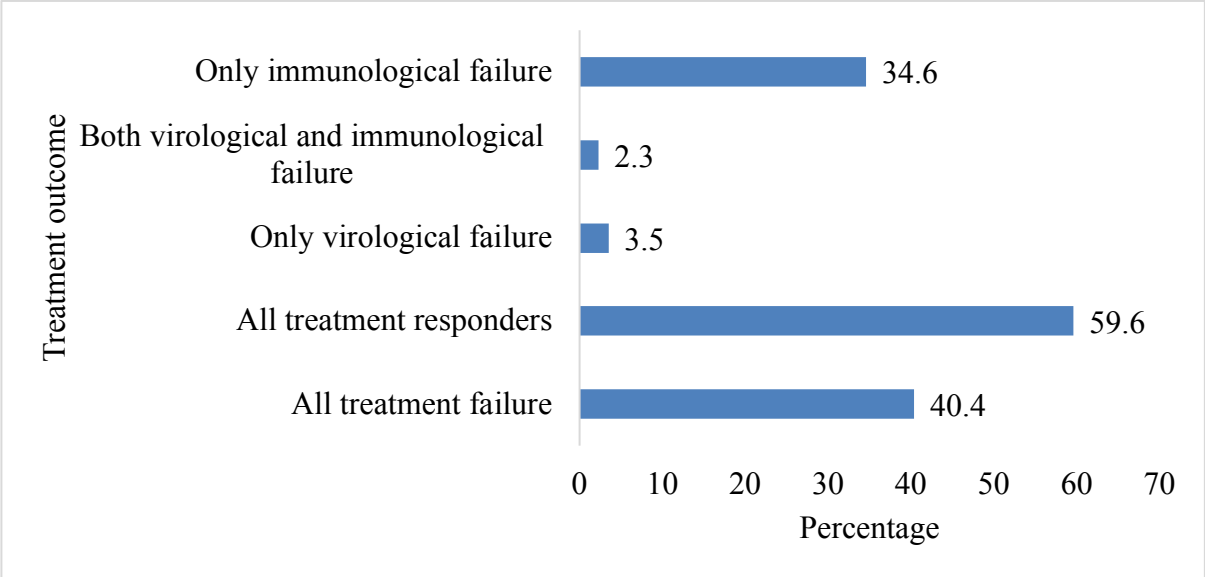


Figure 4.1: Prevalence of immunological and virological failure among study patients

The majority 361(60.3%) of patients were female, among which, 217(60.8%) and 144(59.5%) were responding and failing treatment respectively. The median age of patients during start of ART use was 41 years (IQR 35-47 years). There was no significant difference in age between treatment responders and failures (p = 0.862). The duration (median) infected with HIV among all the patients was 6 years (IQR 4 – 8 years). Patients responding to ART medication had been infected with HIV longer than those failing ART medication (p = 0.017). Although the bantus were more than other ethnic groups (more than 64% of the enrolled patients), there was no significant difference regarding patients' ethnic groups between patients' responders and failures (p = 0.524).

Table 4.1: Comparative analysis of demographic and medical characteristics of the treatment responders and non-responders

Parameters	Unit	Treatment outcomes			
		All patients (n = 599)	Responder (n = 357)	Failure (n = 242)	P value
		n (%)	n (%)	n (%)	
Gender	Male	238 (39.7)	140 (39.2)	98 (40.5)	
	Female	361 (60.3)	217 (60.8)	144 (59.5)	0.799
Age (Years)	Median (IQR)	41 (35 - 47)	41(35 - 47)	40(33.7 - 47)	
	21-25	19 (3.2)	11 (3.1)	8 (3.3)	
	26-30	53 (8.8)	29 (8.1)	24 (9.9)	0.862
	31-40	225 (37.6)	137 (38.4)	88 (36.4)	
	>40	302 (50.4)	180 (50.4)	122 (50.4)	
Education	None	7 (1.2)	6 (1.7)	1 (0.4)	
	College	193 (32.2)	117 (32.8)	76 (31.4)	
	Primary	180 (30.1)	108 (30.3)	72 (29.8)	0.524
	Secondary	219 (36.6)	126 (35.3)	93 (38.4)	
Ethnicity	Bantu	387 (64.6)	231 (64.7)	156 (64.5)	
	Cushite	9 (1.5)	6 (1.7)	3 (1.2)	0.929
	Nilot	203 (33.9)	120 (33.6)	83 (34.3)	
Years HIV positive	Mean (± SD)	6.4 (3.1)	6.7(3.1)	6.1(3)	
	Median (IQR)	6 (4 - 8)	7(4 - 8)	6 (4 - 8)	
	<5	255(42.6)	135(37.8)	120(49.6)	
	6-10	291 (48.6)	188(52.7)	103(42.6)	**0.017**
	<10	53(8.8)	34(9.5)	19(7.9)	
Current ARV regimen	ABC/3TC/NVP	1(0.2)	0	1 (0.4)	
	TDF/3TC/NVP	159 (26.5)	81 (22.7)	78 (32.2)	
	AZT/3TC/NVP	102 (17.1)	53 (14.8)	49 (20.2)	
	D4T/3TC/NVP	1 (0.2)	1 (0.3)	0	**0.002**
	ABC/3TC/EFV	1 (0.2)	1 (0.3)	0	
	TDF/3TC/EFV	210 (35.1)	133 (37.3)	77 (31.8)	
	AZT/3TC/EFV	125 (20.9)	88 (24.6)	37 (15.3)	
CD4 (cells/ml)	Median (IQR)	288 (138 - 410)	305(210 - 409)	206(94 - 419)	
	<500	509 (85)	307(85.9)	202(83.5)	**0.001**
	>501	90 (15)	50(14.1)	40(16.5)	
VL (copies/ml)	Mean (Range)	2644.7(1 - 367728)	41.8(1 - 160)	6484(1 - 367728)	
	<1000	509 (85)	307(85.9)	202(83.5)	**0.001**
	>1000	90 (15)	50(14.1)	40(16.5)	
ALT (U/L)	Median (IQR)	16.9 (11 - 23.9)	18(10.7 - 24)	15.7(11.5 - 22.2)	
	<56	585 (97.7)	350(98)	235(97.1)	0.583
	>56	14 (2.3)	7(2)	7(2.9)	
AST (U/L)	Median (IQR)	17 (11 - 24)	18(11 - 24)	15.5(11 - 21.6)	
	<40	567 (94.7)	336(94.1)	231(95.5)	0.58
	>40	32 (5.3)	21(5.9)	11(4.5)	
Creatinine (mg/dL)	Median (IQR)	0.9 (0.6 - 1.2)	0.9(0.6 - 1.2)	0.9(0.7 - 1.1)	
	<08	219 (36.6)	137(38.4)	82(33.9)	0.3
	>0.8	380 (63.4)	220(61.6)	160(66.1)	
HB (g/dL)	Median (IQR)	12.6 (11 - 14.3)	12.8(11.3 - 14.6)	12.2(10.7 - 13.9)	
	< 13	338 (56.4)	190(53.2)	148(61.2)	0.198
	> 13	261 (43.6)	167(46.8)	94(38.8)	
WBC (10^3/mm^3)	Median (IQR) X 10^3	4.6 (3.6 - 5.8)	4.6(3.6 - 5.8)	4.6(3.6 - 5.9)	
	≤ 4.3 X 10^3	281 (46.9)	166(46.5)	115(47.5)	0.868
	>4.3 X 10^3	318 (53.1)	191(53.5)	127(52.5)	
Plateletes (10^9/L)	Median (IQR) X 10^9/L	289 (219 - 350)	288(214 - 339)	290.5(216.5 - 360)	
	<150 X 10^9	504 (84.1)	300(84)	204(84.3)	0.98
	>150 X 10^9	95 (15.9)	57(16)	38(15.7)	
Lymphocytes (10^9/L)	Median (IQR) X 10^9	2.2 (1.8 - 2.8)	2.2(1.8 - 2.9)	2.1(1.8 - 2.7)	
	<4 X 10^9	584 (97.5)	347(97.2)	237(97.9)	0.791
	>4 X 10^9	15 (2.5)	10(2.8)	5(2.1)	

4.1.3.2. Hematological, and biochemical characteristics of study patients

Patients responding to ART treatment was marked by higher median CD4 cell count of (305 cells/ml [IQR 210-409 cells/ml] compared to those failing treatment 206 cells/ml [IQR 94-419 cells/ml]) ($p = 0.001$). Further, the HIV viral load of patients responding to treatment was lower 41.8 copies/ml (range 1-160 copies/ml) compared to the mean viral load of patients failing treatment (6484 copies/ml (range 1-367728) copies/ml ($p = 0.001$). There was no significant difference in the median baseline ALT, AST, hemoglobin (HB), white blood cell level, platelets and lymphocytes as shown in Table 4.2.

Table 4.2: Baseline Immunological, virological, hematological and biochemical characteristics of the study patients

Variables	All patients (n = 599)	Treatment outcomes: Responders (n = 357)	Failures (n = 242)	*p* value
	n (%)	n (%)	n (%)	
CD4 (cells/ml)				
Median (IQR)	288 (138 - 410)	305(210 - 409)	206(94 - 419)	
<500	509 (85)	307(85.9)	202(83.5)	0.001
>501	90 (15)	50(14.1)	40(16.5)	
VL (copies/ml)				
Mean (Range)	2644.7(1 - 367728)	41.8(1 - 160)	6484(1 - 367728)	
<1000	509 (85)	307(85.9)	202(83.5)	0.001
>1000	90 (15)	50(14.1)	40(16.5)	
ALT (U/L)				
Median (IQR)	16.9 (11 - 23.9)	18(10.7 - 24)	15.7(11.5 - 22.2)	
<56	585 (97.7)	350(98)	235(97.1)	0.583
>56	14 (2.3)	7(2)	7(2.9)	
AST (U/L)				
Median (IQR)	17 (11 - 24)	18(11 - 24)	15.5(11 - 21.6)	
<40	567 (94.7)	336(94.1)	231(95.5)	0.58
>40	32 (5.3)	21(5.9)	11(4.5)	
Creatinine (mg/dL)				
Median (IQR)	0.9 (0.6 - 1.2)	0.9(0.6 - 1.2)	0.9(0.7 - 1.1)	
<08	219 (36.6)	137(38.4)	82(33.9)	0.3
>0.8	380 (63.4)	220(61.6)	160(66.1)	
HB (g/dL)				
Median (IQR)	12.6 (11 - 14.3)	12.8(11.3 - 14.6)	12.2(10.7 - 13.9)	
< 13	338 (56.4)	190(53.2)	148(61.2)	0.198
> 13	261 (43.6)	167(46.8)	94(38.8)	
WBC (10^3/mm^3)				
Median (IQR) X 10^3	4.6 (3.6 - 5.8)	4.6(3.6 - 5.8)	4.6(3.6 - 5.9)	
≤ 4.3 X 10^3	281 (46.9)	166(46.5)	115(47.5)	0.868
>4.3 X 10^3	318 (53.1)	191(53.5)	127(52.5)	
Plateletes (10^9/L)				
Median (IQR) X 10^9/L	289 (219 - 350)	288(214 - 339)	290.5(216.5 - 360)	
<150 X 10^9	504 (84.1)	300(84)	204(84.3)	0.98
>150 X 10^9	95 (15.9)	57(16)	38(15.7)	
Lymphocytes (10^9/L)				
Median (IQR) X 10^9	2.2 (1.8 - 2.8)	2.2(1.8 - 2.9)	2.1(1.8 - 2.7)	
<4 X 10^9	584 (97.5)	347(97.2)	237(97.9)	0.791
≥4 X 10^9	15 (2.5)	10(2.8)	5(2.1)	

n - absolute numbers; % percentages; IQR - interquartile range; TDF-Tenofovir;
kg-kilogram; ml – Milliliter; U – Units; L – Liter; mg-milligrams; dL – Deciliters; g – Grams and mm –Millimeter.

4.1.3.3. Changes in the clinical outcomes 12-month post ART initiation

The changes in clinical laboratory parameters are described in Table 4.3. In the 12 months into ART treatment, a significant increase CD4 cell count (301.7 ± 199.4 baseline to 329.4 ± 305.8 month 12; $p < 0.05$) as well as the AST level (19.5 ± 11.9 baseline to 31.5 ± 22.7 month 12; $p < 0.05$). Similarly, there was a significant increase in the hemoglobin count from 12.4 ± 2.7 at baseline to 14.1 ± 2.5g/dL at month 12 ($p <0.001$). The total WBC increased from 4.8 ± 1.9$10^3$/mm^3 at baseline to 5.1 ± 1.8 10^3/mm^3 at month 12 ($p<0.001$), with the absolute lymphocyte counts increasing from 2.2 ± 0.89 10^9/L at baseline to 2.4 ± 0.84 10^9/L at month 12 ($p <0.001$. Absolute platelets level also increased from 293.1 ± 109.5 10^9/L at baseline to 297.6 ± 100.6 10^9/L) ($p <0.001$ at month 12 post ART initiation.

Table 4.3: Changes in the immunological, hematological and hepatic enzyme levels from baseline to 12 months post ART initiation

Variables	Baseline	Month 12	*p value*
CD4+ (cell/µL) Median (IQR)			
All (n = 599)	288 (138 - 410)	308 (98 - 493)	**0.001**
Responders (n = 357)	305 (211 - 409)	439 (316 - 563)	**0.001**
Failure (n = 242)	206.5 (94 - 418)	89 (59 - 112)	**0.028**
ALT (U/L) Median (IQR)			
All (n = 599)	16.9 (11 - 23.9)	25 (18 - 37)	0.495
Responders (n = 357)	18 (10.7 - 24)	25 (19 - 37)	0.361
Failure (n = 242)	15.7 (11.5 - 22.2)	25 (17 - 37)	**0.017**
AST (U/L) Median (IQR)			
All (n = 599)	17 (11 - 24)	27 (20 - 38)	**0.001**
Responders (n = 357)	18 (11 - 24)	27 (20 - 38)	**0.001**
Failure (n = 242)	15.5 (11 - 21)	27 (20 - 40)	**0.001**
Creatinine (mg/dL) Median (IQR)			
All (n = 599)	0.9 (0.6 - 1.2)	0.9 (0.7 - 1.1)	0.092
Responders (n = 357)	0.9 (0.6 - 1.2)	0.9 (0.6 - 1.1)	0.816
Failure (n = 242)	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.1)	0.602
Hb (g/dL) Median (IQR)			
All (n = 599)	12.6 (11 - 14.3)	14.3 (12.8 - 15.6)	**0.001**
Responders (n = 357)	12.3 (11.3 - 14.3)	14.3 (12.8 - 15.6)	0.206
Failure (n = 242)	12.2 (10.7 - 13.9)	14.3 (12.8 - 15.6)	0.095
WBC (10^3/mm^3) Median (IQR)			
All (n = 599)	4.6 (3.6 - 5.8)	4.88 (3.7 - 6.2)	**0.001**
Responders (n = 357)	4.6 (3.6 - 5.8)	4.8(3.8 - 6.2)	**0.001**
Failure (n = 242)	4.6 (3.6 - 5.8)	4.75(3.7 - 6.2)	**0.001**
Lymphocytes (10^9/L) Median (IQR)			
All (n = 599)	2.2 (1.8 - 2.8)	2.4 (2 - 2.9)	**0.001**
Responders (n = 357)	2.2 (1.8 - 2.9)	2.5 (2 - 3)	**0.001**
Failure (n = 242)	2.1 (1.8 - 2.7)	2.4 (2 - 2.9)	**0.001**
Plateletes (10^9/L) Median (IQR)			
All (n = 599)	289 (219 - 350)	289 (225 - 359)	**0.001**
Responders (n = 357)	288 (219 - 339)	283 (225 - 359)	**0.001**
Failure (n = 242)	290.5 (219 - 360)	289 (225 - 359)	**0.001**

SD - standard deviation; kg-kilogram; ml – Milliliter; U – Units; L – Liter; mg-milligrams; dL – Deciliters; g – Grams and mm –Millimeter.

4.1.3.3. occurrence of liver, kidney and blood abnormalities 12 months into ART treatment

The changes in the clinical chemistry and blood levels 12 months into ART treatment are shown in Figure 4.2. Compared to the baseline levels, both the ALT and AST levels were higher 12 months into ART treatment (2.5% to 10.5%) ALT and (5.3% to 23.4%) AST. Creatinine elevated levels were common at month 12 (68.8%) compared to baseline (63.4%). Further, there was a slight increase in the cases of lymphocytosis at month 12 compared to baseline.

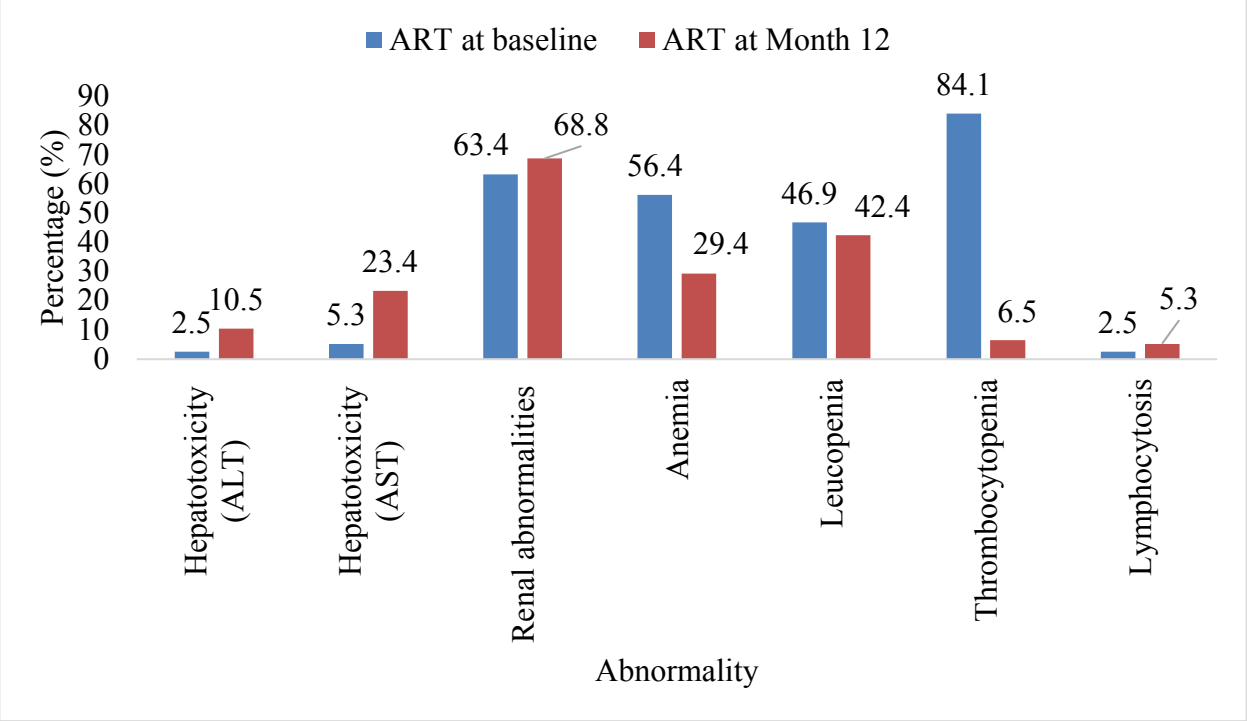


Figure 4.2: Distribution of biochemical and hematological abnormalities among study patients.

4.1.3.4. Factors predicting clinical outcomes 12-month post ART

Multivariable regression analysis describing the factors associated with clinical chemistry and blood abnormalities are shown in Table 4.4. The month 12 CD4 levels were significantly associated duration living with HIV infection (years) (beta 10.1 cells/µL, 95% CI, 1.707 to 18.484 cells/µL, p=0.018) and baseline CD4 cell count (p = 0.001). The month 12 AST was influenced by baseline AST levels (p=0.001). The creatinine concentration at month 12 was

associated with ART regimen type (p =0.048). The study further showed the significant influence of baseline HB, lymphocytes, and platelets on the month 12 HB, lymphocytes, and platelets concentration (Table 4.4).

Table 4.4: Independent factors predicting changes in month12 laboratory parameters

Model	Unadjusted β	(95% CI)		Adjusted β	t	P-value
CD4 cell count						
Baseline CD4	0.44	0.31	0.57	0.27	6.50	**0.001**
Duration with HIV	10.10	1.71	18.48	0.10	2.36	**0.018**
AST (U/L)						
Baseline AST	0.711	0.56	0.86	0.37	9.12	**0.001**
Creatinine (mg/dL)						
ART regimen	0.088	0.00	0.17	0.09	1.98	**0.048**
Hb (g/dL)						
Baseline HB	0.122	0.04	0.20	0.13	3.02	**0.003**
WBC (10^3/mm^3)						
Baseline WBC	0.851	0.82	0.89	0.91	49.50	**0.001**
Lymphocytes (10^9/L)						
Baseline LYMP	0.885	0.85	0.92	0.93	51.24	**0.001**
Duration with HIV	-0.011	-0.02	0.00	-0.04	-2.39	**0.017**
Plateletes (10^9/L)						
Baseline PLT	0.833	0.80	0.87	0.91	48.28	**0.001**
Baseline weight	0.377	0.05	0.70	0.04	2.28	**0.023**

4.1.3. Discussion

The data presented in this objective revealed that both CD4 cell count and HIV viral load responds robustly among patients receiving ART care and treatment. Additionally, occurrence of toxicities and treatment adverse reactions such as liver and kidney induced injuries were higher 12 months into treatment compared to at initiation. The prevalence blood abnormalities such anemia, leucopenia and thrombocytopenia were lower compared 12 months into ART management compared to baseline. The outcome of this study showed that consistent and prolonged use of ART portends significant clinical benefits.

Studies in several backgrounds developing and developed have reported a significant and consistent improvements in the CD4 cell counts especially in patients on ART over extended period of times in (Lok et al., 2010; Nash et al., 2008; Rajasekaran et al., 2009). In this study, there was a noticeable increase in CD4 cell count by about 27.7 cell/ul greater compared to the. baseline. The study also reported close to a quarter of the patients (43.7%) had CD4 cell count above 350 cell/ul (at the time, above the WHO recommended levels for ART initiation). Worth noting is that, there were only 5.7% of the patients had virologic failure month 6 sustained at month 12 into ART treatment. This study demonstrates that ART contributes to significant immunological and virological response. This current study reiterates the progressive virological and immunological response to HAART, consistently reported in other studies (Opravil et al., 2002; Waters et al., 2004). Although other reports showed occurrence of plateau in CD4 cell count response two years into ART use (Tarwater et al., 2001). On the contrary, some reports have also shown suppression of viral load and maintenance of CD4 cell counts is evident over extend years of ART use even beyond a decade especially among patients achieving and maintaining viral suppression (Hunt et al., 2003; Nash et al., 2008).

In regression models, both the duration infected with HIV and the baseline CD4 cell count significantly predicted the current CD4 count as well as in the preceding months. This observation has been documented in other reports both in developed countries (Kaufmann et al., 2002, 2003) and resource-limited settings (Kabugo et al., 2005; Nash et al., 2008). Generally, higher baseline CD4 counts is paramount in achieving a higher CD4 count in the subsequent periods. The baseline CD4 count and medication adherence, have also been

reported as key dictate in the clinical progression and existence after ART initiation (Badri et al., 2006; Etard et al., 2006).

Studies evaluating the toxicities and adverse drug reactions has been a major focus for virtually all ARVs in the market. Hepatotoxicity, neuropathy, renal injuries and pancreatitis are among the major focus of these investigations (Dieterich et al., 2004). Hepatic ARV induced injuries contributes significantly to the proportion of non-AIDS causes of mortality in HIV infection (Smith, 2010; Kovari & Weber, 2011). Additional, in the course of effective ART treatment, 18% of all mortalities in HIV infected patients are caused by hepatic complications (Smith, 2010; Kovari & Weber, 2011). In this study a significant increase in the mean AST and ALT were observed at 12 months into treatment. Studies have shown varied prevalence of hepatotoxicity ranging from 7% to 15% of HIV-infected patients who have been on ART treatment for at least six months (Sulkowski et al., 2002; Abdulahi et al., 2005; Ibeh et al., 2013).

The month 12 concentration of liver enzymes (AST and ALT) were greatly predicted by their corresponding baseline levels. Susceptibility to ART induced hepatotoxicity among HIV patients on ART has been shown to occur owing to the interaction between the effects of the ART regimens and the related risk factors, including substance use, underlying diseases, and concomitant drugs (DeLeve & Kaplowitz, 2000). Guidelines therefore commends regular and systematic monitoring of these levels especially among patients on prolonged administration of ART minimize the potential drug induced liver injuries.

Kidney response to injuries is generally determined using creatinine levels as a excellent bio-marker In regression analysis, ART regimen types significantly influenced creatinine serum concentration. Similar to this study the lack of association between baseline creatinine level and month 12 creatinine levels may signifies a normal functional and intact kidney (Sulkowski et al., 2002; Abdulahi et al., 2005; Ibeh et al., 2013).

Immunopathogenesis, a process by which disease development must induce an immune response is commonly caused to varying extent by HIV infection (Watkins et al., 1990). HIV infection is associated with numerous blood abnormalities. These blood complications during HIV infection may be due to; directly due to viral infection, malignancies and tumors, sequel due to HIV opportunistic diseases, and aftermath of ART treatment (Moyle, 2002). The current study observed cases of the following blood abnormalities including; anemia, thrombocytopenia, lymphocytosis and leucopenia. Various reports have shown significant divergence in the occurrence in the hematological complications in HIV infection, with low hemoglobin levels being most common reported in 1.3 to 95% of the HIV patients (Belperio & Rhew, 2004; Owiredu et al., 2011; Dhurve & Dhurve, 2013).

During the current study 12 months into ART treatment, there was a reduction in the prevalence of leucopenia, thrombocytopenia, low hemoglobin level, while a marginal increase was noted with regards to lymphocytosis. These findings are in agreement with those of Ibeh et al., (2013) and Assefa et al., (2015) that reported a substantial decreases in hematological complications during ART management. Although administration of Zidovudine (AZT) based ART has been associated with anemia (Baroncelli et al., 2011), on the contrary, this study reported reduced cases of anemia 12 months into treatment including in patients receiving AZT based regimen. Johannessen et al., (2011) showed the benefits of long-term ART treatment in the reversal of HIV induced anemia. The reversal of anemia during ART management has been associated with the decrease or elimination of opportunistic infections and the reduction of tumor necrosis factor (TNF) involved in the suppression of erythropoiesis are among the suggested mechanisms (Redig & Berliner, 2013).

4.1.3.6. Conclusion

The prevalence of immunological and virological failure was 40.9%. Patients with immunological and virological failures had a lower CD4 at baseline and a higher viral load. Patients who were failing ART treatment were also more likely to be on treatment with NVP based regimen. ART use was associated with improvement of all hematological indices resulting in a significant decrease in the prevalence of adverse effects including kidney, liver and blood complications 12 months into treatment. Nevertheless, a considerable percentage of

patients continuously experienced hepatotoxicity and hematological abnormalities 12 months into ART treatment, reiterating the necessity for regular screening of biochemical and full blood consequences; and examination of the principal causes and establishing fitting treatment and management approaches to alleviate the adverse effects of ART.

4.2. EFAVIRENZ AND NEVIRAPINE PLASMA LEVELS 12 MONTHS INTO TREATMENT

4.2.1. Introduction

The optimum clinical outcome with NNRTI treatment requires optimal drug exposure. Suboptimal exposure to NVP and EFV presents various jeopardies to the achievement of recommended treatment outcomes (Muro et al., 2005). Previous reports have pointed out the predictive nature of low NNRTIs concentrations to virological failure while highly drug-resistant viruses rapidly emerging when NNRTIs are administered in suboptimal regimens (Fabbiani et al., 2011). In Africa, studies have reported extensive inter-individual heterogenicity in plasma NNRTI concentrations in HIV infected population experiencing treatment failure, majority of whom experiencing either sub or supra therapeutic drug plasma concentration (Gunda et al., 2013; Kimulwo et al., 2017). Current literature shows the relationship between NVP plasma concentrations and treatment outcomes (Baxi et al., 2015), yet therapeutic drug monitoring (TDM) quantifications is not part of the current HIV patients ART management in several low and middle income countries (LMICs) including Kenya (Kimulwo et al., 2017). Studies have shown the importance of patient's characteristics in determining the plasma NNRTI exposure. The host factors, also referred to as pharmacoecologic factors, are those that influence the individual's day-to-day concentration of drugs and they maybe dynamic over time (Pavlos & Phillips, 2012). These include factors relating to lifestyle, body weight, gender, ART adherence, drug interactions, prevailing organ complication (Phillips & Mallal, 2009; Pavlos & Phillips, 2012).

4.2.2. Methods

The methods used in this section are presented in chapter three sections 3.7.3.4 while data management and statistical analysis are presented in section 3.7.5 and 3.7.6

4.2.3 Results

4.2.3.1 The Liquid Chromatography with tandem mass spectrometry method validation

4.2.3.1.1 Selectivity

The LC-MS/MS methodologies are designed to select the ion of the precursor product of the substance or analyte being tested using multiple reaction monitoring (MRM). In this study, six (6) distinct bathes of blanks and spiked samples at LQC levels were evaluated to discriminate the endogenous plasma constituents. This study recorded insignificant hindrances at the retention times of analytes and internal standards. Chromatograms illustrating the blank plasma spiked with analytes and internal standards is represented in Figure 4.3. This study reported no substantial direct interference of both NVP and EFV in the MRM channel because they identified with a distinct peak at respective retention time both in spiked plasma samples and in patients' samples. This demonstrated that the method which was robust, faster and easy was selective to specific drug in question.

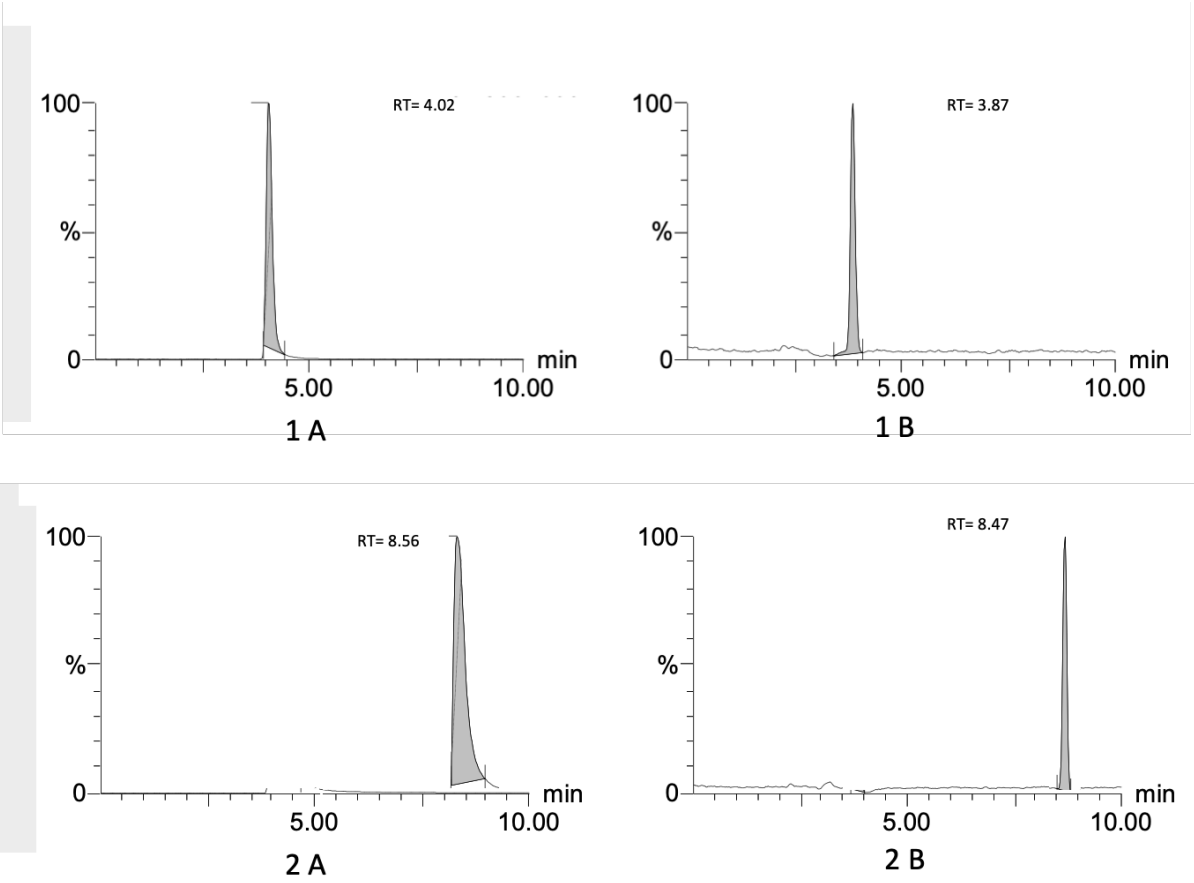


Figure 4.3. Chromatograms of Nevirapine (1A) and its internal standards (1B) and Efavirenz (2B) and its internal standards (2B).

The MS/MS response of nevirapine 1A (m/z = 267 > 226, Rt = 4.02) and its internal standard (1B) (m/z = 237 > 194, Rt = 3.87). The chromatogram of the response of efavirenz (2A) (m/z = 316 > 272, Rt = 8.56) and its internal standard (2B) (m/z = 320 > 276, Rt = 8.47).

4.2.3.1.2 Matrix effects

The matrix effects results of NVP and EFV in the five separate plasma samples at the quality control concentrations are summarized in Table 4.5. For both NVP and EFV, the low and middle quality control values were slightly <100% while the high-quality control values for the two drugs were >100% for spiked plasma samples after extraction. These ME were comparable to the concentration ranges of the QC and was not dependent on analyte

concentration. In this study, the chromatographic and extraction conditions showed weak ionization enhancement or suppression for NVP and EFV (93.1–101.3%). In the course of analytical period, this ionization did not influence the calibration curves' slopes and linearity.

4.2.3.1.3 Method recovery and Linearity

The data for absolute recovery for NVP and EFV for five replicates at low, middle and high-quality control (LQC, MQC and HQC respectively) levels are shown in Table 4.5. The absolute recovery for NVP and EFV were higher than 80%, indicating this method was suitable to analyze NVP and EFV. The calibration curves correlation coefficients (r) for NVP and EFV were >0.99 each determined using linear analysis with a $1/x^2$ regression over a concentration range of 62.5-10000µg/L for NVP and 40-6400µg/L for EFV. The results showed that the linearity range of this method is wide and suitable for analysis of samples from Kenya.

Table 4.5. The method validation outcomes: The matrix effect data, limits of detection, limits of quantitation and Absolute recovery for determination of efavirenz and nevirapine in plasma

Analytes	Method validation		
	Matrix Effect (%)		
	Plasma samples (n = 5)		
Nevirapine	Low quality control	96.7	
	Middle quality control	97	
	High quality control	101.3	
Efavirenz	Low quality control	97.3	
	Middle quality control	93.1	
	High quality control	101.2	
	Plasma samples (n = 3)		
Nevirapine	Limits of detection (µg/L)	0.2	
	Limits of quantitation (LOQ) (µg/L)	0.5	
Efavirenz	Limits of detection (µg·L-1)	1	
	Limits of quantitation (LOQ) (µg·L-1)	2	
	Plasma samples (n = 3)		
	Concentration (µg/L)	Recovery (%)	Precision (%)
Nevirapine	80	105.4	10.4
	640	99.8	8.9
	5120	101.2	8.7
	Concentration (µg·L-1)	Recovery (%)	Precision (%)
Efavirenz	80	89.7	6.6
	640	87.1	7.9
	5120	93.3	8.7

4.2.3.1.4 Method accuracy and precision

The LC-MS/MS accuracy and precision at LQC, MQC and HQC was assessed using analysis conducted within the same day and at different days using both NVP and EFV as summarized in Table 4.6. The intra-day accuracy for NVP varied from 98.5% to 103.5% and inter- day accuracy from 97.3% to 100.2% respectively. For EFV, intra-day accuracy ranged from 92.1% to 102% and inter- day 96.7% to 101.4% respectively. The NVP intra-day precision ranged from 5.9% to 9% with an inter-day variation from 7% to 8.1% respectively while intra-day precision for EFV ranged from 5.1% to 7.7% with an inter-day variation of 6.9% to 9.2% respectively. The method accuracy and precision of both NVP and EFV were within the FDA guideline.

Table 4.6. The test accuracy and precision of nevirapine and efavirenz in plasma

		Intraday			Interday		
Analytes	Concentration (µg/L)	**Mean**	**Accuracy (%)**	**Precision (%)**	**Mean**	**Accuracy (%)**	**Precision (%)**
Nevirapine	80	79.6	99.4	5.9	80.1	100.2	7.6
	640	662.8	103.5	9	658.3	102.9	7
	5120	5044	98.5	6.1	4981.3	97.3	8.1
Efavirenz	80	73.7	92.1	7.1	77.4	96.7	9.2
	640	651	102	7.7	648.7	101.4	8.4
	5120	5179	101.1	5.1	5190	101.4	6.9

4.2.3.2. The Efavirenz and Nevirapine plasma concentration

Out of the 599 enrolled patients (566; 94.5% responses rate) had all the nevirapine and efavirenz plasma levels and were subsequently evaluated in this section. The mean (SD±) nevirapine plasma concentration was 7211.2± 4407.4 ng/ml with a median (interquartile range – IQR) of 6237.5 (4518 – 8964) ng/ml. For efavirenz plasma concertation the mean (SD±) was 5633.7 ± 19361.6 ng/ml with a median (interquartile range – IQR) of 2739.5 (1878 - 4891.5)

ng/ml (Table 4.7). Steady-state plasma nevirapine and efavirenz plasma concentrations were tested for normal distribution using the Shapiro- Wilk test. Both NVP and EFV were not normally distributed and hence handled as log_{10}-transformed data (Shapiro- Wilk test = 0.81529; v = 33.970; p < 0.01) nevirapine plasma concentrations and (Shapiro- Wilk test = 0.14367; v = 188.898; p < 0.01) efavirenz plasma concentrations (Figure 4.4).

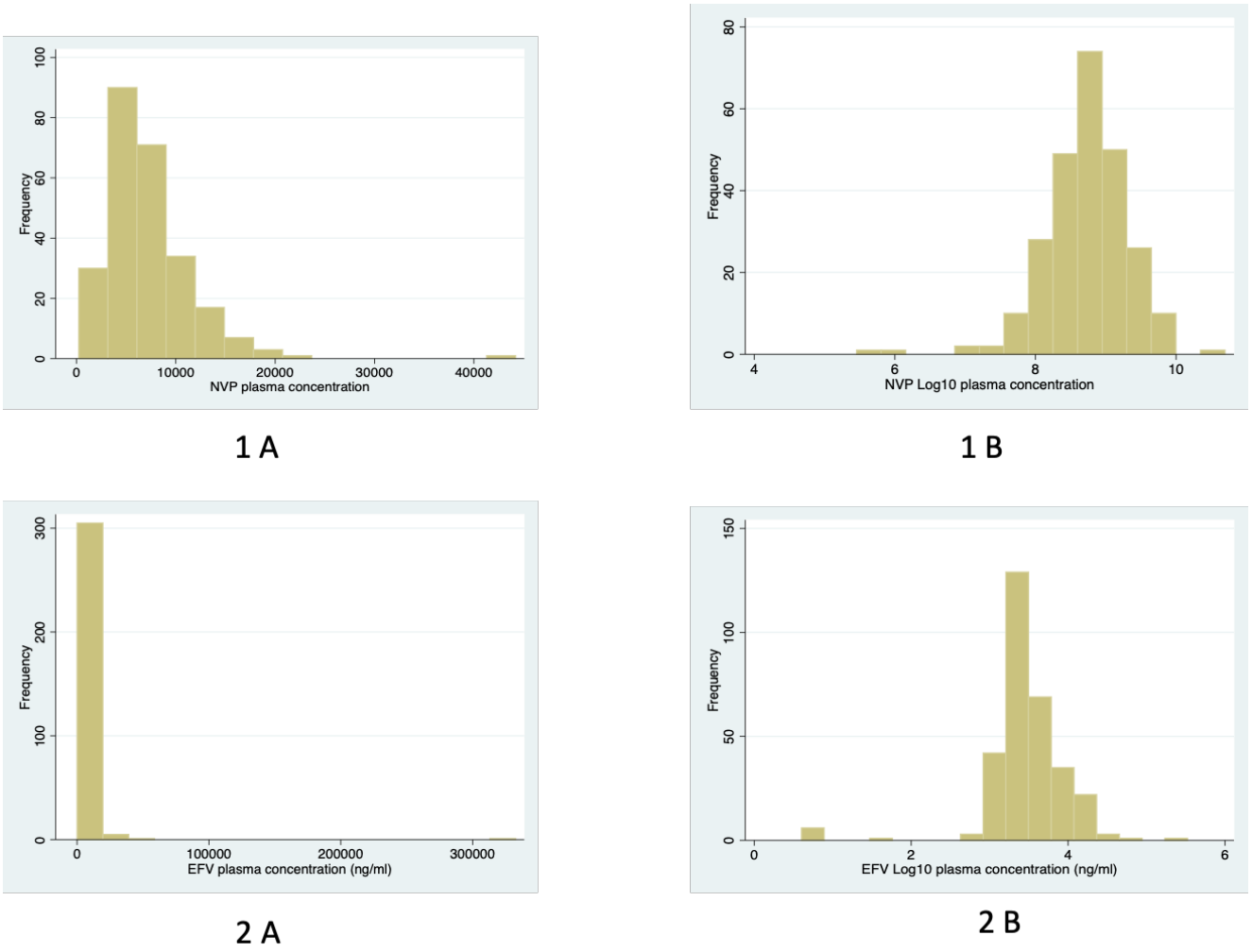


Figure 4.4: Distribution of nevirapine and efavirenz plasma concentration. 1A and 2A are normal plasma concentrations while 1B and 2B are the Log $_{10}$ transformed plasma concentrations

Among the patients on nevirapine based regimens, majority 138 (54.3%) had plasma levels of >6000 ng/ml levels considered to confer durable viral suppression. There were 80 (31.5%) patients with NVP levels between 3400 to 6000ng/ml levels considered for viral mutant

selection windows and the least 36 (14.2%) who had NVP plasma concentration of <3400 ng/ml which are levels considered for poor viral suppression (p = 0.0001).

With regards to patients receiving EFV based ART regimen, the majority 199 (63.8%) had plasma concentrations between 1000 to 4000ng/ml levels which is the window for viral mutant selection with some 14 (4.5%) who had plasma concentrations of <1000 ng/ml levels considered the poor viral suppression window (p = 0.0001). Fewer patients on NVP achieved therapeutic concentrations compared to those on EFV (31.5% vs. 63.8%) (Table 4.7)

Table 4.7: Distribution of nevirapine and efavirenz plasma concentration by therapeutic ranges

Therapeutic Level	Nevirapine plasma concentration(ng/ml)	N	%	*p*	Efavirenz plasma concentration(ng/ml)	N	%	*p*
	Mean (SD)	7223.3 (4391.2)			Mean (SD)	5633.7 (19361.6)		
	Median (IQR)	6237.5 (4518 - 8964)			Median (IQR)	2739.5 (1878 - 4891.5)		
	Range	43973(234 - 44207)			Range	332697 (4 - 332701)		
Sub-therapeutic	< 3400	36	14.2		< 1000	14	4.5	
Therapeutic	3400-6000	80	31.5	**0.0001**	1000-4000	199	63.8	**0.0001**
Supratherapeutic	>6000	138	54.3		>4000	99	31.7	
	n	**254**			**n**	**312**		

4.2.4. Discussion

The success of ART encompasses a multiple throng approaches which targets attaining optimal therapeutic plasma ART concentrations. The prolonged ART use or suboptimal ART plasma concentrations are shown to jeopardize ART management success (Veldkamp et al., 2001; Muro et al., 2005). Multiple factors generally synergize to impact the outcomes of ART treatment. Whereas therapeutic ARV plasma concentrations predict treatment outcomes (both immunological and virologic outcomes), limited HIV programs incorporate therapeutic drug monitoring or pharmacologic assessment into HIV care and treatment monitoring (Aurpibul et al., 2014). Even in patients with acceptably rates of adherence, a significant proportion of them still demonstrate sub-optimal ARV plasma concentrations that restricts interpretation and utilization of adherence data, often largely due to wide interpatient genetic variation (L'Homme et al., 2008). The established intra-interpatient therapeutic drug variation in part owing to dissimilarities in host genetics and drug metabolism, encourages the necessity for integrating TDM in HIV treatment programs to positively boost clinical management of HIV patients (Holzinger et al., 2012). The extended elimination half-life of both nevirapine and efavirenz and their low genetic barrier to drug resistance may reduce the long-term therapeutic efficacy of these ARVs (L'Homme et al., 2006; Yilmaz et al., 2012). It is imperative therefore, for HIV patients on ART treatment to attain and retain optimal plasma drug concentrations to avoid viral rebound. Against this backdrop, in this objective we determined the concentrations and patterns of NVP and EFV among patients in Nairobi.

The NVP plasma concentration ranged between 450 to 44207 ng/mL with a mean (SD±) concentration of 7211.2± 4407.4 ng/ml and a median (interquartile range – IQR) of 6237.5 [4518 – 8964] ng/ml. The majority (54.3%) of patients on NVP based regimen had plasma levels of >6000 ng/ml considered levels for durable viral suppression. There were 80 (31.5%) patients with NVP levels between 3400 to 6000ng/ml considered levels for viral mutant selection windows and the least 36 (14.2%) who had NVP plasma concertation of <3400 ng/ml considered levels for poor viral suppression and the least ($P < 0.05$). The EFV plasma concentration ranged between 4 to 332701 ng/mL with a mean (SD±) concentration of 5633.7 ± 19361.6 ng/ml with a median (IQR) of 2739.5 [1878 - 4891.5] ng/ml. The majority (63.8%) of patients on efavirenz based regimen had plasma concentrations between 1000 to 4000ng/ml

considered levels for viral mutant selection windows followed by 99(31.7%) who had >4000 ng/ml considered levels for durable viral suppression and the least 14(4.5%) had plasma concentrations of <1000 ng/ml considered levels for poor viral suppression window (P <0.05).

This study reported higher NVP plasma concentration compared to other studies both in Kenya and elsewhere. Example in 2012, Oluka (2012) reported NVP plasma level ranging between 0.64-11.8 µg/mL and 1.68-23.12 µg/mL. Further, the same author in 2015 reported a steady-state plasma NVP levels among women in the Costal Kenya portrayed by extensive inter-individual variation, extending from 1.68 µg/mL to 23.12 µg/mL (Oluka et al., 2015). In South Africa, Mazanderani *et al*., (2019) reported slightly higher NVP plasma levels among infants 6 weeks post-delivery 4020 to 11549 µg/mL. In Italy, Giacomelli *et al*., (2018) reported higher NVP plasma ranges of 1571 to 14,189 ng/mL (median = 5063 ng/mL, interquartile range = 3915–6854). In Tanzania Gunda *et al*., (2013) reported lower NVP plasma concentrations of median (IQR) 4915 [2326–7044] ng/ml. Comparable results were reported in India (Gopalan et al., 2017) who reported median (IQR) NVP plasma concentration of 8.4 (5.5-11.5) µg /ml 4 weeks into treatment. In Tanzania, Tabb *et al*., (2018) reported slightly lower NVP plasma concentration of median (IQR) of 54.9 ng/mL [41.9 – 75.3 ng/mL] compared to this study. In Liverpool, United Kingdom, Stöhr *et al*., (2008) reported higher NVP concentration of median (IQR) of 4,081 ng/mL [3,076–5,680 ng/mL]. These difference in NVP plasma levels could be due to different pharmacogenetics and pharmacoecological factors some could e unique to the Kenyan population.

With regards to EFV with a median (IQR) of 2739.5 [1878 - 4891.5] ng/ml, was higher than those reported in Liverpool, UK by Stöhr *et al*., (2008) who reported EFV concentration of media (IQR) of 1,918 ng/mL [1,334–2,896 ng/mL] as well as those reported in Tanzania, by Tabb *et al*., (2018) with a median (IQR) EFV plasma levels of 4.9 ng/mL [3.1 – 8.5 ng/mL]. further, in Tanzania Sungi *et al*., (2018) reported median [IQR] EFV level of 2.56 [IQR = 1.5–4.6] µg/mL higher than levels reported in our study. Other studies such as by Marzolini *et al*., (2001) in Swaziland reported higher EFV plasma concentration ranging from 125 to 15 230 µg /l (median 2188 µg /l). Further, Jackson *et al*., (2013) in London, UK reported EFV plasma level with a median (IQR) of 3747 [3345 to 4661] ng/ml. The variation in both NVP and EFV

plasma levels is observed in various countries and settings. These variations have been attributed to various pharmacoecological and pharmacogenetic factors.

Founded on the reference ARVs therapeutic ranges, outlined as 1000–4000 ng/ml for EFV and 3400–8000 ng/ml for NVP (Duong et al., 2004), three classes of ARV plasma drug levels may be defined: sub-therapeutic (lower than therapeutic range), therapeutic (within the therapeutic range), and supra- therapeutic (greater than therapeutic range) (Duong et al., 2004). The proportion of patients who had NVP plasma concentrations within therapeutic range was 218 (85.8%). There were 36 (14.2%) patients with NVP levels of <3100 ng/ml considered sub-therapeutic levels associated with reduced viral suppression. This finding agrees with previous studies in Kenya which showed numbers of participants with sub-therapeutic nevirapine levels are few. In a study by Shapaya (2014), 6.25% of the participants had NVP plasma levels below 3000ng/mL that is associated with viral suppression failure. Oluka (2012) reported even fewer patients with NVP levels considered poor viral suppression. In India, Gopalan *et al*., (2017) described suboptimal nevirapine trough plasma level in 65% of children sampled. In Tanzania Gunda *et al*., (2013) stated a higher (35.4%) proportion of patients with suboptimal nevirapine trough plasma levels. Nevirapines plasma levels of 3.0 µg/mL and above offers viral suppression, a concentration of 3.0-4.3 µg/mL leads in viral mutant selection. The number of patients who had NVP plasma concentrations below 4.3 µg/mL were 53 (22.1%) with 38 (15.8%) having concentrations that result in mutant selection. Nevirapine plasma concentrations greater than 4.3 µg/mL provides extensive long-term viral suppression and 187 (77.9%) patients in this study had levels exceeding this cut-off. These proportions were comparable with previous study done in Kenya (Oluka, 2012).

Even though the general efavirenz median plasma levels in this study was within the commended therapeutic range 199 (63.8%), significantly wider inter-individual EFV plasma variation was observed. There were 14 (4.5%) patients with suboptimal efavirenz plasma levels of <3100 ng/ml considered levels for poor viral suppression and 31.7% had supra therapeutic EFV plasma levels associated with toxicities. These findings are lower than 27.4%, proportion of patients with suboptimal EFV plasma levels reported in Netherlands among virologic failing patients (Gopalan et al., 2017) and in Tanzania (28.3%) (Gunda et al., 2013). The proportion

of suboptimal levels in the current study were further lower than those reported in Uganda and Italy, with a general suboptimal EFV plasma levels observed in 14.3% and 16.9% respectively (Ahoua et al., 2009; Fabbiani et al., 2011). In Swaziland Marzolini *et al*., (2001) in reported slightly larger proportion of participants 7.7% with sub-therapeutic EFV levels. Furthermore, the prevalence of sub-therapeutic EFV plasma levels reported in Columbia was higher observed in 41.8% of HIV patients with lower CD4 cell count of 50 cells/ml (Alexander *et al*., 2003). This difference in prevalence of the sub-therapeutic EFV levels might be due to the fact these studies could have been conducted among participants with immunological or virological failures.

In this study 63.8% of the HIV patients had therapeutic efavirenz plasma levels. This percentage was similar to those reported in other studies which observed between 60 to 71% of HIV patients on ART treatment had attained commended therapeutic plasma levels (Mutwa et al., 2012; Puthanakit et al., 2009; Sungi et al., 2018). A systematic analysis of previous results by Bouazza *et al*., (2017) revealed that the likelihood of patients attaining the commended therapeutic range was diverse and ranging from 56 to 60% irrespective of the EFV regimen combination. These outcomes are further confirmed by studies of Gunda *et al*., (2013) and Sungi *et al*., (2018) both of which were conducted in Tanzania.

Further, the supratherapeutic EFV plasma levels were identified in 31.7% of the study patients. The reported supratherapeutic rates were higher than that observed in Uganda in which 23.9% of the study participants had EFV plasma levels in supra-therapeutic ranges (Ahoua et al., 2009), and in Tanzania 21.7% (Gunda et al., 2013). This study observed a slightly wider variation in patients EFV plasma levels with 36.2% of them having concentrations outside the optimal therapeutic ranges between 1000 to 4000 ng/mL. Consistent to this study, wide inter-patients variation in EFV plasma levels have been observed in other settings (Fabbiani et al., 2011; Gunda et al., 2013; Sungi et al., 2018). Just like in the case of NVP plasma concentration, this variability in EFV plasma levels may be attributed to a host of pharmacoecological and pharmacogenetic factors.

4.2.4. Conclusion

There were 54.3% and 14.2% patient on NVP based regimen with supra and sub-therapeutic levels respectively. On the other hand, fewer patients, 31.7% and 4.5% on EFV, had supra and sub-therapeutic levels respectively. This study showed wide inter-patients' variation in NVP and EFV plasma levels. This study established the importance of therapeutic drug monitoring in the ARV treatment.

4.3. RELATIONSHIP BETWEEN EFAVIRENZ AND NEVIRAPINE PLASMA CONCENTRATIONS AND HOST PHARMACOECOLOGICAL FACTORS

4.3.1. Introduction

Patients factors are equally important determinants of ARV drug plasma concentrations and are also key in the individualization of ART (Parra et al., 2011). Other than pharmacogenetic factors, pharmacoecologic factors are those that impact the day-to-day concentration of drugs and may be dynamic over time equally play a significant role (Phillips & Mallal, 2009). The pharmacoecologic factors include factors relating to lifestyle and ART adherence, drug-drug interactions, pre-existing organ abnormalities including liver and kidney diseases, or during pregnancy (Pavlos & Phillips, 2012). Patients demographic factors such as age, gender (Wyen et al., 2008; Swaminathan et al., 2011), nutritional status such as body weight, growth rate (Schipani et al., 2011) have been shown to influence the treatment outcomes credited to the difference in body size and drug elimination between genders (Gopalan et al., 2017).

Despite the first case of HIV/AIDS was detected over 30 years ago in Kenya, this infection is still feared by many because of misinformation about the disease and hence the stigma and exclusion associated with the infection (Kose et al., 2012). As a result of the stigma accompanying HIV infection, the patient and their family may experience social psychological complications in the course of disease diagnosis and ART treatment, thus hindering adherence which is associated with poor treatment outcomes. Social support and disclosure are also shown significantly impact adherence to therapy and thus treatment outcomes in many settings (Kose et al., 2012).

In this objective, the study examined the influence of socio- demographic variables (such as age, sex, education level), sexual behavior, HIV stigma, disclosure, social support and nutritional status on adherence to ART and hence the steady-state plasma concentrations of nevirapine and efavirenz among the study patients. The potential predictors that were considered was classified as socio-demographic, sexual behavior, exposure to stigmatization and disclosure status. Very few studies if any have reported the effects of disclosure, stigmatization and sexual behavior on levels of antiviral drugs.

4.3.2. Methods

The methods used in this section are presented in chapter three sections 3.7.2, 3.7.2.1 and 3.7.3.4 while data management and statistical analysis are presented in section 3.7.5 and 3.7.6

4.3.3. Results

4.3.3.1. Baseline characteristics of study participants

Table 4.8 summarizes the baseline socio-demographic, sexual behavior, social psychological related factors. Out of the 599 enrolled patients (566; 94.5% responses rate) had all the nevirapine and efavirenz plasma levels and were subsequently evaluated in this section. The results came from the 254/269 (94.4%) and 312/330 (94.5%) patients on NVP and EFV respectively. Other than age ($p = 0.046$) and life time number of sexual partners ($p = 0.019$), there other baseline characteristics were not statistically different between patients on NVP and those on EFV. The study patients' median age was 41 years (IQR; 35 – 47 years), duration since first tested HIV positive median of 5 years (IQR; 1 – 11years) and duration post ART initiation median of 3 years (IQR; 1 – 8 years). Among these patients 342 (60.4%) were female, 202 (35.7%) were aged between 41-50 years, 379 (67%) were married, 367(64.8%) were Bantus, 106 (18.2%) had a previous partner who died. Only 3.5% and 5.8%; 19.7% and 17.3% (on NVP and EFV respectively) were currently smoking and taking alcohol respectively. Out of 254 and 312 patients on NVP and EFV respectively, there were 65.4% and 65.7%; 55.9% and 64.7% with age of sexual debut <18 years and had had >1 number of lifetime sexual partner respectively. Out of 254 patients on NVP and 312 on EFV, majority (74.4% and 73.3% respectively) stated the difficulties disclosing their HIV status. On the contrary, the majority (79.1% and 75.9%); (68.1% and 65.4%) (on NVP and EFV respectively) did not feel guilty or immoral for being HIV positive, respectively. Majority of the patients did not feel ashamed or worthless for being HIV positive and were very ready to tell their primary sexual partner about their HIV status.

Table 4.8. Socio-demographic, sexual behavior, HIV stigma and HIV disclosure characteristics of study population

Variable		All Patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		*p Value*
		n	(%)	n	(%)	n	(%)	
Age (years)	**Median (IQR)**	41	(35 - 47)	42	(36 - 48)	40	(34 - 47)	
	20-30	66	11.7	25	9.8	41	13.1	
	31-40	210	37.1	84	33	126	40.4	**0.046**
	41-50	202	35.7	106	41.7	96	30.8	
	>51	88	15.5	39	15.4	49	15.7	
Gender	Female	342	60.4	163	64.2	179	57.4	0.102
	Male	224	39.6	91	35.8	133	42.6	
Marrital status	Married	379	67	165	65.0	214	68.6	
	Single	154	27.2	72	28.4	82	26.3	
	Divorced	26	4.6	14	5.5	12	3.9	0.703
	Widow	7	1.2	3	1.2	4	1.3	
Occupation	Employed	193	34.1	80	31.5	113	36.2	
	Unemployed	102	18	44	17.3	58	18.9	0.354
	Self employed	271	47.9	130	51.2	141	45.2	
Ethnicity	Bantu	367	64.8	161	63.4	206	66.0	
	Nilotes	190	33.6	91	35.8	99	31.7	0.256
	Cushites	9	1.7	2	0.8	7	2.2	
Education level	Primary	174	30.7	69	27.2	105	33.4	
	Secondary	203	35.9	102	40.2	101	32.4	0.17
	Tertiary	182	32.2	81	31.9	101	32.4	
	Non-formal	7	1.2	2	0.8	5	1.6	
Previous partner died	Yes	106	18.7	46	18.1	60	19.2	0.747
	No	460	81.3	208	81.9	252	80.8	
Cigarette smoking	Yes	27	4.8	9	3.5	18	5.8	0.24
	No	539	95.2	245	96.5	294	94.3	
Alcohol consumption	Yes	104	18.4	50	19.7	54	17.3	0.099
	No	462	81.6	204	80.3	258	82.7	
Age of sexual debut (Years)	**Median (IQR)**	18	(17 - 20)	18	(17 - 19)	18	(17 - 20)	
	<18	371	65.6	166	65.4	205	65.7	0.929
	>18	195	34.5	88	34.7	107	34.3	
Lifetime sexual partners	**Median (IQR)**	2	(1-5)	2	(1 - 4)	3	(1 - 5)	
	None	3	0.5	2	0.8	1	0.3	
	1	214	37.8	110	43.3	104	33.3	**0.019**
	>1	349	61.7	142	55.9	207	66.4	
Difficult to tell others about my HIV infection	Agree	418	73.8	189	74.4	229	73.4	0.848
	Disagree	148	26.2	65	25.6	883	26.6	
Being HIV positive makes me feel guilty	Agree	189	33.4	81	31.9	108	34.6	0.531
	Disagree	377	66.6	173	68.1	204	65.4	
Being HIV positive makes me feel worthless	Agree	137	24.2	55	21.7	82	26.3	0.236
	Disagree	429	75.8	199	78.4	230	73.7	
Hide HIV status from others	Agree	403	71.2	186	73.2	217	69.5	9.352
	Disagree	163	28.8	68	26.8	95	30.5	
Disclose HIV status to anyone	Yes	539	95.2	244	96.1	295	94.6	0.435
	No	27	4.7	10	3.9	17	5.4	
Disclosed HIV status to partner or spouse	Yes	446	78.8	204	80.3	242	77.8	
	No	63	11.1	25	9.8	38	12.2	0.665
	Not applicable	57	10.1	25	9.8	32	10.3	
Disclosed HIV status to family members	Yes	349	61.7	166	65.4	183	58.7	
	No	212	37.5	87	34.4	125	40.1	0.178
	Not applicable	5	0.9	1	0.4	4	1.3	
Disclosed HIV status to the public	Yes	12	2.1	5	1.9	7	2.2	
	No	513	90.6	231	90.4	282	90.4	0.965
	Not applicable	41	7.2	18	7.1	23	7.4	

Table 4.9 summarizes the baseline characteristics of the study patients with regards to HIV social support and nutritional profile. Out of 254 patients on NVP and 312 on EFV, majority (85% and 78.2%) got as much useful advice as they would like about important things in life ($p = 0.022$). Similarly, the majority of these patients got as much as possible a chance to talk to someone about work/household problems, about personal/family problem, and people who cared about their situations and got as much love and affection. Majority of the patients also got emergency financial and transportation support but this was not statistically different by ART regimen.

The median body mass index for all patients was 24.9 (21.8 – 28.9) kg/m^2 while patients on NVP and EFV had BMI of 25.4 (22.2 – 28.4) kg/m^2 and 24.6 (21.5 – 29.2) kg/m^2 respectively. Out of 254 patients on NVP and 312 on EFV, majority 43.7% and 48.7% had a body mass index considered normal (18.5 to 24.9 kg/m^3). Further, the majority of these patients had regular access to staple food, and regularly took porridge. For the majority, barrier to food intake was not due to poor appetite, diarrhea, pain, constipation and nausea. The majority of the patients gained weight during their second clinic visit and did not show any sign of wasting.

Table 4.9. The HIV social support and nutritional status characteristics of study population

Variable		All Patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		*p Value*
		n	(%)	n	(%)	n	(%)	
Get useful advice about important things in life	As much as I wouild like	460	81.3	216	85.0	244	78.2	
	Less than I wouild like	79	13.9	33	12.9	46	14.7	
	Much less than I wouild like	11	1.9	1	0.4	10	3.2	**0.022**
	Never	16	2.8	4	1.6	12	3.9	
Get financial help during emergency	As much as I wouild like	337	59.5	162	63.8	175	56.1	
	Less than I wouild like	92	16.3	40	15.8	52	16.7	
	Much less than I wouild like	44	7.8	12	4.7	32	10.3	**0.066**
	Never	93	16.4	40	15.8	53	16.9	
Get transportation help when needed	As much as I wouild like	357	63.1	169	66.5	188	60.3	
	Less than I wouild like	81	14.3	38	14.9	43	13.8	
	Much less than I wouild like	45	7.9	15	5.9	30	9.6	0.19
	Never	83	14.7	32	12.6	51	16.4	
Get general help when sick	As much as I wouild like	456	80.6	212	83.5	244	78.2	
	Less than I wouild like	67	11.8	27	10.6	40	12.8	
	Much less than I wouild like	18	3.2	6	2.4	12	3.9	0.437
	Never	25	4.4	9	3.5	16	5.2	
BMI (Kg/m^2)	**Median (IQR)**	24.95	(21.8 - 28.9)	25.4	(22.2 - 28.4)	24.6	(21.5 - 29.2)	
	<18.5 (Underweight)	20	3.5	7	2.7	13	4.2	
	18.5 to 24.9 (Normal weigh)	263	46.5	111	43.7	152	48.7	0.325
	25 to 29.9 (Overweight)	167	29.5	84	33.1	83	26.6	
	≥30 (Obese)	116	20.5	52	20.5	64	20.5	
Regular access to staple food	Yes	494	87.3	222	87.4	272	87.2	
	No	72	12.7	32	12.6	40	12.8	0.937
Regular uptake of porridge	Yes	397	70.1	169	66.5	228	73.1	
	No	169	29.9	85	33.4	84	26.9	**0.06**
Food intake in the last 5-10 days	None	85	15	36	14.2	49	15.7	
	Poor	478	84.5	216	85.0	262	83.9	0.637
	Moderate/Adequate	3	0.5	8	0.8	1	0.4	
Barriers to food indake due to pain	Yes	4	0.7	2	0.8	2	0.6	
	No	562	99.3	252	99.2	310	99.4	0.836
Barriers to food indake due to constipation	Yes	4	0.7	3	1.2	1	0.3	
	No	562	99.3	251	98.8	311	99.7	0.331
Wasting	Mild	109	19.3	48	18.9	61	19.6	
	Moderate	71	12.5	21	8.3	50	16.1	
	Severe	2	0.4	1	0.4	1	0.3	**0.023**
	Not Applicable	384	67.8	184	72.4	200	64.1	
Feeding syndrom risk	Low	155	27.4	70	27.6	85	27.2	
	High	67	11.8	21	8.3	46	14.7	**0.052**
	Not applicable	344	60.8	163	64.2	181	58.1	

4.3.3.2. Relationship between Nevirapine and efavirenz plasma levels and ART drug adherence

Because most of these factors that influences the drug plasma level act by affecting drug adherence, the study first assessed their relationship with ART drug adherence and only those factors found significant were then evaluated against drug plasma levels. In this study, patients'

nonadherence was determined as the percentage of overdue dose at refill, averaged over a four-month period and used to assign adherence as good (< = 5% dose skipped), fair (6–15% dose skipped) or poor (> 15% dose skipped). Among all the study patients 371(n = 566; 65.6%); 164(n = 254; 64.6%) on NVP and 207(n = 312; 66.4%) on EFV reported non-adherence in the last 120 days. Adherence was generally poor across the two regimen and the prevalence of was similar across the two treatment regimens (Figure 4.5).

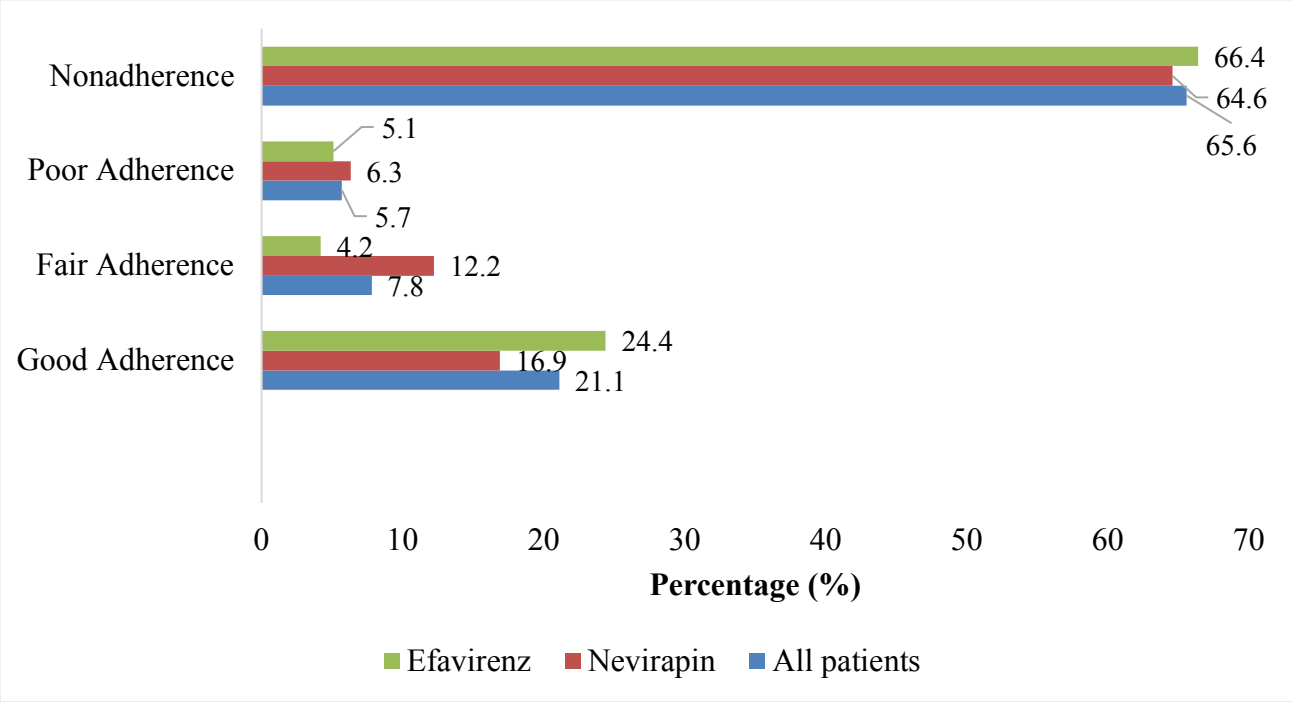


Figure 4.5: Patients ART adherences in the past 30 days by type of regimen

Table 4.10 shows the relationship between host pharmacoecological factors including demographic, sexual behavior, HIV stigma and disclosure, social support and nutritional intake and ART adherence. Only factors significantly associated with ART drug adherence were assessed for association with NVP and EFV plasma level. Among these factors included, gender, age, substance abuse, age of sexual debut, number of sexual life partners, number of sexual acts 3 months ago and the nature of sexual relationship. HIV stigma related factors assessed included; feeling guilty for being HIV positive, hiding HIV status from others and feeling confident to tell primary sexual partner about HIV status. HIV disclosure related factors

included being able to disclose HIV status to anyone and disclosing the HIV status to family members.

Table 4.10. Exploratory data analysis for the relationship between socio-demographic, sexual behavior, HIV stigma and disclosure factors and ART drug adherence

Variable	FISHER'S EXACT TEST ART drug Adherence	
	Nevirapine *P - values*	Efavirenz *P - values*
Socio-demographic		
Gender	0.318	0.253
Age	0.393	0.129
Marital status	0.06	0.368
Occupation	0.952	0.565
Religion	0.785	0.689
Education	0.611	0.124
Vacational schooling	0.482	0.209
Living with partner	0.363	0.871
Had more than one partner	0.97	0.533
Previous partner died	0.919	0.953
Smoking	0.725	0.798
Alcohol use	**0.011**	**0.011**
Sexual behaviors		
Age of sexual debut	**0.028**	**0.024**
Number of sexual life partners	**0.0001**	**0.0001**
Number of sexual acts in the past 3 months	**0.002**	**0.0001**
Number of sexual acts with a spouse in the past 3 months	**0.034**	**0.002**
Number of sexual acts with a steady partner in the past 3	**0.005**	**0.0001**
Number of sexual acts with a casual partner in the past 3 months	0.265	**0.007**
HIV stigma		
Difficult to tell others about my HIV infection	0.234	0.281
Being HIV positive makes me feel immoral	0.260	**0.005**
Being HIV positive makes me feel guilty	**0.035**	0.314
Being HIV positive makes me feel ashamed	0.570	0.794
Being HIV positive makes me feel it worthless	0.750	0.344
Being HIV positive makes me feel it is my own fault	0.111	0.318
Hide HIV status from others	**0.005**	0.605
Feel confident to tell primary sexual partner being HIV positive	**0.0001**	**0.0001**
HIV disclosure		
Disclose HIV status to anyone	0.332	**0.033**
Disclosed HIV status to partner or spouse	0.197	0.578
Disclosed HIV status to family members	0.570	0.730
Disclosed HIV status to friends	0.908	0.383
Disclosed HIV status to neighbor	0.306	0.202
Disclosed HIV status to employers	0.217	0.579
Disclosed HIV status to religious leaders	0.362	0.582
Disclosed HIV status to the public	0.748	0.331
Number disclosed about HIV status in the family	0.185	**0.055**

Table 4.11 summarizes the relationship between patient's HIV social support and nutritional intake and ART adherence. Only factors significantly associated with ART drug adherence were assessed for association with NVP and EFV plasma level. Among these factors included, gender, age, substance abuse, age of sexual debut, number of sexual life partners, number of sexual intercourse 3 months ago and the nature of sexual relationship. Among HIV social support related factors assessed included; getting useful advice about important things in life, getting chance to talk to someone about work or household problems, getting love and affection, being helped with household duties, getting financial and transportation help. Nutritional related factors included: regular access to staple food, regular uptake of porridge, access to food supplements, Food intake in the last 5-10 days, presence of wasting and feeding syndrome risk.

Table 4.11. Exploratory data analysis for the relationship between socio-demographic, sexual behavior, HIV stigma and disclosure factors and ART drug adherence

Variable	FISHER'S EXACT TEST ART drug Adherence	
	Nevirapine *P - values*	Efavirenz *P - values*
HIV social support		
Get useful advice about important things in life	**0.022**	**0.005**
Get chance to talk to someone about work or household problems	**0.005**	**0.001**
Get chance to talk to someone about personal or family	0.071	**0.002**
I have people who cares about what happens to me	0.256	**0.038**
I get love and affection	**0.0001**	**0.008**
Help with household duties	**0.007**	**0.001**
Get financial help during emergency	**0.005**	**0.045**
Get transportation help when needed	**0.001**	**0.014**
Get general help when sick	0.138	**0.009**
Nutritional related factors		
Body mass index	0.317	0.461
Regular access to staple food	**0.03**	**0.0001**
Regular uptake of porridge	0.108	**0.024**
Receiving food supplements	**0.04**	**0.0001**
Food intake in the last 5-10 days	**0.0001**	**0.0001**
Barriers to food indake due to poor appetite or anorexia	0.88	0.665
Barriers to food indake due to diarrhoea	0.458	0.476
Barriers to food indake due to pain	0.665	0.113
Barriers to food indake due to constipation	0.287	0.337
Barriers to food indake due to fever	0.354	0.337
Barriers to food indake due to nausea or vomiting	0.665	0.991
Weight gain for revisiting patients	0.922	0.128
Wasting	**0.001**	0.541
Presence for complications	0.829	0.712
Poor dietary practice or food insecurity as the cause of wasting	0.136	**0.002**
Uncontrolled barriers to food intake due to poor appetite	0.584	0.087
Feeding syndrome risk	0.223	**0.036**

4.3.3.3. Variation of NVP and EFV plasma levels across socio-demographic, sexual behavior, HIV stigma and HIV disclosure variable

Table 4.12 summarizes the variation in the median NVP and EFV plasma concentration and socio-demographic, sexual behavior, HIV stigma and disclosure characteristics. Patients with higher median (IQR) NVP plasma concentration were those who did not feel guilty by being HIV positive (6511, IQR = 4607–9863 ng/mL) as compared to patients with felt guilty for being HIV positive (5557, IQR = 4247–7633 ng/mL; $p = 0.016$). Patients with higher median (IQR) NVP plasma concentration were those disclosing their HIV sero-positivity to sexual partner or spouse (6402.5, IQR = 4564.5–9180.5 ng/mL) as compared to patients who lacked this spousal HIV status disclosure (4853, IQR = 3450–6202 ng/mL; $p = 0.036$).

No significant variation was reported among patients with regards to median (IQR) EFV plasma concentration across socio-demographic, sexual behavior, HIV stigma and HIV disclosure variable.

Table 4.12. Variation in median nevirapine and efavirenz plasma concentration and socio-demographic, sexual behavior, HIV stigma and disclosure

Variable	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
	n	Median	(IQR)		P	n	Median	(IQR)		P
Age group (Years)										
20-30	25	6034	4448	7817		41	2961	1679	4603	
31-40	84	6207	4558	8946.5	0.667	126	2698.5	1918	5976	0.476
41-50	106	6368	4599	9784		96	2685.5	1950.5	4282.5	
>51	39	6011	4518	8843		49	2754	1833	4074	
Gender										
Male	91	5917	4449	8638	0.387	133	2747	1918	5336	0.728
Female	163	6364	4558	9293		179	2712	1868	4647	
Alcohol use number of times										
Never	204	6351	4407	9194		258	2636	1857	4744	
1 time	33	6180	5128	8573	0.758	45	3093	2068	5787	0.373
≥2 times	17	5773	4935	8034		9	2747	2086	5988	
Age of sexual debut										
<18 Years	166	6402.5	4558	8964	0.306	205	2709	1857	5308	0.869
≥ 19Years	88	6042.5	4390	9274		107	2763	1960	4487	
Number of sexual life partners										
None	2	5508.5	1154	9863		1	4740	4740	4740	
1	110	6596	5128	8843	0.096	104	3082	2006.5	5513.5	0.163
≥ 1	142	5957.5	4247	9163		207	2570	1784	4713	
Being HIV positive makes me feel guilty										
Agree	81	5557	4247	7633	**0.016**	108	2645.5	1895	5171.5	0.927
Disagree	173	6511	4607	9863		204	2854	1869	4839.5	
Disclosed HIV status to anyone to partner or spouse										
Yes	204	6402.5	4564.5	9180.5		242	2759.5	1886	5204	
No	25	4853	3450	6202	**0.036**	38	2991	1918	5336	0.565
Not applicable	25	6273	4577	9909		32	2556	1750	3488	
Disclosed HIV status to anyone to neighbor										
Yes	13	5239	3631	7009		22	3079	1917	7572	
No	234	6237.5	4558	9095	0.210	280	2739.5	1902	4837	0.088
Not applicable	7	7966	6372	9909		10	2027.5	857	2961	

4.3.3.4. Quantile regression analysis of nevirapine and efavirenz plasma concentration and socio-demographic, sexual behavior, HIV stigma and disclosure

Table 4.13 is a summary of quantile regression analyses estimating the relationships between NVP and EFV plasma levels and socio-demographic, sexual behavior, HIV stigma and disclosure characteristics

On bivariate analysis the factors associated with NVP plasma concentrations included feeling guilty for being HIV positive (unadjusted β -954, 95% confidence interval (CI) 26.7 to 1881.3; p =0.044) or feeling worthless for being HIV positive (unadjusted β 1268, 95% CI 2156.6 to 2156.6; p =0.005) and disclosing patient's HIV status to neighbors (unadjusted β 1675, 95% CI 137.5 to 3212.5; p =0.033).

On multivariate quantile regression analysis factors independently associated with plasma concentrations of NVP were: having vocational training (adjusted β -1029, 95% CI, -2068.4 to -10.4; p = 0.042); feeling guilty for being HIV positive (adjusted β 954, 95% CI 192.7 to 2156.6; p =0.014) or feeling worthless for being HIV positive (adjusted β 852, 95% CI 64.3 to 1639.7; p =0.034) and disclosing patient's HIV status to neighbors (adjusted β 1731, 95% CI 376 to 3086; p =0.012).

On bivariate regression analysis being confident about telling the primary sexual partner about HIV positive status (unadjusted β -426, 95% CI -24.3 to -827.7; p =0.038) was negatively associated with EFV plasma levels. On multivariate quantile regression analysis factors that were independently associated with EFV plasma concentrations was disclosing HIV positive status (adjusted β 363, 95% CI, 97.9 to 628.1; p = 0.007).

Table 4.13. Socio-demographic, sexual behavior, HIV stigma and disclosure factor associated with nevirapine and efavirenz plasma concentrations

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β	(95% CI)		P-value	Unadjusted β	(95% CI)		P-value
Age	-14	-56.2	28.2	0.307	-13.7	-38.7	11.4	0.284
Gender	447	-545.5	1439.5	0.376	-35	-536.5	466.5	0.891
Alcohol use number of times	-198	-680.7	284.7	0.42	330	-534.4	1194.4	0.453
Age of sexual debut	-364	-1385.8	657.8	0.484	54	-459.7	567.7	0.836
Number of sexual life partners	-600	-1285.7	85.7	0.086	-557	-918	-196	**0.003**
Number of sexual acts in the past 3 months	-46.5	-748.9	655.9	0.896	-106	-648.9	436.9	0.701
Difficult to tell others about my HIV infection	141	-958.8	1240.8	0.801	-126	-703.9	451.9	0.668
Being HIV positive makes me feel guilty	954	26.7	1881.3	**0.044**	210	-281.3	701.3	0.401
Being HIV positive makes me feel it worthless	1268	379.4	2156.6	**0.005**	-33	-744.7	678.7	0.927
Feel certain to tell primary sexual partner being HIV positive	372	-453.2	1197.2	0.376	426	24.3	827.7	**0.038**
Disclose HIV status to anyone	-539	-1578.8	500.8	0.308	983	-1058.04	3024.04	0.344
Disclosed HIV status to anyone to family members	1051.5	-541.5	2644.5	0.195	134	-381.761	649.761	0.61
Disclosed HIV status to anyone to neighbor	1675	137.5	3212.5	**0.033**	-445	-1441.05	551.047	0.38
Disclosed HIV status to anyone to employers	-112	-1203.3	979.3	0.84	-489	-1037.24	59.2426	0.08
Disclosed HIV status to anyone to religious leaders	1609	-98.7	3316.7	0.065	-410	-907.916	87.9159	0.106

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β	(95% CI)		P-value	Adjusted β	(95% CI)		P-value
Age	0.4	-71.7	72.5	0.991	-15.5	-52.5	21.6	0.412
Gender	172	-1010.5	1354.5	0.775	-40.4	-832.7	751.9	0.92
Alcohol use number of times	-162.5	-810.953	485.953	0.622	398	-431.2	1227.2	0.346
Age of sexual debut	-1008.1	-2745.4	729.1	0.254	563.5	-424.6	1551.6	0.263
Number of sexual life partners	-988	-2156.8	180.8	0.097	-845.7	-1315	-376.4	**0.0001**
Number of sexual acts in the past 3 months	-2180.8	-5358.2	996.6	0.178	487.3	-3224.2	4198.8	0.796
Difficult to tell others about my HIV infection	-528.5	-1633.9	576.9	0.347	-177	-1021.3	667.3	0.68
Being HIV positve makes me feel guilty	954	192.7	1715.3	**0.014**	347.7	-153.4	848.7	0.173
Being HIV positve makes me feel it worthless	852	64.3	1639.7	**0.034**	-143.3	-759.2	472.5	0.647
Feel certain to tell primary sexual partner being HIV positve	341.5	-1357	2040	0.692	363	97.9	628.1	**0.007**
Disclose HIV status to anyone	-1042.9	-2597.4	511.6	0.188	1342	1653.6	4337.6	0.379
Disclosed HIV status to anyone to family members	812.9	-483.3	2109.1	0.218	245	-365.8	855.8	0.431
Disclosed HIV status to anyone to neighbor	1731	376	3086	**0.012**	-251	-1714.1	1212.1	0.736
Disclosed HIV status to anyone to employers	-393.5	-1586.1	799.1	0.516	-505	-1410.3	400.3	0.273
Disclosed HIV status to anyone to religious leaders	241.6	-1675.6	2158.7	0.804	29	-1120.3	1178.3	0.96

4.3.3.5. Variation of nevirapine and efavirenz plasma levels and HIV social support and nutritional variables

Table 4.14 summarizes the variation in the median NVP and EFV plasma concentration and HIV social support related factors. Patients who did not consumed porridge regularly had higher median (IQR) NVP plasma concentration (7188.5, IQR = 5448–10452.5 ng/mL) compared to patients who did consume porridge on a regular basis (5917, IQR = 4336–8323 ng/mL; $p = 0.0031$). The variation between other HIV social support and nutritional variables across both NVP and EFV were not significant.

Table 4.14. Variation in median nevirapine and efavirenz plasma concentration and HIV social support and nutritional characteristics

Variable	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
	n	Median	(IQR)		*P*	n	Median	(IQR)		*P*
Get financial help during emergency										
As much as I wouild like	162	6365.5	4558	8964		175	2836	1918	4911	
Less than I wouild like	40	5468.5	4275	8191.5	0.492	52	2309.5	1789.5	5038	0.797
Much less than I wouild like	12	7275.5	6056.5	9583		32	2747.5	1615.5	8797.5	
Never	40	5710.5	4046	9867.5		53	2872	2043	4241	
Get transportation help when needed										
As much as I wouild like	169	6538	4571	9198		188	2821.5	1895	5223.5	
Less than I wouild like	38	5527.5	4336	8382	0.550	43	2462	1818	4872	0.917
Much less than I wouild like	15	6202	4180	6868		30	2670	1679	6875	
Never	32	5635.5	3955.5	8750		51	2786	1942	3875	
Get general help when sick										
As much as I wouild like	212	6351	4448.5	9129		244	2796.5	1895	4977.5	
Less than I wouild like	27	5911	4990	9411	0.970	40	2569	1999.5	5589.5	0.534
Much less than I wouild like	6	7039	5729	8405		12	2447.5	911	4693	
Never	9	5692	5457	7009		16	2931.5	1613.5	4168	
BMI (Kg/m^2)										
<18.5 (Underweight)	7	5439	4211	8001		13	3095	2462	4867	
18.5 to 24.9 (Normal weigh)	111	6291	4336	8889	0.729	152	2949.5	1933	5171.5	0.077
25 to 29.9 (Overweight)	84	6308	4524	10138		83	2094	1760	3867	
≥30 (Obese)	52	6251.5	5006	8836.5		64	3134	1996.5	6696	
Regular access to staple food										
Yes	222	6126.5	4503	8889	0.533	272	2625	1862	4680	0.091
No	32	6850	5348	9479		40	3349	2182.5	6033	
Regular uptake of porridge										
Yes	169	5917	4336	8323		228	2924	1962.5	5171.5	
No	80	7188.5	5448	10452.5	**0.003**	83	2244	1604	4074	0.125
Not applicable	5	2650	2630	6809		1	2276	2276	2276	
Barriers to food indake due to pain										
Yes	2	3149	2746	3552	0.056	2	2057	1838	2276	0.362
No	252	6282	4545	9029.5		310	2750.5	1886	4911	
Barriers to food indake due to constipation										
Yes	3	12278	7238	13164	0.055	1	2872	2872	2872	0.916
No	251	6180	4503	8889		311	2732	1870	4911	
Not applicable										
Wasting										
Mild	48	6322.5	3886	8783		61	2992	2127	4647	
Moderate	21	6698	6011	8875	0.688	50	2615.5	1777	5336	0.288
Severe	1	9909	9909	9909		1	1368	1368	1368	
Not Applicable	184	6109.5	4525	9180.5		200	2720.5	1835.5	4977.5	
Feeding syndrom risk										
Low	70	5765	3794	8323		85	2836	1960	4050	
High	21	6738	6051	9935	0.146	46	2755	1639	6116	0.890
Not applicable	163	6202	4558	9293		181	2610	1857	5139	

4.3.3.6. Quantile regression analysis of nevirapine and efavirenz plasma concentration and HIV social support factors

Table 4.15 describes linear regression analysis estimating the relationships between NVP and EFV plasma levels and HIV social support related factors. In bivariable linear regression analysis factors associated with NVP plasma concentrations included; access to transportation to hospital whenever needed (unadjusted β -300, 95% CI -599.7 to -87.9; p =0.006), barrier to food intake due to pain (unadjusted β 3545, 95% CI 2567.7 to 4522.3; $p < 0.001$), due to fever (unadjusted β -6098, 95% CI -8717.8 to -3478.2; $p < 0.001$), nausea or vomiting (unadjusted β 3527, 95% CI 3004.9 to 4049.1; $p < 0.001$), diarrhea (unadjusted β -2566, 95% CI -3036.9 to -2095.1; $p < 0.001$).

On multivariate analysis factors that independently associated with NVP plasma concentration were; getting transportation to hospital whenever needed (adjusted β *-1143.3,* 95% CI -1914.3 to -372.4; p =0.004), regular uptake of porridge (adjusted β 1780, 95% CI 121.4 to 3438.6; p =0.036). Others included barrier to food intake due to pain (adjusted β 2261, 95% CI 919.2 to 3602.8; p =0.001), constipation (adjusted β -6042, 95% CI -7175.2 to -4908.8; $p < 0.001$), diarrhea (adjusted β -2092, 95% CI-3787.4 to -396.6; p =0.016) and pain (unadjusted β 2261, 95% CI 1244.4 to 3277.6; $p < 0.001$).

In bivariable linear regression analysis the most import determinant of EFV plasma concentrations included: food intake in the last 5-10 days (unadjusted β -592, 95% CI -1119.6 to -64.4; p =0.028), barrier to food intake due to diarrhea (unadjusted β -787, 95% CI -1120.7 to -453.3; $p < 0.001$) and fever (unadjusted β -2012, 95% CI -2377.2 to -1646.8; p = 0.0001). In multivariate quartile regression analysis food intake in the last 5-10 days (adjusted β -629, 95% CI -1135 to -123; p =0.015), barrier to food intake due to fever (adjusted β -2405, 95% CI-2676.5 to -2133.5; $p < 0.001$), presence of complication (adjusted β -822.5, 95% CI -1641.4 to -3.6; p =0.049) and poor dietary practice or food insecurity as the cause of wasting (adjusted β -1042, 95% CI -1910.2 to -173.8; p =0.019) remained significantly associated with EFV plasma concentration.

Table 4.15. Social support and nutritional related factors associated with nevirapine and efavirenz plasma concentrations

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β	(95% CI)		*P-value*	Unadjusted β	(95% CI)		*P-value*
Get useful advice about importat things in life	-539	-1778.4	1303.7	0.762	-134.3	-483.8	215.1	0.45
Get financial help during emergency	-124.7	-541.9	292.6	0.557	19	-189.8	227.1	0.86
Get transportation help when needed	-300	-512.1	-87.9	**0.006**	-5	-177.6	167.6	0.955
Get general help when sick	-217	-599.7	165.7	0.265	-158	-562.3	246.3	0.442
Regular access to staple food	729	-590.1	2048.1	0.277	723	-746.8	2192.8	0.334
Food intake in the last 5-10 days	-331	-1392	730	0.54	-592	-1119.6	-64.4	**0.028**
Barriers to food indake due to diarrhoea	367	-112.1	846.1	0.133	-787	-1120.7	-453.3	**0.0001**
Barriers to food indake due to pain	3545	2567.7	4522.3	**0.0001**	478	-33.3	989.3	**0.067**
Barriers to food indake due to constipation	-6098	-8717.8	-3478.2	**0.0001**	-14	-471.6	191.6	0.407
Barriers to food indake due to fever	3527	3004.9	4049.1	**0.0001**	-2012	-2377.2	-1646.8	**0.0001**
Presence for complications	2037	-228.2	4302.2	0.078	-135	-1199.1	929.1	0.803
Uncontrolled barriers to food intake due to diarrhoea	-2566	-3036.9	-2095.1	**0.0001**		Omitted		
Uncontrolled barriers to food intake due to pain	2721	2282.6	3159.4	**0.0001**		Omitted		
Uncontrolled barriers to food intake due to	-6962	-12969	-955	**0.023**		Omitted		
Uncontrolled barriers to food intake due to fever	6273	5845.2	6700.8	**0.0001**		Omitted		
Feeding syndrom risk	75	-545.2	695.2	0.812	-89	-535.4	357.4	0.695

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β	(95% CI)		*P-value*	Adjusted β	(95% CI)		*P-value*
Get useful advice about importat things in life	-112.7	-1430	1204.6	0.866	16.4	-400.5	433.4	0.938
Help with household duties	-315.2	-1460	829.6	0.588	-226.4	-556.1	103.4	0.178
Get financial help during emergency	779.3	-291.9	1850.6	0.153	245	-304.7	794.7	0.381
Get transportation help when needed	-1143.3	-1914.3	-372.4	**0.004**	-6.6	-377.8	364.7	0.972
Get general help when sick	212.3	-560.5	985.1	0.589	74.1	-478.3	626.5	0.792
Regular access to staple food	1030	-540.7	2600.7	0.198	979	-115.9	2073.9	0.08
Regular uptake of porridge	1780	121.4	3438.6	**0.036**	-138	-775.1	499.1	0.67
Barriers to food indake due to poor appetite or anorexia	673	-2712.6	4058.6	0.696	-629	-1135	-123	**0.015**
Barriers to food indake due to diarrhoea	233	-381.8	847.8	0.456	-1421	-2857	15	0.052
Barriers to food indake due to pain	2261	919.2	3602.8	**0.001**	700	-674.1	2074.1	0.317
Barriers to food indake due to constipation	-6042	-7175.2	-4908.8	**0.0001**	-104	-1092.9	884.9	0.836
Barriers to food indake due to fever	-361	-5710.1	4988.1	0.894	-2405	-2676.5	-2133.5	**0.0001**
Presence for complications	2171	-816.1	5158.1	0.154	-822.5	-1641.4	-3.6	**0.049**
Poor dietary practice or food insecurity as the cause of wasting	-729	-2063.8	605.8	0.283	-1042	-1910.2	-173.8	**0.019**
Uncontrolled barriers to food intake due to diarrhoea	-2092	-3787.4	-396.6	**0.016**		Omitted		
Uncontrolled barriers to food intake due to pain	2261	1244.4	3277.6	**0.0001**		Omitted		
Feeding syndrom risk	163	-587.3	913.3	0.669	-124	-529.756	281.756	0.548

4.3.4. Discussion

This study is probably the first to explore how nutritional and social factors impacts on NVP and EFV drug levels. In this study, vocational training was associated with a lower NVP plasma level. The importance of education in determining treatment outcomes among HIV patients varies. Burch *et al*., (2016) showed the non-university education, a measure of poorer socioeconomic rank, strongly predicted ART non-adherence and virologic failure. The machinery employed by educational level to impact HIV treatment outcomes is multifaceted and intermediated by aspects related to patients HIV status disclosure, admittance and retention into care and treatment, and treatment adherence (Cohen et al., 2014). Education levels affects health knowledge and impact the health seeking behavior vital in attaining optimum treatment results (Kagee et al., 2011). A broad literature exploration divulged a scantiness of information or data on the association between patients' education levels and HIV treatment outcomes including NNRTI plasma concentrations. Some studies showed an association while others drawing the opposite conclusion (Belo et al., 2011; Cohen et al., 2014).

In this study there was no association between age, gender and efavirenz and nevirapine plasma concentration. Although not significant, the patients advanced in age had greater median NVP and EFV plasma level than young adults. This is consistent with other studies which have indicated that nevirapine metabolism in younger patients is generally faster compared to older patients, with younger patients requiring higher nevirapine doses to attain therapeutic concentrations (Swaminathan et al., 2011; Gopalan et al., 2017). Further, female gender had slightly increased median EFV and NVP concentrations compared to males. These results are comparable to those of Shapaya (2014) done in Kenya as well as by Shiau *et al*., (2014) done in South Africa. These two studies showed women had increased median NVP plasma concentrations than men. This variance in ARV plasma concentrations by gender is credited to the difference in body size and drug elimination between genders.

This study found a negative association between the number of sexual life partners and age of sexual debut with both NVP and EFV plasma levels. In the US, Goldsamt *et al*., (2011) showed an association between an increased frequency of resistance to ARVs and high risk sexual behavior. The study further related the HIV transmission between sexual partners through the

high-risk sexual behaviors. The presence of HIV drug resistance has been demonstrated to affect the NNRTI plasma levels (Vardhanabhuti et al., 2013). Study have also shown the increased risk of sexual transmission of multi-drug-resistant HIV, rates of which is dependent on risky sexual behavior (van Kesteren et al., 2007). The frequency of HIV drug-resistant infection in recently diagnosed and/or treatment-naïve HIV infected population range between 5% to 17% (Johnson et al., 2008). Increased occurrence of unprotected anal sex and more numbers of parallel sexual partners has been linked to infection with more frequency of HIV drug resistance (Gorbach et al., 2008). Further, the risk of HIV superinfection; infection with a second HIV strain is higher when HIV patients engages in unprotected sexual activities (Piantadosi et al., 2008). HIV superinfection apart from demonstrating a failure of the early immune reaction to guard against succeeding infection, has implication in HIV management and vaccine development.

HIV self-stigma related factors such as feeling guilty and feeling worthless for being HIV positive were associated with higher median NVP plasma levels. This relationship was probably mediated by ART adherence. For patients on EFV based regimens, those who were confident to tell their primary sexual partner about their HIV status had higher median EFV plasma levels. Stigma and discrimination continue to be the greatest challenge faced by People Living with HIV/AIDS (StigmaIndex, 2013). HIV-related stigma described as discrimination against HIV infected individuals (Goffman, 2009). Stigma negatively impact HIV infected individuals (Chambers et al., 2015). HIV-related stigma is worldwide societal occurrence that exhibits within diverse social spheres, such as in healthcare systems comprising treatment withdrawal and denial, mandatory HIV testing, infringements of confidentiality and inhuman and demeaning treatment by healthcare professionals (Sears, 2008). Studies have shown impact HIV related stigma impacting patients physical and mental health wellbeing (Rueda et al., 2016). Stigma related to HIV secondarily also influence healthcare seeking behavior, utility of health and social services and ART adherence (Rao et al., 2012; Rueda et al., 2016). Inevitably, these negative outcomes of stigma are bound to affect the overall treatment outcomes in terms of therapeutic monitoring. This is an area that needs more attention especially in longitudinal studies.

HIV status disclosure in our study was associated with better ART adherence with a concomitant higher median NVP and EFV plasma concentration regardless whether the disclosure was to a spouse, family member, religious leaders and employers. In Thailand, Sirikum *et al*., (2014) found no difference in adherence to ART and immunological and virologic response between HIV patient who disclosed their status and those who had not. Studies have shown HIV disclosure to have two possible treatment outcomes (Medley et al., 2004). Disclosing HIV infection to husbands or spouses is a fundamental objective in preventing HIV transmission stressed by CDC and WHO's guidelines for HIV testing and counselling (Johns et al., 2016). Some of the key advantages of HIV status disclosure on an individual and common public includes lessening of worry and increases social support (Medley et al., 2004; Johns et al., 2016). Further, , HIV status disclosure improves admittance and inclusivity into HIV prevention and treatment programs, access to various risk cutting programs and improved access to avenues to help infected patients plan their future. Further, status disclosure increases the understanding of HIV risk to un-dragonized partners, leading to better acceptance of HIV counselling and testing and informed risky behavior change (Medley et al., 2004; Johns et al., 2016). Data shows that dramatic changes in risky sexual behaviors occurs among partners who are aware of their HIV serostatus (Medley et al., 2004; Johns et al., 2016). Among couples, HIV disclosure also equip them to decide form knowledgeable point of view on reproductive health choices (Medley et al., 2004).

Inevitably, HIV status disclosure is marked with significant potential risks especially among HIV-infected female such as economic hardships, blame, rejection, bodily and emotional abuse, discrimination and interference of family relations (Medley et al., 2004; Johns et al., 2016). These risks often hinder HIV status disclosure especially with close and extended family circles. Consequently, prevention of any new infections are thwarted with reduced opportunities for women to gain admittance to prompt and proper care, treatment and supportive services widely available (Medley et al., 2004; Johns et al., 2016). Data are unavailable directly linking disclosure to NNRTI levels and other ARV plasma levels and longitudinal studies are suggested to probe this relationship.

Tied to disclosure, our study showed that one of the benefits of disclosure is the availability of social support (Medley et al., 2004; Johns et al., 2016). In South Africa, Brittain *et al*., (2017) showed an interrelationship between social support and stigma which were associated with symptoms of depression. Elevated depression scores which were prevalent in many developing nations are risk factors for poor treatment outcomes (Brittain et al., 2017). In our study, patients who got social support such as getting useful advice about important things in life, having a chance to talk to someone about work, household, personal or family problems, accessing people who cares for them, getting love and affection as much as they needed, had higher median NVP and EFV plasma concentration than patients who never had such social support. Accruing data demonstrates the far reaching benefits of community support networks in enriching societal relationships that demystify stigma associated with HIV (Campbell et al., 2007; Zachariah et al., 2007).

A study in Kenya revealed that membership in community support networks expressively enhanced ART adherence and clinical treatment results (Ochieng et al., 2015). Another study in Kenya showed that patients actively involved in community support networks inclined towards attaining optimum NVP plasma concentrations within the first three to four hours post-dosing, with higher NVP plasma levels compared to patients not linked to any community support networks. Countless studies provide data associating improved adherence to ARV medication and better clinical outcomes to social support (Gonzalez et al., 2004). Available data demonstrates the positive impact of social support and protection on some HIV induced outcomes including reduced risky sexual behaviors (Handa et al., 2014; Cluver et al., 2016), mental well-being and family relations (Bhana et al., 2014; Kilburn et al., 2016). The available proof associating social protection with reduced HIV-risk (Pettifor et al., 2016) is reflected by numerous policy guidelines generated by international health organizations such as WHO, PEPFAR-USAID, UNAIDS and UNICEF focusing on preventing HIV transmission among pediatric and adolescent and young adults (UNAIDS, 2014; PEPFAR, 2015). Data are skewed directly linking social support for HIV patients to NNRTI and other ARV plasma levels and longitudinal studies are suggested to probe this relationship.

In the multivariate analysis this study showed that availability and adequate nutritional uptake was associated with higher NVP and EFV plasma concentration. On the contrary, any barrier to nutritional uptake was associated with lower NVP and EFV plasma concentration. Further, patients who reported wasting due to poor dietary practice or food insecurity had also lower NVP and EFV plasma concentration. Consistent to other studies, in Kenya, body size was significantly associated with NVP pharmacokinetic parameters (Vreeman et al., 2014). In Uganda, Bartelink *et al*., (2015) showed the interdependent between nutritional profile and lowered EFV bioavailability with an increased NVP bioavailability. Among women, an earlier study by Bartelink *et al*., (2013) in Uganda showed a consistent trend with food insecurity lowering EFV plasma levels and increasing NVP plasma bioavailability (Bartelink et al., 2013). Contrary reports are also available; malnourished pediatric PK-studies do not evidently show a relationship between malnutrition and ARV plasma levels. Further, in Uganda children taking EFV based regimen, no significant association between height-for-age Z-score or weight-for-age Z-score and EFV plasma concentration was observed (Fillekes et al., 2011). In Malawi, Pollock *et al,* (2009) in multivariate analysis observed no significant relationship between malnutrition and NVP plasma levels. In Zambia, Ellis *et al.,* (2007) on the other hand observed decreased NVP plasma levels among stunted children, but improved exposure among children who were malnourished or wasted.

One of the suggested mechanism by which nutritional profile affects ARV plasma concentration is that, malnutrition is thought to lower albumin or α-acid glycoprotein levels, hence increasing the free portion of highly protein bound drugs including EFV allowing greater drug metabolism and elimination (Bartelink et al., 2015). Malnutrition is also known to lower autoinduction of NVP metabolism explaining the increased NVP plasma levels (Boullata, 2010; Raiten, 2011). The diet deficient of fats or the general nutritional deficiency has been shown to affect majorly the protease inhibitor's absorption and cellular transportation (Griffin et al., 2011; Lamorde et al., 2012). Our study further showed though not significant, obesity was related to slightly higher NVP and EFV plasma concentration compared to patients who were underweight. Data relate good nutritional status supporting immunity and physical functioning. Losing weight due to poor diet enhanced by (loos of appetite, ulcer of digestive track, food insecurity), deformed-absorption, and distorted metabolism, occurs commonly

among patients infected with HIV. Weight gain more so muscle mass among HIV patents, demands for the uptake of ART, management of all opportunistic diseases, intake of balanced diet, physical exercise and prevention of adverse effects. Correcting nutritional status among HIV patients requires significantly more effort in the course of HIV/AIDS disease progresses (De Pee & Semba, 2010). The pathways and mechanisms nourishment affect the metabolisms of ARV is a promising future and could contribute to personalization of ART treatment considering other factors such as age groupings, gender and ethnic diversity.

4.3.4. Conclusions

Demographic characteristics such as age, gender, education levels are important specific pharmacoecological variables influencing NNRTI plasma levels by affection adherence to ART. Sexual behavior such as sexual life partners, age of sexual debut influences ART adherence which in turn is associated to NNRTI plasma levels. The risky sexual practices are key to infection and transmission of HIV drug resistance virus which has been demonstrated to affect the NNRTI plasma levels. HIV stigma, disclosure, social support which are interlinked with each other influences medication adherence and better clinical outcome**.** Nutritional status is important determinant affecting NNRTI exposures.

4.4. ASSOCIATION BETWEEN EFAVIRENZ AND NEVIRAPINE PLASMA CONCENTRATIONS AND ART DRUG ADHERENCE AND ACCESSTO THERAPY

4.4.1. Introduction

Realizing constructive HIV treatment outcomes is a major bottle-neck, predominantly due to poor ART adherence and occurrence of drug resistance mutations (Pefura-Yone et al., 2013). Set by WHO, periodic quantification of viral load has been the approach adopted to monitor treatment outcomes in HIV patients receiving ART treatment (WHO, 2016). The WHO recommendations states that a patient is categorized as experiencing virologic failure if maintain a viral load > 1000 copies/ml after 6 and 12 months on ART treatment (WHO, 2016). The persistent high viral load is majorly attributed to lack of ART adherence (WHO, 2016). Several reports from developing countries associates highly poor ART adherence to virologic failure rather than with the immunological and clinical outcomes (Chendi et al., 2019). In a larger Kenyan HIV population, Ochieng *et al*., (2015) showed that 35% of patients on NVP, were failing treatment mainly due to poor adherence. In Cameroon, Chendi et al., (2019) associated virologic failure to non- adherence, interruption of the long term ART management and through loss to follow-up and inconsistent utilization of healthcare services. In other African and Asian populations, studies have reported highly heterogenous virologic failure rates across different population sites; an observation that may be associated with variable treatment and adherence behavior (Aghokeng et al., 2014). Additionally, in developing countries poor prescribing practices, ART stock outs, delayed refill pickup and poor healthcare access are factors that significantly affect adherence and treatment outcomes (Bennett et al., 2008; Kimulwo et al., 2017). The general rule regarding ART treatment is achieving ideal drug concentration; consistent long term uptake, or subtherapeutic exposure to, ART, particularly NVP and EFV (Veldkamp et al., 2001; Muro et al., 2005).

In spite of the knowledge that adherence is an important element in ART treatment impacting treatment outcomes, there are no normalized procedures for its quantification (Nachega et al., 2014; Fonsah et al., 2017). Patients' self-reporting is commonly used method to measure adherence because its cheaper and easy to implement. However, this method is exceedingly biased and has been shown to overestimate adherence rates when compared to objective

methods (Vreeman et al., 2014). Total pill numbers, pharmacy top-ups, and electronic monitoring systems are more objective measures and have shown to predict strongly the virologic outcomes. These methods are however expensive and are unable to reveal actual number of medicine consumed (Kagee & Nel, 2012). Measurements of drugs levels in plasma or urine are more consistent quantifications of drug adherence; nevertheless, the method is restricted because it's able to quantify a narrow window (one to two days) of drug adherence apart from being very costly (Yan et al., 2016). Quantification of HIV- load may offer a slightly cheaper and greater insight into adherence (Tabb et al., 2018).

The WHO worldwide 90-90-90 target (90% of individuals with HIV diagnosed, 90% of tested initiated into ART treatment, and 90% of treated attain virologic suppression) is greatly hampered by the high rates of virologic failure and occurrence of HIV drug resistant mutations due to partly poor ART adherence (WHO, 2017). Identifying the optimal ART adherence rates needed to attain optimal virologic levels is a concern of many researchers (Tabb et al., 2018). In principle, rates of adherence of $\geqslant$95% are often cited as required for optimum treatment outcomes for antiretroviral medications (Gulick et al., 2006). A study by Maggiolo *et al*., (2005), showed patients on NNRTI-based treatment achieved higher adherence rates compared to those receiving PI-based regimens (94% vs. 90%). Further, lower adherence (>75%) to NNRTI-based regimen was associated with sustained virologic suppression compared to patients on PI-based regimen who required at least 85% adherence rate to sustain virologic suppression at equal time interval. The Maggiolo *et al*., (2005), study established that NNRTI-based regimens were "more forgiving" compared to PI-based regimens. In this objective, the examined the association between healthcare access and ART adherence and nevirapine and efavirenz plasma concentrations among the study patients.

4.4.2. Methods

The methods used in this section are presented in chapter three sections 3.7.2, 3.7.2.1 and 3.7.3.4 while data management and statistical analysis are presented in section 3.7.5 and 3.7.6

4.4.3. Results

4.4.3.1. Comparison of healthcare access, utilization and adherence of patients on nevirapine and efavirenz

Table 4.16 summarizes the measures of access and utilization of healthcare and adherence that were evaluated. Out of the 599 enrolled patients (566; 94.5% responses rate) had all the nevirapine and efavirenz plasma levels and were subsequently evaluated in this section. The median (IQR) time taken to ART clinic was 80(IQR 60-120) minutes for all the patients; 90(IQR 60-120) minutes for patients on NVP and was shorter for patients on EFV (75 (IQR 60-120) minutes ($p = 0.0001$). The majority of the patients had not been admitted in the hospital for HIV related complications. However, patients on NVP were more likely to have been admitted compared to those on EFV ($p = 0.083$). There were 29.2% of the patients who had visited a clinic in the past 3 months due to an acute illness. There were no significant differences in clinic visits among patients on NVP and EFV. The majority of patients (285 out of 566, 50.4%) were receiving one ARV pill per day while patients 273 patients were taking 2 ART pills. This distribution was not significantly different between patients on NVP and EFV ($p =0.286$). The ART non-adherence rate for all patients was 65.6%, 64.6% for patients on NVP and 66.4% patients on EFV which was not significantly different among patients on NVP and EFV ($p = 0.658$).

Table 4.16. Measure of access, utilization of Healthcare facilities and adherence among study patients

Variable		All Patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		*p Value*
		n	(%)	n	(%)	n	(%)	
Time to HIVclinic (Minutes)	**Median (IQR)**	80	(60 - 120)	90	(60 - 120)	75	(60 - 120)	
	≤60 Min	270	47.7	116	45.7	154	49.4	
	61-120 Min	141	24.9	45	17.7	96	30.8	**0.0001**
	≥121 Min	155	27.3	93	36.6	62	19.9	
Hospital admission past 3 months	Yes	29	5.1	18	7.1	11	3.5	
	No	537	94.9	236	92.9	301	96.5	0.083
Number of times admitted to the hopsital	None	531	93.8	238	93.7	293	93.9	
	1 time	26	4.6	12	4.7	14	4.5	0.991
	≥2 times	9	1.6	4	1.6	5	1.6	
Number of times spent lying down due to an illness in the past 3 months	None	531	93.8	239	94.1	292	93.6	
	1 time	15	2.7	5	1.9	10	3.2	0.57
	≥2 times	20	3.5	10	3.9	10	3.2	
Number of times visited medical clinic due to an acute illness in the past 3 months	None	401	70.6	181	71.3	220	70.5	
	1 time	135	23.9	61	24.1	74	23.7	0.898
	≥2 times	30	5.3	12	4.7	18	5.8	
Missed scheduled HIV medical visit in the past 3 months	Yes	46	8.1	21	8.3	25	8.1	
	No	520	91.9	233	91.7	287	91.9	0.912
Reasons for missing scheduled HIV medical visit in the past 3 months	Not applicable	520	91.9	233	91.7	287	91.9	
	No money	11	1.9	6	2.4	5	1.6	
	No childcare	3	0.5	1	0.4	2	0.6	
	Working in the field	21	3.7	10	3.9	11	3.5	0.99
	Forgot	7	1.2	3	1.2	4	1.3	
	No transport	3	0.5	1	0.4	2	0.6	
	No enough food	1	0.2	0	0	0	0	
No of ARV Pills taken per day	1	285	50.4	119	46.9	166	53.2	
	2	273	48.3	132	51.9	141	45.2	0.286
	3	8	1.4	3	1.2	5	1.6	
Missed taking current ARV in the past 30 days	Yes	371	65.6	164	64.6	207	66.4	0.658
	No	195	34.5	90	35.4	105	33.7	
ART adherence in the past 30 days	Good	119	21	43	16.9	76	24.4	
	Fair	44	7.8	31	12.2	13	4.2	**0.001**
	Poor	32	5.7	16	6.3	16	5.1	
	Non-adherence	371	65.7	164	64.6	207	66.4	

4.4.3.2. Relationship between healthcare access and ART utilization and ART drug adherence

Table 4.17 shows the relationship between host healthcare access and ART utilization and adherence. Only factors significantly associated with ART drug adherence for the past 30 days were assessed for association with NVP and EFV plasma level. These factors included, time taken to healthcare facility, number of times spent lying down due to HIV related illness in the past 30 days, number of times taking current ART, number of ARV pills taken per day and source of adherence information. In summary, measure of the pill burden was associated with adherence.

Table 4.17. Exploratory data analysis for the relationship between healthcare access and ART utilization and ART drug adherence

Variable	FISHER'S EXACT TEST ART drug Adherence	
	Nevirapine *P - values*	Efavirenz *P - values*
Health care access and utilization		
Time to health facility (Min)	**0.023**	**0.001**
Hospital admission past 3 months		
Number of times admitted to the hopsital	0.123	0.345
Number of times spent lying down due to an illness in the past 3 months	0.219	0.886
Number of times spent lying down due to an illness in the past 30 days	**0.05**	0.655
Number of times visited medical clinic to see a doctor in the past 3 months	0.983	0.296
Number of times visited medical clinic due to an acute illness in the past 3 months	0.528	0.529
Missed schedulled HIV medical visit in the past 3 months	0.342	0.512
Reasons for missing schedulled HIV medical visit in the past 3 months	0.905	0.112
ART drug utilization		
Previous ART regimen	0.069	0.882
Current ART regimen	0.699	0.935
No of times taken ARV per day	**0.02**	**0.0001**
No of ARV Pills taken per day	**0.001**	**0.0001**
ARV pill information from pill bottle	**0.0001**	**0.0001**
ARV pill information from medical records	**0.0001**	**0.0001**
ARV pill information from selfreport	**0.001**	**0.0001**
ARV pill information from other sources	0.659	0.476

4.4.3.3. Variation in nevirapine and efavirenz plasma levels and healthcare access, utilization and adherence among study patients

Table 4.18 summarizes the variation in the median NVP and EFV plasma concentration with measure of healthcare access, utilization and ART adherence. Patients who spent ≥2 number of times laying down due to HIV related illness in the past 3 months had higher median (IQR) NVP plasma concentration (6860, IQR = 4204–8405 ng/mL) compared to patients who did not spent time laying down (6273, IQR = 4558–9198 ng/mL) or spent one time laying down due to an illness in the past 3 months (2821, IQR = 2746–2960 ng/mL; $p = 0.049$).

Patients with higher median (IQR) EFV plasma concentration were those who took 2 ARV pills per day (3216, IQR = 1994–6116 ng/mL) compared to those who took 3 ARV pills (2008, IQR = 1868–2396 ng/mL; $p = 0.032$) and those patients who took one ARV pill per day (2594, IQR = 1838–4911 ng/mL; $p = 0.032$).

Table 4.18. Variation in median nevirapine and efavirenz plasma concentration and healthcare access and ART drug adherence variables

	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
Variable	n	Median	(IQR)		*P*	n	Median	(IQR)		*P*
Time to health facility (Min)										
≤60 Min	116	6191	4894	9029.5		154	2563.5	1838	4264	
61-120 Min	45	5734	3859	8575	0.385	96	2828.5	1852.5	5071.5	0.066
≥121 Min	93	6372	4359	9906		62	3137.5	2011	6677	
Hospital admission past 3 months										
Yes	18	4970.5	3008	7690	0.083	11	3095	2183	4264	0.770
No	236	6317	4564.5	9180.5		301	2732	1870	4911	
Number of times admitted to the hopsital										
None	238	6317	4558	9095		293	2712	1868	4911	
1 time	12	4970.5	2877	7979.5	0.286	14	3947	2276	7085	0.102
≥2 times	4	5968.5	4225.5	11055		5	2539	1149	2992	
Number of times spent lying down due to an illness in the										
None	239	6273	4558	9198		292	2645.5	1862	4775.5	
1 time	5	2821	2746	2960	**0.049**	10	4812	2276	14273	0.137
≥2 times	10	6860	4204	8405		10	3043.5	2396	5586	
Number of ARV Pills taken per day										
1	166	6393.5	4571	9906		217	2594	1838	4911	
2	73	5813	4370	7509	0.429	78	3216	1994	6116	**0.032**
3	15	6051	3739	8798		17	2008	1868	2396	
Missed taking current ARV in the past 30 days										
Yes	164	6151	4545	9129	0.774	207	2592	1838	4603	0.266
No	90	6365.5	4370	8875		105	2992	1935	5586	
ART adherence in the past 30 days										
Good	43	6390	5439	10881		76	3020.5	1992.5	5935.5	
Fair	31	6538	4117	7814	0.672	13	2872	1868	3607	0.499
Poor	16	6142	4660	10360.5		16	2698	1861.5	5087.5	
NonAdherence	164	6151	4545	9129		207	2592	1838	4603	

4.4.3.4. Quantile regression analysis of nevirapine and efavirenz plasma concentration and ART adherence factors

Table 4.19 describes quantile regression analysis estimating the relationships between NVP and EFV plasma levels and ART adherence variables. On bivariable linear regression analysis the most import determinant of NVP plasma concentrations included current ART regimen type (unadjusted β -800, 95% CI -1569.8 to -30.2; p =0.042) and obtaining ARV pill uptake information from other sources (unadjusted β -2393, 95% CI -4634.5 to -151.5; p =0.036). On multivariate regression analysis the only ART adherence related factor that independently influenced NVP plasma level was the current ART regimen type (adjusted β -828, 95% CI, -1304.5 to -351.5; p = 0.0001).

On multivariate regression analysis ART adherence related factors that independently influenced EFV plasma levels included previous ART regimen type (adjusted β -289.2, 95% CI, -483.2 to -95.1; p = 0.004), obtaining ARV pill uptake information from self-reports (adjusted β -7348.8, 95% CI, -7895.1 to -6802.4; p = 0.0001) and obtaining ARV pill uptake information from other sources (adjusted β 18421.3, 95% CI, 16291.5 to 20551.2; p = 0.0001).

Though there was a negative association between non-adherence and plasma levels, the association was not statistically significant on both bivariate and multivariate analysis.

Table 4.19. Regression analysis between nevirapine and efavirenz plasma concentrations and healthcare utilization and ART adherence variables

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β (95% CI)			*P-value*	Unadjusted β (95% CI)			*P-value*
Time to health facility	101.5	-388.95	591.95	0.684	260.5	-54.154	575.15	0.104
Hospital admission past 3 months	1353	-727.86	3433.9	0.202	-363	-2086.5	1360.5	0.679
Number of times admitted to the hopsital	-521.75	-3097.4	2053.9	0.69	122.5	-753.92	998.92	0.783
Number of times spent lying down due to an illness in the past 3 months	268	-1107.7	1643.7	0.702	15.125	-280.34	310.59	0.92
Missed schedulled HIV medical visit in the past 3 months	-910	-2821.4	1001.4	0.349	-415	-1860.7	1030.7	0.573
Previous ART regimen	-70	-609.41	469.41	0.798	-223.2	-402.7	-43.702	**0.015**
Current ART regimen	-800	-1569.8	-30.24	**0.042**	318	-161.24	797.24	0.193
No of times taken ARV per day	-399	-1251.6	453.56	0.358	-346	-783.06	91.055	0.12
No of ARV Pills taken per day	-466	-1329.1	397.1	0.289	-31	-639.12	577.12	0.92
ARV pill information from pill bottle	499	-469.29	1467.3	0.311	-54	-733.38	625.38	0.876
ARV pill information from medical records	507	-85.848	1099.8	0.093	501	200.43	801.57	**0.001**
ARV pill information from selfreport	356	-480.4	1192.4	0.403	547	141.73	952.27	**0.008**
ARV pill information from other sources	-2393	-4634.5	-151.5	**0.036**	-7837	-8114.9	-7559.1	**0.001**
Non Adherence	-211	-1156.4	734.43	0.661	421	-224.77	1066.8	0.201

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β (95% CI)			*P-value*	Adjusted β (95% CI)			*P-value*
Time to health facility	-1.38	-10.196	7.4308	0.758	3.858703	-1.6029	9.3203	0.165
Hospital admission past 3 months	1467.5	-8782.4	11717	0.778	-58.21451	-2196.6	2080.1	0.957
Number of times admitted to the hopsital	1240.1	-106445	108925	0.982	-13.92151	-1050.2	1022.4	0.979
Number of times spent lying down due to an illness in the past 3 months	-981	-6313.9	4352	0.717	-128.1996	-786.81	530.41	0.702
Missed schedulled HIV medical visit in the past 3 months	378.8	-6939.2	7696.9	0.919	-2315.321	-6862.3	2231.6	0.317
Previous ART regimen	-132	-512.54	248.46	0.495	-289.1667	-483.2	-95.138	**0.004**
Current ART regimen	-828	-1304.5	-351.54	**0.001**	250.9167	-157.7	659.53	0.228
No of times taken ARV per day	-775.0	-2070.9	520.91	0.24	-281.25	-761.85	199.35	0.25
No of ARV Pills taken per day	-854.0	-2024.3	316.39	0.152	-203.25	-560.51	154.01	0.264
ARV pill information from pill bottle	729.4	-610.34	2069.1	0.284	-58.33333	-627.84	511.17	0.84
ARV pill information from medical records	277.4	-1106.8	1661.5	0.693	347	-9.5385	703.54	**0.056**
ARV pill information from selfreport	384.8	-1088.5	1858.1	0.607	-7348.75	-7895.1	-6802.4	**0.001**
ARV pill information from other sources	-3570.1	-8079.3	939	0.12	18421.33	16291	20551	**0.001**
Non Adherence	4287.9	-665.32	313.99	0.481	176.0491	-1440.1	1792.2	0.83

4.4.3.5. Comparison of reasons for non-adherence among patients on nevirapine and efavirenz

Table 4.20 summarizes the reasons stated for lack of adherence to ART among study participants. Among the reasons stated for stopping taking current ART treatment, was fatigue 2.3% of patients who mentioned fatigue as the main reason which was not significantly different between patients on NVP and EFV (p = 0.402). Hand and feet pain were stated by 1.4% of the patients as the reason for stopping current ART medication which was not significantly different between patients on NVP and EFV (p = 0.769). Experiencing skin problems such as rash and dryness and experiencing depression was stated by 2.3% of the study patients each. A total of 222 (39.2%) of the patients stated experiencing bodily pain of various degree as the main reason for non-adherence. Among the patients 7.1% and 1.9% experienced moderate and severe bodily pain hindering ART adherence. There were about 4.5% of the patients who reported their ill health limited their ability to cook or wash their own cloths with patients on EFV affected slightly more than those on NVP (p = 0.088).

Table 4.20. Comparison of reasons for non-adherence among study patients on nevirapine and efavirenz

Current (month 12) Variables		All Patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		*p Value*
		n	(%)	n	(%)	n	(%)	
Stopped or interrupted taking current medication due to fatigue	Yes	13	2.3	4	1.6	9	2.9	
	No	553	97.7	250	98.4	303	97.1	0.402
Stopped taking current medication due to pain in the hands and feet	Yes	8	1.4	4	1.6	4	1.3	
	No	558	98.6	250	98.4	308	98.7	0.769
Stopped taking current medication due to nausea	Yes	3	0.5	2	0.8	1	0.3	
	No	563	99.5	252	99.2	311	99.7	0.59
Stopped or interrupted taking current medication due to skin problems such as rash, dryness	Yes	13	2.3	3	1.2	10	3.2	
	No	553	97.7	251	98.8	302	96.8	0.158
Stopped taking current medication due to depression	Yes	13	2.3	7	2.8	6	1.9	
	No	553	97.7	247	97.2	306	98.1	0.579
Bodily pain in the past 30 days	None	344	60.8	159	62.6	185	59.3	
	Very mild	107	18.9	48	18.9	59	18.9	
	Mild	64	11.3	24	9.5	40	12.8	0.472
	Moderate	40	7.1	16	6.3	24	7.7	
	Severe	11	1.9	7	2.8	4	1.3	
Does your health limits ability to perform moderate activities such as cooking, washing cloths	Not Limited at all	541	95.6	246	96.9	295	94.6	
	Limited a Little	13	2.3	2	0.8	11	3.5	0.088
	Limited a Lot	12	2.1	6	2.4	6	1.9	
Does your health keep you from doing certain kinds or amount of work, housework or schoolwork	Not Limited at all	531	93.8	242	95.3	289	92.6	
	Limited a Little	20	3.5	5	1.9	15	4.8	0.202
	Limited a Lot	15	2.7	7	2.7	8	2.6	

4.4.3.6. Relationship between reasons for non-adherence and ART drug adherence

Table 4.21 explores the relationship between reasons for stooping taking current ART medication and ART adherence among patients on NVP and EFV. Experiencing bodily fatigue was associated with ART non-adherence among patients on EFV ($p = 0.031$) and not patients on NVP ($p = 0.61$). Experiencing bodily pains in the past 30 days were found to significantly affect ART adherence for both patients on NVP ($p = 0.002$) and those on EFV ($p = 0.006$). These were the only factors assessed for the association with NVP and EFV plasma level in subsequent analysis.

Table 4.21. Exploratory data analysis for the relationship between reasons for non-adherence and ART drug adherence

Variable	FISHER'S EXACT TEST ART drug Adherence	
	Nevirapine *P - values*	Efavirenz *P - values*
Medical outcomes		
Stopped taking current medication due to fatigue	0.66	**0.031**
Stopped taking current medication due to dizziness	0.829	0.35
Stopped taking current medication due to headache	0.913	0.057
Stopped taking current medication due to pain in the hands and feet	0.66	0.304
Stopped taking current medication due to nausea	0.125	0.476
Stopped taking current medication due to vomiting	0.939	0.552
Stopped taking current medication due to bloating	0.665	0.553
Stopped taking current medication due to weight loss or wasting	0.3	0.552
Stopped or interrupted taking current medication due to fat deposit or weight gain	0.458	0.991
Stopped taking current medication due to skin problems such as rash, dryness	0.939	0.173
Stopped taking current medication due to nervousness or anxiety	0.659	0.553
Stopped taking current medication due to depression	0.427	0.101
Categorization of general health condition	0.399	0.665
Bodily pain in the past 30 days	**0.002**	**0.006**
How much pain interfered with normal work in the past 30 days	0.677	0.913
Does your health limits ability to perfome vigorous activities such as digging or splitting firewood	0.637	0.172
Does your health limits ability to perform moderate activities such as cooking, washing cloths	0.215	0.564
Does your health limits ability to walk up a hill	0.365	0.706
Does your health limits ability to bend or lift light objects	0.72	0.98
Does your health limits ability to walk a distance of 100 meters	0.226	0.665
Does your health limits ability to eat, dress or bathe	0.357	0.878
Does your health keep you from working at a job, attending school	0.848	0.714
Does your health keep you from doing certain kinds or amount of work, housework or schoolwork	0.275	0.079

4.4.3.7. Quantile regression analysis of nevirapine and efavirenz plasma concentration by reasons for non-adherence

Table 4.22 describes quantile regression analysis estimating the relationships between NVP and EFV plasma levels and reasons for non-adherence among study participants. On bivariable regression analysis factors associated with NVP plasma concentrations included stopping taking current ARVs due fat deposition or weight gain (unadjusted β -697, 95% CI -1111.1 to -282.9; p =0.001), stopped taking current medication due to skin problems such as rash (unadjusted β 1490, 95% CI 73 to 3053; p =0.062) as well as the stopped taking current medication due to severity of skin problems such as rash (unadjusted β 719, 95% CI 17 to 1429; p =0.045). On multivariate regression analysis factors that independently were associated with NVP plasma concentrations comprised; patients health conditions limiting their ability to walk up a hill (adjusted β 4200.5, 95% CI, 870.8 to 7530.2; p = 0.014) and health conditions limiting their ability to bend or lift light objects (adjusted β -1959, 95% CI, -3663 to -255; p = 0.024).

A common practice during data analysis is to incorporate in multivariate analysis only variables that are statistically important in bivariate analysis. This practice has some risk given that a number of variables not statistically significant in bivariate analysis is known to turn significant on multivariate evaluation majorly due to the presence of interaction between variables. This study witnessed the confounding factors associated with NVP plasma levels. Thus, on multivariate regression analysis ART side effect related factors independently associated with NVP plasma level included stopping taking current ARVs medication due to pain in the hands and feet (adjusted β -11495, 95% CI, -16059.8 to -6930.2; p = 0.0001), stopping taking current ARVs medication due to nausea (adjusted β 5356, 95% CI, 483.8 to 10228.2; p = 0. 031), stopping taking current ARVs medication due to weight loss or wasting (adjusted β -3636, 95% CI, -6209.2 to -1062.8; p = 0.006), stopping taking current ARVs medication due fat deposit or weight gain (adjusted β 10869, 95% CI, 6080 to 15658; p = 0.0001), stopping taking current ARVs medication due to skin problems such as rash (adjusted β -3864, 95% CI, 2078.3 to 5658; p = 0.0001), stopping taking current ARVs medication due to nervousness or anxiety (adjusted β -8169, 95% CI, -15399.6 to -938.4; p = 0.027) and stopping taking current ARVs medication due to depression (adjusted β 12485, 95% CI, 7696 to 17274; p = 0.0001).

On bivariable regression analysis factors associated with EFV plasma concentrations included; stopping taking current ARVs due to nausea (unadjusted β 653, 95% CI 312 to 994; p =0.001) and stopped taking current medication due to skin problems such as rash (unadjusted β 788, 95% CI 140.4 to 1435.6; p =0.017).

On multivariate regression analysis factors associated with EFV plasma concentrations included stopping taking current ARVs due nausea (adjusted β 713, 95% CI 425.5 to 1000.5; $p < 0.001$) and stopped taking current medication due to skin problems such as rash (adjusted β 1847, 95% CI 137.9 to 3556.1; p =0.035) and the presence of body pain in the past 30 days (adjusted β 475, 95% CI, 117.2 to 832.8; p = 0.009).

Table 4.22. Regression analysis of nevirapine and efavirenz plasma concentrations by reasons for non-adherence

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β	(95% CI)		P-value	Unadjusted β	(95% CI)		P-value
Stopped taking current medication due to nausea	5823	-442.3	12088.3	0.066	653	312	994	**0.001**
Stopped or interrupted taking current medication due to fat deposit or weight gain	-697	-1111.1	-282.9	**0.001**	826	-10143.1	11795.1	0.882
Stopped taking current medication due to skin problems such as rash, dryness	1490	-73	3053	**0.062**	788	140.4	1435.6	**0.017**
Stopped taking current medication due to severity of skin problems such as rash	719	-41.8	1479.8	0.064	374.5	-16.6	765.6	0.06
Does your health limits ability to walk up a hill	331	-1510	2172	0.724	-367	-1030.6	295.6	0.276
Does your health limits ability to bend or lift light objects	-603.5	-1185.5	-21.5	**0.042**	-496	-1301.9	308.9	0.226
Does your health keep you from doing certain kinds or amount of work, housework or schoolwork	518	-1066.8	2102.8	0.52	27	-918.5	972.5	0.955

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β	(95% CI)		P-value	Adjusted β	(95% CI)		P-value
Stopped taking current medication due to pain in the hands and feet	-11495	-16059.8	-6930.2	**0.0001**	-1086.5	-20989.3	18816.3	0.915
Stopped taking current medication due to nausea	5356	483.8	10228.2	**0.031**	713	425.5	1000.5	**0.0001**
Stopped taking current medication due to vomiting	775	-1828.2	3378.2	0.558	15987	-40117	72091	0.575
Stopped taking current medication due to bloating	3229	-1844.3	8302.3	0.211	-14329	-50348.4	21690.4	0.434
Stopped taking current medication due to weight loss or wasting	-3636	-6209.2	-1062.8	**0.006**	1696.5	-4069.8	7462.8	0.563
Stopped or interrupted taking current medication due to fat deposit or weight gain	10869	6080	15658	**0.0001**	2802	-22143.9	27747.9	0.825
Stopped taking current medication due to skin problems such as rash, dryness	3864	2078.3	5649.7	**0.0001**	1847	137.9	3556.1	**0.034**
Stopped taking current medication due to severity of skin problems such as rash	-1222	-2833.3	389.3	0.136	-817	-1998.4	364.4	0.175
Stopped taking current medication due to nervousness or anxiety	-8169	-15399.6	-938.4	**0.027**	-3582.5	-17433.9	10268.9	0.611
Stopped taking current medication due to depression	12485	7696	17274	**0.0001**	-829.5	-13763.9	12104.9	0.9
Categorization of general health condition	-91.5	-667.7	484.7	0.755	-56	-473.8	361.8	0.792
Bodily pain in the past 30 days	251.3	-543.6	1046.3	0.534	475	117.2	832.8	**0.009**
Does your health limits ability to walk up a hill	4200.5	870.8	7530.2	**0.014**	-164	-1536	1208	0.814
Does your health limits ability to bend or lift light objects	-1959	-3663	-255	**0.024**	-427	-1942.4	1088.4	0.58

4.4.4. Discussion

Admittance into an ART program is a key step to an efficient HIV management program (Moosa et al., 2019). Optimum ART adherence is also critical too attaining desirable treatment outcomes. Data indicates that ART adherence rate of ≥95% adherence essential to attaining and retaining viral suppression (Paterson et al., 2000). Although, depending on the ART regimen type, period into ART treatment and prior ART exposure, recent data indicates that positive virologic response may still be realized with < 95% ART adherence levels (Ammassari et al., 2012). Additionally, recurrent ART adherence rates of < 100% and treatment disruptions are associated with greater risk in the emergence of HIV drug resistance strains to practically all ARVs (Haberer et al., 2015; Kimulwo et al., 2017). Poor ART adherence may cause diverse adverse effects on both individual and public HIV healthcare including. First, patients with poor ART adherence who develop resistance mutations to first line NNRTI-based regimens are swapped to either second or third-line ARV regimens which are expensive, require higher pill burden, higher dosing frequency and frequently have tolerated side effects (Snedecor et al., 2013). Second, the ease of transmitting drug resistant HIV strains to newly infected ART-naïve patients or to those treatment experienced infected patients, heightens ART treatment failures in the populations (Cambiano et al., 2013). Third, treatment failure related non-adherence is connected to a huger risk of advancement to Acquired Immune Deficiency Syndrome (AIDS) and death (Lima et al., 2009).

Assessment of ART adherence rates is best done in observational surveys involving close monitoring of patients' medication uptake, this might reflect definite clinical practice (Cherry et al., 2009). Rate of adherence could be erroneously higher in randomized controlled trials because of the nature of this design including meticulous patients selection or/and the more rigorous follow-up; nevertheless, randomized controlled trials may generate vital data relating ART rates of adherence on treatment outcomes such as virologic failures and ART plasma concentration (Van Onzenoort et al., 2011). In this aim, the study reports the level of adherence and association with treatment outcomes in Kenya. The non-adherence rate for a whole day was 55(21.7%) for patients on NVP and 72(23.1%) for patients on EFV. The non-adherence rate for past 30 days was 164 (64.6%) for patients on NVP and 207 (66.3%) for patients on

EFV. In a previous study in Kenya, Kimulwo *et al*., (2017) reported 22.4% of patients had poor adherence. In Malawi among patients partaking ART, there were 47% of them who reported poor adherence to ART treatment (Van Oosterhout et al., 2005).

This study showed the of number of pills taken per day is related to NVP and EFV plasma concentrations. A previous study in Kenya Kimulwo *et al*., (2017) showed a two-fold higher mean NVP plasma concentration for patients reporting good or fair rate of adherence compared to poor adherence. Further, among HIV-infected Kenyan children Tu *et al*., (2017) showed poorer adherence which was significantly associated with reduced NVP plasma levels and lower CD4% count. In India, Gopalan *et al*., (2017) reported an adherence rate of ≥95% in study patients which was associated with median nevirapine concentrations of 4.8 μg/ml considered supratherapeutic. In Tanzania, Mugusi *et al*., (2019) reported varied adherence rates among orphaned children ranging from 72.2%, 79.6% to 82.9% established by NVP plasma levels, parents self-report and by healthcare worker respectively. The study further reported significant association between non- adherence, poor ART healthcare access and poor immunological outcomes with reduction in NVP plasma levels (< 3 μg/mL) among study children. On the contrary, Oluka *et al*., (2015) reported no relationship between NVP plasma levels and pill count as a measure of adherence. The study by Oluka *et al*., (2015) concurs with this current findings.

Among patients on EFV, Guo *et al*., (2019) showed that among the Chinese HIV-infected adults, improved adherence over time was associated with higher median plasma efavirenz concentration. On the contrary, In Cambodia Borand *et al*., (2014) and Uganda, Mukonzo *et al*., (2013), reported no relationship between adherence and EFV plasma levels. In South Africa Johnston *et al*., (2019). No relationship between hair efavirenz concentrations failure or adherence measured using an electronic adherence was shown. This is in line with the findings of this study where there was no association between adherence and EFV levels after adjusting for confounding. However, there was an association on bivariable analysis.

This study showed that ARV regimen was an important factor affecting NVP and EFV plasma concentration. Patients receiving NVP/3TC and TDF combination had higher median NVP plasma concentration than those on NVP/3TC/ZDV and NVP/3TC/d4T. On the contrary,

patients on EFV/ 3TC/ZDV had higher EFV plasma levels than those on EFV/3TC/ TDF. Patients on d4T had significantly lower NVP plasma level. The use of d4T has been discontinued in many developed countries majorly due to increased toxicities (Gilks et al., 2006), and in Kenya it has been phased-out (Ochieng et al., 2015). Because data link TDF to kidney injury (Ryom et al., 2013) while ABC to cardiovascular injuries (Desai et al., 2015), limiting their uptake by some specific patients may be prudent. A study done in Kenya by Kimulwo *et al*., (2017) comparing the regimen with NVP plasma concentration found association between level of AZT and D4T. during the current study, TDF was a major component of first line ART regimen both for patients initiated into treatment or replacing regimen. Due to the linkages of TDF use to kidney injury (Ryom et al., 2013) it might be prudent for renal injuries be rigorously interrogated among this population. For beneficial ART clinical outcomes interrogating both previous/initiation ART type and current ART combination is important because it has bearing on the NVP and EFV plasma concentration.

Healthcare access and utilization is a known important factor affecting NVP and EFV plasma concentration and if not checked could impose a significant impediment to treatment outcomes. History of hospital admission, time taken to reach the ARV dispensing clinic, the frequency in the visitation of medical clinic to see a doctor, number of times visited medical clinic due to an acute illness or missing scheduled HIV medical visit are some of the health access variables probed but were not found associated with NVP and EFV plasma levels. Studies however shows the importance of accessing ART clinics or healthcare influencing treatment outcomes. Sustaining a good ART adherence requires early initiation and retention, consistent monitoring of treatment outcomes, and continuous psychosocial support to the patient (Nosyk et al., 2015). Lack of quick access to ART dispensing facilities and lack of means of transport are also a major obstacles to health facilities access and retention in HIV treatment programs in rural settings in developing countries (Skovdal et al., 2011; Wakibi et al., 2011). Selke *et al*., (2010) reported limited healthcare access as one of the key challenges for attaining required adherence level of >95% suitable for achieving positive HIV.

This study showed a significant association between NVP and EFV plasma concentration with stopping taking medication due to pain in the hands and feet, nausea, weight loss or wasting,

fat deposit or weight gain, skin problems such as rash, nervousness or anxiety and depression. Consistent to the current study, Mbuagbaw *et al*., (2016) reported an association between EFV and the occurrence of nervousness or anxiety and depression than reported among patients on NVP. These results also agreed with those of Kaimal *et al*., (2018). Oluka *et al*., (2015) on the other hand did not show an association between rash and NVP plasma concentration. Studies in Africa shows that nevirapine induces hypersensitivity reactions between 6% to 10% of patients, comprising of liver injuries, severe skin rash, Stevens–Johnson syndrome and toxic epidermal necrolysis (Carr et al., 2017). Mollan *et al*., (2014) reported adverse mental health associated with EFV. The study showed the suicidality incidence was 2-fold higher among efavirenz receiving group and compared to efavirenz-free. This study also shown the importance of wasting, fat deposit or weight gain is associated with NVP and EFV plasma levels. Herrmann *et al*., (2013) also reported abnormal fat distribution/lipodystrophy due to ARVs and this affects patients Health-related Quality of life by decreasing medication adherence. These results re-affirm the importance of NVP and EFV induced adverse medical outcomes which must always be monitored among HIV patients on these regimens.

This study also evaluated the connection between NVP and EFV plasma levels and outcomes of physical and daily activities. Inability to walk up a hill was connected with increased NVP plasma levels, while inability to bend or lift light objects was associated with lower NVP plasma level. For efavirenz the presence of body pain in the past thirty days was linked to a higher EFV plasma levels. Herrmann *et al*., (2013) showed the HIV infected persons often experience episodes of tiredness requiring to take a break often from daily chores and experiencing difficulties in executing daily tasks such as walking short distances or carrying light weight.

4.4.4. Conclusions

This study found no significant association between adherence and NVP and EFV plasma levels. Pill burden however affected NVP and EFV plasma levels. The measure of no-adherence that was found consistently associated with drug plasma level was failing to take medicine for a whole day. This study showed that ARV regimen was an important factor affecting NVP and EFV plasma concentrations. The effects of non-adherence on drug levels

was more pronounced for NVP levels compared to EFV. In the case of EFV, the key determinant was prior regimen. Access to healthcare had no bearing on drug plasma levels

4.5. RELATIONSHIP BETWEEN EFAVIRENZ AND NEVIRAPINE PLASMA CONCENTRATIONS AND HIV DRUG RESISTANCE AND SELECTED SIDE EFFECTS

4.5.1. Introduction

The two NNRTI drugs used in Kenya, nevirapine and efavirenz disrupts HIV replication within host cells by inhibition of the HIV reverse transcriptase (RT) enzyme. Both are potent, safe and convenient to use in several clinical trials (Van Leth et al., 2004). As a result, NVP and EFV are frequently used as the basis of HAART for management of both treatment-naive and treatment-experienced patients. During this study, these two drugs were expansively utilized in resource poor countries especially NVP due its efficiency, obtainability, reduced cost and application in PMTCT (WHO, 2016), while efavirenz is greatly tolerated taken as a single pill with an extended plasma half-life (Thompson *et al*., 2010). Unfortunately, the use of NVP is marked by higher occurrence of skin rashes (some are severe and life-threatening including Stevens-Johnson syndrome) (Van Leth et al., 2004). Nevirapine use other than being linked to life-threatening risk of liver injuries (Aizire et al., 2012) the drug has a low genetic barrier impeding its 100% use (Clutter et al., 2016). Likewise, efavirenz based regimen marked by severe adverse effects including dyslipidaemia, central nervous system (CNS) toxicity, including dizziness and the factor that it is also a low genetic barrier drug threatens to limit it is global use (Poeta et al., 2011). Even though considerable inter-individual variation (due to drug-drug interactions, gene alterations, ethnicity and adherence rates) in both EFV and NVP plasma level has been recorded, data relates plasma levels of these two drugs toxicity and emergence of HIV drug resistance strains especially due to termination of ART treatment (Van Leth et al., 2004; Aizire et al., 2012). In this objective, the study determined the association between NVP and EFV plasma concentration and HIV drug resistance, hepatic, renal and hematological side effects.

4.5.2. Methods

The methods used in this section are presented in chapter three sections 3.7.2.1, 3.7.3.1, 3.7.3.2, 3.7.3.3, 3.7.3.4 and 3.7.3.6 while data management and statistical analysis are presented in section 3.7.5 and 3.7.6

4.5.3. Results

4.5.3.1. Prevalence of HIV drug resistance among study patients

Table 4.23 describes the ARV drug resistant mutations by to the regimen type. Out of the 599 enrolled patients (566; 94.5% responses rate) had all the nevirapine and efavirenz plasma levels and were subsequently evaluated in this section. None of the patients had HIV viruses resistant to major proteases inhibitors drugs. Of the 254 patients on NVP regimen and 312 patients on EFV, 24 (9.4%) and 13(4.2%) had resistant mutations respectively to reverse transcriptase inhibitors drugs (NNRTI and NRTI) ($p = 0.002$). The most common NNRTI mutations reported included K103N among 19 patients followed by Y181C among 13 patients, G190ASEQ among 9 patients and one patient had Y188FY mutation. The most common NRTI mutation was Nucleoside Analogue Mutations (NAMS), which included 32 patients with M184V, 14 patients with K65R, 8 patients with K70R and 2 patients with Y115F. There were some patients who harbored Thymidine Analogue Mutations (TAMs) mutations, including 9 patients with K70R, 9 patients with T215Y mutation, 7 patients had D67N mutation and 2 patients with M41L mutation (Figure 4.6).

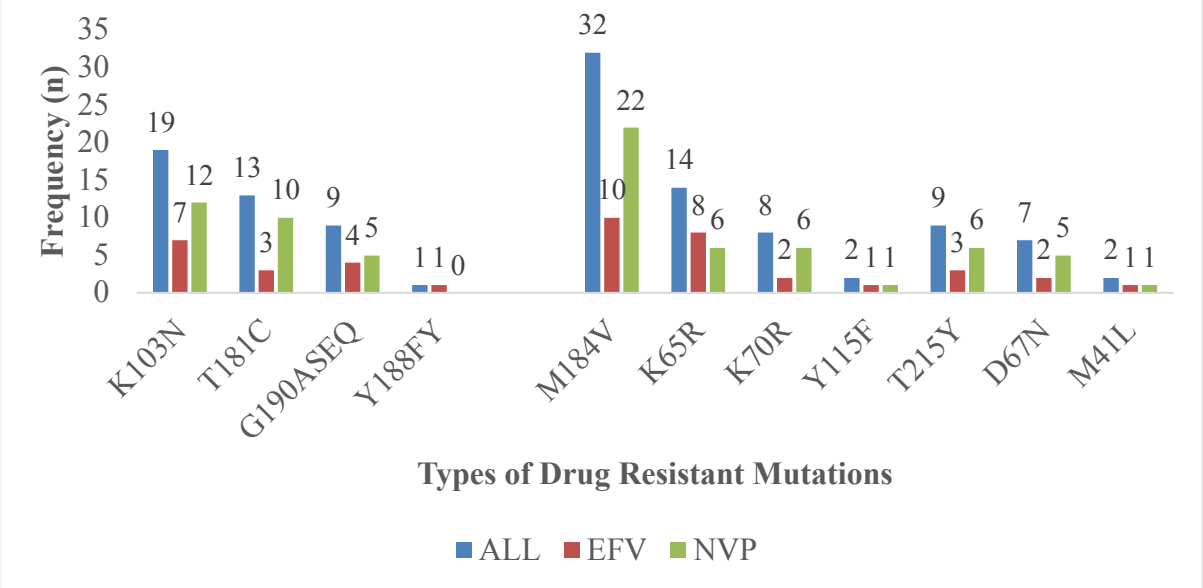


Figure 4.6: Prevalence of types of drug resistant mutations among study patients

Table 4.23 Types of Resistance Mutations identified and patients viral load

Regimen	Viral load	HIV	Protease inhibitors Mutation		Reverse-transcriptase inhibitors Mutations	
		Subtype	MAJOR	MINOR	NRTI	NNRTI
EFV	**44,225**	**AE**	Susceptible	L10V	Susceptible	G190S
EFV	**74,352**	A	Susceptible	L10V	K65R	K101E, V179T, Y181C, G190S
EFV	**12,229**	C	Susceptible	Susceptible	K65R, Y115F, M184V	L100I, K103N, Y181CY
EFV	**77,383**	AE	Susceptible	L10V	K65R, M184V	K101P, K103N, E138AEKT, V179L
EFV	**46,978**	A	Susceptible	Susceptible	Susceptible	Susceptible
EFV	**13,252**	A	Susceptible	Susceptible	M41LM, M184V, T215F	K101EK, Y181C
EFV	**16,284**	G	Susceptible	K20I	Susceptible	Susceptible
EFV	**7,130**	A	Susceptible	L10I	Susceptible	Susceptible
EFV	**40,828**	AE	Susceptible	Susceptible	D67N, K70R, M184V, T215F, K219E	V90I, K101E, V108I, E138Q, G190A
EFV	**4,384**	AE	Susceptible	L10V	M184IMV	K103N, M230LM
EFV	**86,661**	AE	Susceptible	Sucesptible	K65R, V75I	L100I, K103N
EFV	**4,686**	AE	Susceptible	Sucesptible	A62V, K65R, M184V	K103N, P225H
EFV	**93,249**	A	Susceptible	Susceptible	K65R, M184I	L100I, K103N, Y188FY, M230L
EFV	**917**	A	Susceptible	Susceptible	K65R, M184V	K101P, K103N, E138AEKT, V179L
EFV	**393**	A	Susceptible	Susceptible	A62V, K65R, M184V	K103N, P225H
EFV	**430**	G	Susceptible	Susceptible	Susceptible	Susceptible
EFV	**23,333**	AE	Susceptible	L10I	D67N, K70R, M184V, T215F, K219E	V90I, K101E, V108I, E138Q, G190A
NVP	**2,298**	A	Susceptible	Susceptible	M184V	Y181C, H221Y
NVP	**367,728**	A/D	Susceptible	A71T	M41L, K70KN, V75M, M184V, L210W, T215Y	K103N, V108IV
NVP	**545**	**A**	Susceptible	L10IL	D67DN, K70KR, M184V, T215F, K219EK	V90I, V179FI, Y181C
NVP	**123,404**	D	Susceptible	L10IL	M184V	K103N
NVP	**201,408**	**AE**	Susceptible	L10IL	D67N, K70R, M184V, T215F, K219Q	A98G, K103N, K238T
NVP	**10,775**	A	Susceptible	K20I	A62V, K65R, M184I	V90I, Y181V
NVP	**2,882**	D	Susceptible	L10I, A71T	D67DG, M184V, T215IT	V106M, E138K, H221Y, F227C, M230L
NVP	**31,910**	A	Susceptible	Susceptible	M41L, D67N, M184V, L210W, T215F	A98AG, K101E, G190A
NVP	**21,398**	A	Susceptible	L10I	K70E, M184V	V108I, Y181C, H221Y
NVP	**19,714**	A	Susceptible	Susceptible	Susceptible	Susceptible
NVP	**4,624**	A	Susceptible	Susceptible	D67N, K70R, M184V, T215F, K219E	K103N, Y318F
NVP	**3,740**	D	Susceptible	Susceptible	K65R, M184V, K219EK	K101E, Y181C, G190AG
NVP	**126,326**	D	Susceptible	Susceptible	A62AV, K65KR, T69N, M184V	Y181C, G190A
NVP	**253,278**	A	Susceptible	Susceptible	K65R, K70KT, M184V	K103N, Y181C
NVP	**12,559**	A	Susceptible	Susceptible	M184V	V90I, K103N
NVP	**901**	**D**	Susceptible	L10I, Q58E	M184V	K101EK, G190A
NVP	**1,343**	C	Susceptible	Susceptible	M184V	K103N, E138Q
NVP	**2,200**	**A**	Susceptible	L10I	M184V	Susceptible
NVP	**25,107**	AE	Susceptible	L10I	K65R, Y115F	Y181C
NVP	**41,652**	AE	Susceptible	Susceptible	Susceptible	Y181C, H221Y
NVP	**3,337**	A	Susceptible	Sucesptible	K65KR, M184V	K103NS, Y181C
NVP	**2,376**	D	Susceptible	L10V	K65R, M184V, K219Q	K103N
NVP	**1,329**	A	Susceptible	L10V	M184V	K103N
NVP	**4,624**	AE	Susceptible	L10I	M184V	K103KN, G190AG
NVP	**81,619**	D	Susceptible	Sucesptible	K70E, M184V	K103N
NVP	**6,409**	AE	Susceptible	L10I	Susceptible	Susceptible

4.5.3.2 Prevalence of virologic and immunological failure and side effects among study patients

Table 4.24 summarizes the prevalence of virologic and immunological failure and side effects among study patients. Although not significantly different across regimens (p = 0.928), immunological failures CD4 count <500 cells/mls was the more prevalent (66.9%) than virologic failure (33.1%). There were 6.1% of 566 patients who experienced virologic failure (≥ 1001 cells\ml) with the majority 24 (9.4%) of patients on NVP based regimen and 12 (3.8%) receiving EFV (p =0.009). Leucopenia was the most prevalent hematological side effect followed by anemia (29.1%). The AST elevation was more prevalent than ALT elevation. Renal abnormality was more prevalent than hepatotoxicity. Elevated creatinine was common among patients on EFV 64.7% with 49.2% among patients receiving NVP (p = 0.0001). Although there was no difference among study patients with regards to hepatoxicity, and other blood abnormalities; the study reported 28(11%) and 55(21.7%) versus 33(10.6%) and 52(16.7%) patients on NVP and EFV with elevated ALT and AST. Further, 125(49.2%), 74(29.1%), 111(43.7%), 21(8.3%) and 6(2.4%) on NVP while 202(64.7%), 94(30.1%), 126(40.4%), 15(4.8%) and 6(1.9%) on EFV at month 12 had renal abnormalities, were anemic, had leucopenia, had thrombocytopenia and Lymphocytosis respectively (Figure 4.7).

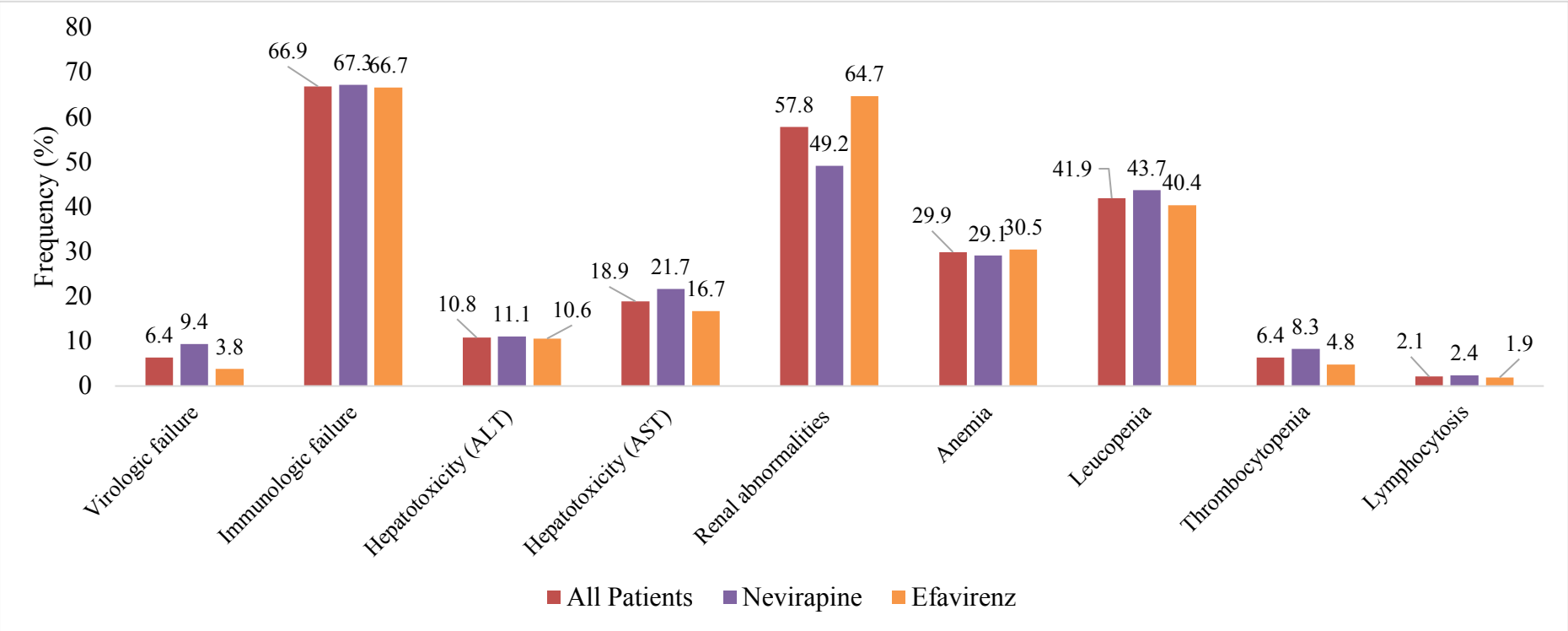


Figure 4.7: Distribution of HIV drug resistant mutation, biochemical and hematological abnormalities among study patients

Table 4.24. Distribution of side effect due to ART among study patients

Current (month 12) Variables		All Patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		*p Value*
		n	(%)	n	(%)	n	(%)	
HIV drug resistant mutation	Yes	34	6.1	24	9.5	10	3.2	
	No	532	93.9	230	90.5	302	96.8	**0.002**
HIV viral load (Cells/mls)	<1000	530	93.6	230	90.6	300	96.2	**0.009**
	≥1001	36	6.4	24	9.4	12	3.8	
CD4+ (cell/µL)	**Median (IQR)**	404	(278 - 545)	404	(287 - 548)	404.5	(273.5 - 543.5)	
	<500	379	66.9	171	67.3	208	66.7	0.928
	≥500	187	33.1	83	32.7	104	33.3	
ALT (U/L)	**Median (IQR)**	25	(18 - 37)	23.5	(17 - 35)	25	(19 - 39.5)	
	<56	505	89.2	226	88.9	279	89.4	0.892
	≥56	61	10.8	28	11.1	33	10.6	
AST (U/L)	**Median (IQR)**	27	(20 - 38)	26	(20 - 40)	28	(20 - 38)	
	<40	459	81.1	199	78.4	260	83.3	0.16
	≥40	107	18.9	55	21.7	52	16.7	
Creatinine (mg/dL)	**Median (IQR)**	0.9	(0.6 - 1.1)	0.9	(0.6 - 1.1)	0.9	(0.7 - 1.1)	
	<0.8	239	42.2	129	50.8	110	35.3	**0.0001**
	≥0.8	327	57.8	125	49.2	202	64.7	
HB (g/dL)	**Median (IQR)**	14.3	(12.8-15.6)	14.3	(12.8 - 15.5)	14.3	(12.8 - 15.7)	
	<13	169	29.9	74	29.1	95	30.5	0.782
	≥13	397	70.1	180	70.9	217	69.6	
WBC (10^3/mm^3)	**Median (IQR)**	4.8	(3.8 - 6.2)	4.7	(3.7 - 6.2)	4.85	(3.8 - 6.2)	
	≤ 4.3 X 10^3	237	41.9	111	43.7	126	40.4	0.442
	>4.3 X 10^3	329	58.1	143	56.3	186	59.6	
Plateletes (10^9/L)	**Median (IQR)**	289	(225 - 359)	287.5	(210 - 358)	289	(230 - 359)	
	<150 X 10^9	36	6.4	21	8.3	15	4.8	0.119
	≥150 X 10^9	530	93.6	233	91.7	297	95.2	
Lymphocytes (10^9/L)	**Median (IQR)**	2.4	(2 - 2.9)	2.4	(2 - 3)	2.4	(2 - 2.8)	
	<4 X 10^9	554	97.9	248	97.6	306	98.1	0.775
	≥4 X 10^9	12	2.1	6	2.4	6	1.9	

4.5.3.4. Variation of nevirapine and efavirenz plasma levels across Pathological and hematological variable

Table 4.25 summarizes the variation in NVP and EFV plasma levels among hematological side effects and other conditions. Patients whose baseline Hemoglobin (Hb) concentration were ≥13 g/dL (6721, IQR = 5092–10471 ng/mL) had higher median (IQR) NVP plasma concentration compared to patients whose baseline HB levels were <13 g/dL (6034, IQR = 4204–8729 ng/mL; p = 0.0272). Further, patients whose health condition limited them a lot from doing certain kinds or amount of work, housework or schoolwork (8798, IQR = 5906–11046 ng/mL) had higher median (IQR) NVP plasma concentration compared to patients whose health condition limited them a little bit from doing certain kinds or amount of work, housework or schoolwork (4990, IQR = 3511–5092 ng/mL; p = 0.037).

Patients with higher median (IQR) EFV plasma concentration were those who did not have ART drug resistant mutation (2758.5, IQR = 1918–5139 ng/mL; p = 0.006), or stop taking

current ARVs due to occurrence of a skin rash (2796.5, IQR = 1923–5091.5 ng/mL; $p = 0.021$). Further, patients whose baseline HB levels were >13 g/dL (2992, IQR = 1949–6120 ng/mL; $p = 0.046$) and those who did not have skin rash (2796.5, IQR = 1923–5091.5 ng/mL; $p = 0.021$) had higher median (IQR) EFV plasma concentration.

Table 4.25. Variation in median nevirapine and efavirenz plasma concentration and pathological and hematological outcomes

Variable	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
	n	Median	(IQR)		*P*	n	Median	(IQR)		*P*
HIV drug resistant mutation										
Yes	24	6062	4119	8786	0.519	10	1373.5	49	2807	**0.006**
No	230	6237.5	4532	9163		302	2758.5	1918	5139	
HIV viral load (Cells/mls)										
<1000	230	6237.5	4532	9095	0.609	300	2764	1919.5	5171.5	**0.002**
≥1001	24	6062	4119	8866		12	1373.5	52.5	2714	
CD4+ (cell/µL)										
<500	171	6359	4577	9198	0.321	208	2739.5	1930	4625	0.911
≥500	83	6114	4143	8323		104	2698.5	1716	6046	
ALT (U/L)										
<56	226	6351	4449	9095	0.749	279	2709	1868	4872	0.584
≥56	28	5691.5	4764	8510		33	3040	2086	5308	
AST (U/L)										
<40	199	6180	4449	8964	0.762	260	2722	1869	5322	0.602
≥40	55	6343	4518	9095		52	2975	1962.5	4184	
Creatinine (mg/dL)										
<0.8	129	6343	4571	8875	0.671	110	2998	1870	5962	0.276
≥0.8	125	6011	4316	9198		202	2585	1886	4461	
Baseline HB (g/dL)										
<13	149	6034	4204	8729	**0.027**	169	2579	1818	4264	**0.046**
≥13	105	6721	5092	10471		143	2992	1949	6120	
HB (g/dL)										
<13	74	5764	4211	7817	0.297	95	2732	1838	4487	0.516
≥13	180	6381	4592	9245.5		217	2747	1904	5204	
WBC (10^3/mm^3)										
≤ 4.3 X 10^3	111	6105	4503	8323	0.267	126	2669	1868	5139	0.782
>4.3 X 10^3	143	6390	4518	9755		186	2758.5	1917	4740	
Lymphocytes (10^9/L)										
<4 X 10^9	248	6237.5	4510.5	9029.5	0.555	306	2750.5	1868	5044	0.909
≥4 X 10^9	6	6771.5	5917	8875		6	2450	2244	3352	

4.5.3.5. Quantile regression analysis of nevirapine and efavirenz plasma concentration by pathological and hematological variables

The quantile regression analysis of NVP and EFV plasma concentration across side effects are shown in Table 4.26. In bivariable linear regression analysis factors associated with NVP plasma concentrations included the current platelets concentration (unadjusted β -3.18, 95% CI -5.9 to -0.5; p =0.02). In multivariate regression current white blood cell concentration (adjusted β 4012.4, 95% CI *1032.2* to 6992.6; $p < 0.001$) independently influenced NVP plasma levels.

In bivariable quantile regression analysis factors associated with EFV plasma concentrations included; the occurrence of HIV drug resistant mutation (unadjusted β = 1388, 95% CI = 484.1 to 2291.9; p =0.003), current viral load concentration (unadjusted β = -1390, 95% CI = -2642.7 to -137.3; p =0.03) and baseline ALT (unadjusted β = -749, 95% CI = -1258.2 to -239.8; p =0.004); stopping taking current ARVs due to nausea (unadjusted β = 653, 95% CI = 312 to 994; p =0.001) and stopped taking current medication due to skin problems such as rash (unadjusted β = 788, 95% CI = 140.4 to 1435.6; p =0.017). None of these factors remained significantly associated with EFV plasma level in multivariate analysis.

Table 4.26. Regression analysis between nevirapine and efavirenz plasma concentrations by pathological and hematological variables

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β	(95% CI)		P-value	Unadjusted β	(95% CI)		P-value
HIV drug resistant mutation	-117	-2064.4	1830.4	0.906	1388	484.1423	2291.858	**0.003**
Current VL (Cells/mls)	117	-2474	2708	0.929	-1390	-2642.732	-137.2677	**0.03**
Baseline CD4+ (cell/µL)	386	-1401	2173	0.671	356	-1703.746	2415.746	0.734
Current CD4+ (cell/µL)	-245	-1024.8	534.8	0.537	21	-630.1351	672.1351	0.949
Baseline HB (g/dL)	687	-480.1	1854.1	0.247	413	-313.3429	1139.343	0.264
Current HB (g/dL)	687	-480.1	1854.1	0.247	15	-437.1669	467.1669	0.948
Baseline ALT (U/L)	1236	-3140.5	5612.5	0.579	-749	-1258.231	-239.7693	**0.004**
Current ALT (U/L)	-555	-2022.6	912.6	0.457	6.9	-0.172292	14.09148	0.056
Baseline AST (U/L)	666	-1536.4	2868.4	0.552	-642	-1713.649	429.6486	0.239
Current AST (U/L)	163	-630.1	956.1	0.686	308	-486.0996	1102.1	0.446
Baseline Creatinine (U/L)	379	-538.4	1296.4	0.417	45	-655.3701	745.3701	0.899
Current Creatinine (U/L)	-332	-1278.7	614.7	0.49	-414	-1005.825	177.8252	0.17
Baseline WBC (103/mm3)	18	-889.2	925.2	0.969	186	-554.3072	926.3072	0.621
Current WBC (103/mm3)	285	-592.6	1162.6	0.523	54	-494.8686	602.8686	0.847
Baseline Lymphocytes (109/L)	1307	-3536.4	6150.4	0.596	-35.85366	-247.921	176.2137	0.74
Current Lymphocytes (109/L)	1236	-817.2	3289.2	0.237	-133	-914.4356	648.4356	0.738
Baseline Plateletes (109/L)	-3.4	-7.3	0.4	0.082	-26	-1813.74	1293.74	0.742
Current Plateletes (109/L)	-3.18	-5.9	-0.5	**0.02**	-75	-2084.477	1934.477	0.942

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β	(95% CI)		P-value	Adjusted β	(95% CI)		P-value
HIV drug resistant mutation	-345.7	-4568.1	3876.6	0.872	-816.1	-6990.2	5358	0.795
Current VL (Cells/mls)	107.4	-4410.2	4624.9	0.963	0.02	-0.04	0.08	0.568
Baseline CD4+ (cell/µL)	726.5	-1255.1	2708.1	0.471	523.5	-1548.3	2595.4	0.619
Current CD4+ (cell/µL)	600.5	-1352.4	2553.4	0.545	-205.4	-1594.1	1183.3	0.771
Baseline HB (g/dL)	1152.3	-997.6	3302.2	0.292	11.9	-1008.8	1032.6	0.982
Current HB (g/dL)	1813.2	-699.7	4326.1	0.156	506.4	-485.7	1498.4	0.316
Baseline ALT (U/L)	-3649.9	-11809	4509.1	0.379	-118.5	-2980.5	2743.6	0.935
Current ALT (U/L)	104.6	-2968.4	3177.5	0.947	-504.4	-1771.5	762.8	0.434
Baseline AST (U/L)	-468.1	-4767	3830.8	0.83	-701.2	-2479.8	1077.3	0.438
Current AST (U/L)	389.5	-2501.2	3280.2	0.791	26.7	-1323.9	1377.2	0.969
Baseline Creatinine (U/L)	-120.0	-1618.7	1378.6	0.875	-112.6	-1299.9	1074.6	0.852
Current Creatinine (U/L)	-494.5	-2312.2	1323.3	0.592	-531	-1549.5	487.4	0.306
Baseline WBC (103/mm3)	169.9	-623.2	963	0.673	24.8	-1739.3	1788.8	0.978
Current WBC (103/mm3)	5071.0	1734.5	8407.5	**0.003**	621.5	-918.7	2161.6	0.428
Baseline Lymphocytes (109/L)	1750.9	-35.8	3537.7	**0.055**	-87.4	-1070.9	896.0	0.861
Current Lymphocytes (109/L)	-838.4	-8279	6602.1	0.824	1514.1	-1519.4	4547.6	0.327
Baseline Plateletes (109/L)	-2011.8	-12238.7	8215.1	0.699	-176.5	-6611.4	6258.3	0.957
Current Plateletes (109/L)	1226.9	-8759.1	11213	0.809	121	-6401.2	6643.2	0.971

4.5.3. Discussion

The study provided a comprehensive relationship between NVP and EFV plasma concentrations and clinical outcomes including HIV drug resistance, hepatic, renal, hematological side effects and medical adverse outcomes. Out of the 254 and 312 patients on NVP and EFV based regimen, 9.5% and 3.2% respectively, were infected with HIV virus resistant to one or more NRTI and NNRTI drugs. This study identified mutations associated with NNRTI for both patients on NVP and EFV as follows: K103N, Y181C, G190S, V106A/M, Y181C, Y188L and P225H. The current results were consistent to the results reported by others (Rhee et al., 2015; De La Cruz et al., 2019). The occurrence of resistant mutation to ARVs did not influence NVP plasma level, even though half 12/24 (50%) of the patients with drug resistant mutation had supra-therapeutic NVP plasma levels with some 3/24 (12.5%) having sub-optimal levels.

On the contrary, drug resistance mutation was linked to lower EFV plasma levels. About of 30% of the 10 patients with drug resistant mutation had sub-optimal plasma level with some 10% having EFV plasma level considered supra-therapeutic levels. Similar results were reported previously among Kenyan women by Oluka *et al*., (2015) which reported no association between presence of HIV drug resistance and NVP plasma levels. Vardhanabhuti *et al*., (2013) on the other hand reported interesting findings where study patients of African lineage harboring HIV resistant to ARVs had a reduced median time to nevirapine IC_{50} of 412 hours compared to Indian women. Our results and those of others could be due to the fact that during the long-term ART treatment, the frequency of specific HIV drug resistant mutations conferring resistance to NVP and EFV (K103N, Y181C and G190A) are dynamic. Vardhanabhuti *et al*., (2013) showed that in the extended life-long ART uptake, the rate of specific HIV drug resistant mutations conferring resistance to NVP and EFV (K103N, Y181C and G190A) were not steady. The study further showed that the frequency of quasi-species with K103N mutation decreases with time, and could even vanish in SGA sequences, while the proportion of Y181C and G190A could increase from 0% to nearly 100%. Further, archival resistance mutations are also key in determining treatment outcomes. In many studies, genotyping proviral DNA establishes that archived HIV drug resistant mutations from previous

ARV types (Derache et al., 2015) in cellular reservoirs (Dalai et al., 2009), with phylogenetic transmission clustering (Kassaye et al., 2009), viral evolution (Zaccarelli et al., 2016), may predict suitability of ARV drug swapping especially in patients responding to treatment (Allavena et al., 2017). Inevitably therefore drug resistant mutations whether transmitted, acquired or archived are crucial in determining the treatment outcomes especially for the NNRTIs which are characterized by low-genetic barrier to development of drug resistance mutation. To develop high-level resistance, for NVP and EFV requires only one DRM and two DMS respectively. Further etravirine requires two DRMs for HIV to develop high-level resistance (Vingerhoets et al., 2010; Melikian et al., 2014). It is therefore paramount to maintain optimum NVP and EFV therapeutic plasma levels, given that a solitary point mutation on the *pol* gene of HIV-1 genome at explicit position may lead to development of high-level resistance mutations to NVP and EFV (Wang et al., 2014). The trough NVP and EFV plasma concentrations (C_{trough}) of ≤3μg/ml and ≤1μg/ml respectively predict greater risk for virologic and clinical failures due to emergence of drug resistant viral variants (Wang et al., 2011).

This study also reported NRTI viral drug resistance that is caused by mutations denoted as either thymidine analogue mutations (TAMs) – (M41L, D67N and T215T/Y), or nucleoside analogue mutations (NAMs) (M184V/I, K65R and K70E). Viruses previously containing multiple TAMs predisposes them to develop extra TAMs than K65R when being managed using TDF or ABC based regimen. Some HIV strains containing several TAMs will also have insertions of double-amino acid at RT position 69 known as T69insertion or T69S_SS. Combined with multiple TAMs, T69S_SS mutation is associated with high-level resistance to tenofovir disoproxil fumarate, abacavir and zidovudine and with intermediate resistance to lamivudine and emtricitabine (Clutter et al., 2016).

The ARV levels in plasma and quantity of ARV excreted into breastmilk has been associated with the rate of ARVs suppression of viral replication as well as the duration of ARV influences viral replication (Davis et al., 2019). Consequently therapeutic drug plasma levels are fundamental to an effective ART (Gunda et al., 2013), given any low drug plasma level detected in patients taking ART is connected to failure to attain speedy virological success and a longer-term immunological failure (Boulle et al., 2008). In this study, patients (n=99) on EFV

regimen with supra therapeutic EFV plasma level had virologic suppression (mean viral load 49.7; SD 440.6 cells/ml) compared to patients (n=199) with therapeutic EFV plasma level (2385.7; SD 14590.6 cells/ml) and patients (n=14) with sub-optimal EFV plasma level who had significant virologic failure (11,628.5; SD 26,931.6 cells/ml). On the contrary, NVP plasma concentration was not associated with viral load; patients (n=37) with sub-optimal NVP had lower mean (SD) viral load (1763.9; SD 7484.8 cells/ml) followed by patients (n =80) with supra therapeutic NVP plasma level (4024.5; SD 25183.1 cells/ml) and those with therapeutic NVP plasma level (9442.9; SD 48418.1 cells/ml). Previously in Kenya, Oluka *et al*., (2015) reported no relationship between NVP plasma level and viral load. Other studies have shown that many HIV patients receiving the efavirenz regimen and have plasma efavirenz concentration of <1.000 mg/L predicts greater risk for virologic failure and an occurrence of HIV drug resistant mutations, while patients with efavirenz concentration above 4.000 mg/L appear to experience many neurological adverse effects (Rotger et al., 2007; Gunda et al., 2013). In Tanzania, Gunda *et al*., (2013) reported a connection between increased frequency of patients with suboptimal NVP and EFV plasma levels with increasing virologic failures and progressing AIDS disease . Concurring observation to the current study was reported in Uganda and Italy (Ahoua et al., 2009; Fabbiani et al., 2011). Inevitably, the occurrence of increased percentages of patients presenting with suboptimal ARV plasma levels is an indication of ever increasing risk of virologic treatment failures and accumulation of HIV resistant strains if circulating in populations (Kasang et al., 2011).

The importance of drug levels has also been shown with regards to immunological outcomes. Several studies have demonstrated the importance of $CD4^+$ T-cell count in predicting progression to AIDS and death (Perrone et al., 2014). Although the current study reported no statistical association between CD4 count and both NVP and EFV plasma levels, there was a tendency towards having higher CD4 counts among patients with sub- therapeutic NVP plasma level and higher CD4 count for patient with supra-therapeutic EFV plasma level. The mean (SD) current CD4 count for sub- therapeutic NVP level was 600.9 (1617.2) cells/μL compared to 313.3(217.9) cells/μL for patients with supra-therapeutic NVP levels. The mean (SD) current CD4 count for sub- therapeutic EFV level was 335.8 (266.4) cells/μL compared to 421.4(190.5) cells/μL for patients with supra-therapeutic EFV levels.

Conflicting results have been reported regarding ARV drugs plasma levels and CD4 count; some studies such as by Oluka *et al*., (2015) detected association between NVP plasma level and greater change in CD4 cell count after ART initiation. Perrone *et al*., (2014) on the other hand reported no differences between NNRTI drug plasma levels and $CD4^+$ T-cell count. Regardless of the results of the current study, the importance of initiating patients testing positive early into ARV treatment, when CD4 cell counts are higher, has been adopted in many countries' HIV treatment guidelines (Lima et al., 2015). Further, mathematical modelling shows the importance test and immediately initiate ART treatment for all persons found infected with HIV. This practices is projected to significantly reduce occurrence of virologic failures and curb rates of new HIV infections in a population (Granich et al., 2009). These evidence led to the new guideline by WHO that require all persons testing HIV positive to be immediately initiated into ART treatment, irrespective of viral load and CD4 cell count (Cohen et al., 2011; Lundgren et al., 2015).

Although this current study found no significant association between NVP and EFV plasma levels and hepatotoxicity (ALT and AST), there was evidence towards increased ALT and AST levels among HIV patients with supra-therapeutic NVP and EFV plasma levels. Data associates patients with NNRTI plasma levels in the supratherapeutic ranges with increased risk of drug complications and toxicities which have been linked to treatment non-compliance and discontinuation (Gunda et al., 2013). The actual association can be best re-confirmed in larger longitudinal studies.

Patients with NVP plasma levels in the supra-therapeutic ranges tended to have anemia, leucopenia, and thrombocytopenia but not lymphocytosis. For patients on EFV had varied relationship with hematologic abnormalities. Patients with sub-therapeutic EFV plasma concentrations tended to have anemia, thrombocytopenia and lymphocytosis while patients with supra-therapeutic EFV plasma concentrations had leucopenia. Moyle (2002) has equated volumes of hematological and biochemical abnormalities to HIV infection. These deformities have been attributed to either; HIV infection, sequel of opportunistic infections, tumors and aftermath of HIV treatment drugs and related illnesses. This area still remains a grey area of

research globally and many patients would benefit significant on the outcome of this monitoring.

Most ARVs especially tenofovir disoproxil fumarate (TDF) linked to kidney injuries and renal adverse effects (McLaughlin et al., 2018). These kidney injuries ranges from higher blood creatinine levels, lowered elimination of blood creatinine, lowed rate of glomerular filtration, nephrolithiasis, urolithiasis, to crystalluria (Gupta et al., 2005). Further although this study reported no association between NVP and EFV plasma level with renal function (creatinine), patients with high NVP plasma levels had higher mean (SD) creatinine level of 0.92(0.4) mg/dL compared to 0.88(0.41) mg/dL to patients with lower NVP plasma levels. On the contrary, patients with higher EFV plasma levels had lower creatinine level compared to patients with lower EFV plasma levels. For all patients, those on 3TC+TDF+ either (NVP/EFV) had creatinine levels above the ≥0.8 mg/dL threshold.

Uptake of EFV based regimen is linked to hypersensitivity reactions that could negatively affect kidney function (McLaughlin et al., 2018). This area patients a significant area of research given that many patients are on these first line combinations therapy in developing countries.

4.5.4. Conclusions

Various mutations conferring resistant to ART were identified. These mutations were associated with virologic outcomes and not with immunological outcomes for patients on EFV. Only anemia was associated with plasma levels. Patients with low levels of EFV had a higher viral load and more mutations.

4.6. ROLE OF CYP2B6 AND CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) ON NEVIRAPINE AND EFAVIRENZ PLASMA CONCENTRATIONS

4.6.1. Introduction

Populations in Sub-Saharan African for extended time frame has gained enormous genetic diversity compared to other global races (Prugnolle et al., 2005). Ethnic groupings are shown to impact population variant alleles distribution leading to variation in drug plasma levels (Ngaimisi et al., 2013). Approximately 70 distinct ethnic groups have been identified in Kenya. These ethnic blocks vary in size ranging from 575 the Dahalo group to over 8 million Kikuyu (KNBS, 2019). The ethnic groups in Kenya is broadly categorized into three linguistic groups namely; the Bantu, Nilotic and Cushites. Although these ethnic groups dwell in close vicinity to each other, there is an extensive environmental and cultural variation between them (KNBS, 2019). Subsequently, there are prevailing extensive host genetic and environmental diversity which have been linked to variation in drug efficiency and adverse reactions occurrence and general treatment outcomes among different African ethnic groups receiving same ART treated regimen (Ngaimisi et al., 2013).

The human cytochrome P450 2B6 enzyme (CYP2B6) is centrally involved in the metabolism of different HIV ARVs drugs. Data has shown the wide polymorphism of CYP2B6 enzyme which impacts the therapeutic response in individuals (Hedrich et al., 2016). Outstandingly, population of African origin show significant degree of diversity in the *CYP2B6* gene (Čolić et al., 2015). The transcription factors pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3) act on genes involved in xenobiotic metabolism and excretion (Faucette et al., 2006; Cortes et al., 2013). EFV has the ability to autoinduce its own metabolism through the activation of PXR and CAR (Faucette et al., 2006).

The hepatic enzyme CYP2B6 has a vital role in both EFV and NVP hydroxylation with NVP also being metabolized by CYP3A4 (Ward et al., 2003). High genetic polymorphism has been observed in the CYP2B6 (chromosome 19) gene with several non-synonymous, synonymous and promoter SNPs identified (Zanger et al., 2007). Currently, about 38 CYP2B6 alleles (*1A

[wild-type] to *38) associated with either increased, decreased or abolished enzymatic activity have been defined (Pharmacogene Variation Consortium; https://www.pharmvar.org/gene/CYP2B6; Zhang et al., 2011). A number of CYP2B6 SNPs impacting EFV and NVP plasma levels such as 516G>T, 785A>G, 983T>C, and 1459C>T, have been studied widely (Hofmann et al., 2008; Zhang et al., 2011; Maimbo et al., 2012; Ngaimisi et al., 2013; Aurpibul et al., 2014). Nonetheless, investigating an individual SNP often do not offer satisfactory data predicting inter and intra personal variations in EFV plasma levels. To provide a more accurate data on the influence of SNPs on ARV plasma levels, studies are recommending evaluating a battery of SNPs that reduce the metabolic function of CYP2B6 (Carr et al., 2010).

The EFV can be metabolized in two phases; Phase 1, EFV is metabolized by majorly by CYP2B6 and to a lesser extent by CYP2A6 (Wyen et al., 2011). Efavirenz is primarily metabolized to 8-hydroxyefavirenz by CYP2B6 (Ward et al., 2003) and to a lesser extent to 7-hydroxy-EFV by CYP2A6 (Ogburn et al., 2010). The direct N glucuronidation of EFV metabolites for excretion by UDP-glucuronosyltransferase (UGT) isoforms (including UGT1A1 and 2B7) represent minor metabolic pathway (Kwara et al., 2009; Ogburn et al., 2010).

The constitutive androstane receptor (CAR) and the preganane X receptor (PXR) control complex metabolizing enzymes involved in phase I and phase II in response to xenobiotics. Prominently, expression of CAR and PXR is associated with the expression of CYP2B6 and CYP2A6 and UGT2B in vivo (Wortham et al., 2007; Wyen et al., 2011). Consequently, CAR is involved in the secondary regulation of the EFV metabolizing enzymes. Studies have since shown the association between CYP2B6 and CAR genetic variations and treatment termination among patients receiving EFV based regimen because of high plasma EFV levels (Wyen et al., 2011). The objective of this objective was to examine the prevalence of thirteen CYP2B6 polymorphisms (329G>T, 341T>C, 444 G>T/C, 15582C>T, 983T>C, 516G>T, 548T>G, 637T>C, 785A>G, 18492C>T, 835G>C, 1459C>T and 21563C>T) and CAR (540C>T) and the impact of these polymorphisms on efavirenz and nevirapine plasma levels. Additionally,

we also investigated the association of CYP2B6 and CAR polymorphisms and haplotypes with efavirenz and nevirapine plasma concentrations.

4.6.2. Methods

The methods used in this section are presented in chapter three sections 3.7.2.1, 3.7.3.1, 3.7.3.3, 3.7.3.4, 3.7.3.5 and 3.7.3.6 while data management and statistical analysis are presented in section 3.7.5 and 3.7.6

4.6.3. Results

4.6.3.1. Allele and genotype frequencies of CYP2B6 and CAR genetic variants

Table 4.27 describes the CYP2B6 and CAR allele and genotype frequencies and their association NVP and EFV plasma levels. Out of the 599 enrolled patients (566; 94.5% responses rate) had all the nevirapine and efavirenz plasma levels and were subsequently evaluated in this section. The most frequently occurring types of mutations at >30% included CYP2B6 516G>T, 785A>G and 21563C>T. Those mutations occurring at a frequency of between 10 to 20% included CYP2B6 18492C>T and CYP2B6 15582C>T. CAR (540C>T) and CYP2B6 983T>C were found at a rate of 5 to 10%. Mutations occurring rarely >0.05% included CYP2B6 1459C>T, CYP2B6 329G>T, CYP2B6 341T>C, CYP2B6 444 G>T/C, CYP2B6 637T>C and CYP2B6 835G>C. The CYP2B6 548T>G SNP was not detected. The genotype and allele frequencies of SNPs were similar, regardless of the ART regimen.

Table 4.27: Frequency distribution of alleles and genotypes of CYP2B6 gene and CAR SNPs across drug regimens

SNPs	Regimen	Allele Frequency - n(%)		Genotype Frequency - n(%)			Hardy Weinberg Eq
		G	**T**	**G/G**	**G/T**	**T/T**	
	All	710(0.6)	422(0.37)	220(0.39)	270(0.48)	76(0.13)	0.65
CYP2B6 516G>T	NVP	315(0.62)	193(0.38)	92(0.36)	131(0.52)	31(0.12)	0.15
	EFV	395(0.63)	229(0.37)	128(0.41)	139(0.45)	45(0.14)	0.47
		A	**G**	**A/A**	**A/G**	**G/G**	
	All	708(0.63)	424(0.37)	218(0.39)	272(0.48)	76(0.13)	0.59
CYP2B6 785A>G	NVP	314(0.62)	194(0.38)	91(0.36)	132(0.52)	31(0.12)	0.14
	EFV	394(0.63)	230(0.37)	127(0.41)	140(0.45)	45(0.14)	0.54
		C	**T**	**C/C**	**C/T**	**T/T**	
	All	710(0.63)	422(0.37)	217(0.38)	276(0.49)	73(0.13)	0.32
CYP2B6 21563C>T	NVP	316(0.62)	192(0.38)	91(0.36)	134(0.53)	29(0.53)	0.062
	EFV	394(0.63)	230(0.37)	126(0.4)	142(0.46)	44(0.14)	0.72
		C	**T**	**C/C**	**C/T**	**T/T**	
	All	916(0.81)	216(0.19)	370(0.65)	176(0.31)	20(0.04)	1
CYP2B6 18492C>T	NVP	405(0.8)	103(0.2)	163(0.64)	79(0.31)	12(0.05)	0.56
	EFV	511(0.82)	113(0.18)	207(0.66)	97(0.31)	8(0.003)	0.57
		C	**T**	**C/C**	**C/T**	**T/T**	
	All	1011(0.89)	121(0.11)	459(0.81)	93(0.16)	14(0.02)	**0.003**
CYP2B6 15582C>T	NVP	451(0.89)	57(0.11)	204(0.8)	43(0.17)	7(0.03)	**0.024**
	EFV	560(0.9)	64(0.1)	255(0.82)	50(0.16)	7(0.02)	**0.03**
		T	**C**	**T/T**	**T/C**	**C/C**	
	All	1055(0.93)	77(0.07)	492(0.87)	71(0.13)	3(0.01)	0.74
CYP2B6 983T>C	NVP	477(0.94)	31(0.06)	224(0.88)	29(0.11)	1(0.004)	1
	EFV	578(0.93)	46(0.07)	268(0.86)	42(0.13)	2(0.01)	0.68
		C	**T**	**C/C**	**C/T**	**T/T**	
	All	1041(0.92)	91(0.08)	480(0.85)	81(0.14)	5(0.01)	0.39
CAR 540C>T	NVP	467(0.92)	41(0.08)	216(0.85)	35(0.14)	3(0.14)	0.21
	EFV	574(0.92)	50(0.08)	264(0.85)	46(0.15)	2(0.01)	1
		C	**T**	**C/C**	**C/T**	**T/T**	
	All	1124(0.99)	8(0.01)	560(0.99)	4(0.01)	2(0.004)	**0.002**
CYP2B6 1459C>T	NVP	503(0.99)	5(0.01)	250(0.98)	3(0.01)	1(0.004)	**0.02**
	EFV	621(0.99)	3(0.005)	310(0.99)	1(0.003)	1(0.003)	**0.005**
		G	**T**	**G/G**	**G/T**	**T/T**	
	All	1128(0.99)	4(0.01)	562(0.99)	4(0.01)	0	1
CYP2B6 329G>T	NVP	504(0.99)	4(0.01)	250(0.98)	4(0.02)	0	1
	EFV			312(1)	0	0	
		T	**C**	**T/T**	**T/C**	**C/C**	
	All	1121(0.99)	11(0.01)	555(0.98)	11(0.02)	0	1
CYP2B6 341T>C	NVP	500(0.98)	8(0.02)	246(0.97)	8(0.03)	0	1
	EFV	621(0.99)	3(0.01)	309(0.99)	3(0.01)	0	1
		G	**T**	**G/G**	**G/T**	**T/T**	
	All	1121(0.99)	1190.01)	555(0.98)	11(0.02)	0	1
CYP2B6 444G>T/C	NVP	500(0.98)	8(0.02)	246(0.97)	8(0.03)	0	1
	EFV	621(0.99)	3(0.01)	309(0.99)	3(0.01)	0	1
		T	**C**	**T/T**	**T/C**	**C/C**	
	All	1131(0.99)	1(0.01)	565(0.99)	1(0.01)	0	1
CYP2B6 637T>C	NVP	507(0.99)	1(0.01)	253(0.99)	1(0.01)	0	1
	EFV	624(1)	0	312(1)	0	0	
		G	**C**	**G/G**	**G/C**	**C/C**	
	All	1120(0.99)	12(0.01)	554(0.98)	12(0.02)	0	1
CYP2B6 835G>C	NVP	500(0.98)	8(0.02)	246(0.97)	8(0.03)	0	1
	EFV	602(0.99)	4(0.01)	308(0.98)	4(0.01)	0	1
		T	**C**	**T/T**	**T/C**	**C/C**	
	All	1132(1)	0	480(0.85)	0	0	
CYP2B6 548T>G	NVP	508(1)	0	216(0.85)	0	0	
	EFV	574(0.92)	0	264(0.85)	0	0	

4.6.3.2 The CYP2B6 and CAR SNPs Allele and genotype distribution across ethnic groups

Table 4.28 summarizes the allele and genotype frequencies of SNPs across ethnic groups. Three major ethno-linguistic groups were recruited in this study including 367 (64.8%) Niger-Congo (Bantus), 190 (33.6%) Nilo-Sahara (Nilotic) and 9(1.6%) Afro-Asiatic (Cushite). The median (IQR) of EFV and NVP was not statistically different across ethnic groups ($p = 0.66$ and $p = 0.772$ respectively). The distribution of viral load, the occurrence of HIV drug resistant mutations, ALT, AST, creatinine, BMI presence of body rash and the number of SNPs were not different across the three ethnic groups.

Sixteen different alleles were classified among this population with wild-type C and mutant T were the most dominant. No statistical difference in the frequency of wildtype and mutant alleles across the ethnolinguistic groups. Further there was no significant difference in the genotype frequency between the three population. However, CYP2B6 15582 C>T was more prevalent amongst the bantus and Cushites (11.7% vs 8.4%). The difference in the prevalence of the 1558C>T was almost statistically significant ($p = 0.14$).

Table 4.28: The CYP2B6 gene and CAR SNPs allele and genotype frequency distribution across ethnicity

SNPs	Ethnicity	Allele Frequency - n(%)		P value	Genotype Frequency - n(%)			P value
		C	**T**		**C/C**	**C/T**	**T/T**	
	Bantu	648 (88.3)	86 (11.7)		292 (79.6)	64 (17.4)	11 (2.9)	
CYP2B6 15582C>T	Nilote	348 (91.6)	32 (8.4)	0.14	161 (84.7)	26 (13.7)	3 (1.6)	0.313
	Cushites	15 (83.3)	3 (16.7)		6 (66.7)	3 (33.3)	0	
		G	**T**		**G/G**	**G/T**	**T/T**	
	Bantu	457 (62.3)	277 (37.7)		138 (37.6)	181(49.3)	48 (13.1)	
CYP2B6 516G>T	Nilote	238 (62.6)	142 (37.4)	0.294	76 (40)	86 (45.3)	28 (14.7)	0.454
	Cushites	15 (83.3)	3 (16.7)		6 (66.7)	3 (33.3)	0	
		A	**G**		**A/A**	**A/G**	**G/G**	
	Bantu	456 (62.1)	278 (37.9)		137 (37.3)	182 (49.6)	48 (13.1)	
CYP2B6 785A>G	Nilote	237 (62.4)	143 (37.6)	0.29	75 (39.5)	87 (45.8)	28 (14.7)	0.458
	Cushites	15 (83.3)	3 (16.7)		6 (66.7)	3 (33.3)	0	
		C	**T**		**C/C**	**C/T**	**T/T**	
	Bantu	592 (80.7)	142 (19.3)		237 (64.6)	118 (32.2)	12 (3.2)	
CYP2B6 18492C>T	Nilote	308 (81.1)	72 (18.9)	0.785	126 (66.3)	56 (29.4)	8 (4.2)	0.857
	Cushites	16 (88.9)	2 (11.1)		7 (77.8)	2 (22.2)	0	
		T	**C**		**T/T**	**T/C**	**C/C**	
	Bantu	689 (93.9)	45 (6.1)		323 (88)	43 (11.7)	1 (0.3)	
CYP2B6 983T>C	Nilote	350 (92.1)	30 (7.9)	0.336	162 (85.2)	26 (13.7)	2 (1.1)	0.328
	Cushites	16 (88.9)	2 (11.1)		7 (77.8)	2 (22.2)	0	
		C	**T**		**C/C**	**C/T**	**T/T**	
	Bantu	456 (62.1)	278 (37.9)		134 (36.5)	188 (51.2)	45 (12.2)	
CYP2B6 21563C>T	Nilote	239 (62.9)	141 (37.1)	0.286	77 (40.5)	85 (44.7)	28 (14.7)	0.267
	Cushites	15 (83.3)	3 (16.7)		6 (66.7)	3 (33.3)	0	
		C	**T**		**C/C**	**C/T**	**T/T**	
	Bantu	731 (99.6)	3 (0.4)		364 (99.2)	3 (0.8)	0	
CYP2B6 1459C>T	Nilote	375 (98.7)	5 (1.3)	0.256	187 (98.4)	1 (0.5)	2 (1)	0.249
	Cushites	18 (100)	0		9 (100)	0	0	
		C	**T**		**C/C**	**C/T**	**T/T**	
	Bantu	675 (91.9)	59 (8.1)		312 (85)	51 (13.9)	4 (1.1)	
CAR 540C>T	Nilote	350 (92.1)	30 (7.9)	0.831	161 (84.7)	28 (14.7)	1 (0.5)	0.804
	Cushites	16 (88.9)	2 (11.1)		7 (77.8)	2 (22.2)	0	

4.6.3.3. Hardy-Weinberg equilibrium of CYP 2B6 and CAR genetic variants

Hardy-Weinberg equilibrium could not be examined for CYP2B6 329G>T, 341T>C, 444 G>T/, 637T>C, 835G>C, due to deficient in homozygous mutant as well as deficient for both heterozygous and homozygous mutants for CYP2B6 548T>G. In five CYP2B6 SNPs including 516G>T, 785A>G, 983C>TA, 18492C>T, and 21563C>T and one CAR (540C>T) analyzed, there was no statistical difference between expected and observed genotype numbers by ARV regime. However, a significant deficit of heterozygosity at CYP2B6 1459C>T was observed in the entire population as well as those on NVP and EFV based regimen ($p > 0.05$). Seven (7) out of the 13 CYP2B6 SNPs and the one CAR SNPs that conformed to Hardy–Weinberg equilibrium and were further analyzed with the SNPStats software (Table 4.27).

4.6.3.4. CYP2B6 and CAR Linkage disequilibrium

Table 4.29 summarizes the pairwise linkage disequilibrium in CYP2B6 and CAR SNPs among study patients. The extent of LD was quantified among the CYP2B6 and CAR SNP pairs in all the study patients. Strong LD, defined by high values for both D' (≥0.8) and r^2 (≥0.5) parameters, were observed between SNP pairs CYP2B6 516–785, 516-21563, 785-2156, 444-341, 835-341 and 835-444. The 516-18492 pair, 785-18492 pair and 18492-21563 pair, 835-329 pair, 444 – 329 pair and 341 – 329 pair had high D' (>0.8) and moderate r^2 (0.12–0.351) values. Other than the CAR 540, all other SNP pairs had highly variable D' (0–0.8) and low r^2 (<0.1) values in all populations (Figure 4.8)

Table 4.29 Pairwise linkage disequilibrium in CYP2B6 and CAR SNPs among study patients

D' statistic

	15582C>T	516G>T	785A>G	18492C>T	983T>C	21563C>T	1459C>T	CAR.540C>T	329G>T	341T>C	444G>T/C	637T>C	835G>C
15582C>T		0.998	0.955	0.9962	0.0262	0.9076	0.9308	0.3865	0.0436	0.9239	0.9239	0.899	0.9599
516G>T			**0.9997**	0.9565	0.9968	**0.981**	0.324	0.0401	0.9429	0.7653	0.7653	0.7581	0.5498
785A>G				0.9572	0.9968	**0.9887**	0.3272	0.0342	0.9432	0.795	0.795	0.7593	0.5785
18492C>T					0.9948	0.9782	0.6556	0.0148	0.8852	0.9574	0.9574	0.5274	0.9775
983T>C						0.9968	0.8913	0.0175	0.135	0.7251	0.7251	0.0238	0.8415
21563C>T							0.324	0.0401	0.9429	0.7653	0.7653	0.7581	0.5498
1459C>T								0.3035	0.0143	0.2389	0.2389	0.0837	0.3023
CAR.540C>T									0.7352	0.8989	0.8989	0.0106	0.9466
329G>T										0.9785	0.9785	0.0869	0.9785
341T>C											**0.9918**	0.0812	**0.9918**
444G>T/C												0.0812	**0.9918**
637T>C													0.0804
835G>C													

r^2 statistic

	15582C>T	516G>T	785A>G	18492C>T	983T>C	21563C>T	1459C>T	CAR.540C>T	329G>T	341T>C	444G>T/C	637T>C	835G>C
15582C>T	.	0.07086	0.06538	0.02799	0.00001	0.05861	0.00074	0.00156	0.00006	0.00100	0.00100	0.00598	0.00118
516G>T			**0.99202**	0.12824	0.04310	**0.96236**	0.00045	0.00024	0.00187	0.00341	0.00341	0.00030	0.00193
785A>G				0.12938	0.04343	**0.97003**	0.00046	0.00017	0.00189	0.00371	0.00371	0.00031	0.00214
18492C>T					0.01703	0.13410	0.01297	0.00008	0.00066	0.00213	0.00213	0.00006	0.00241
983T>C						0.04310	0.00041	0.00025	0.00089	0.00038	0.00038	0.00001	0.00055
21563C>T							0.00045	0.00024	0.00187	0.00341	0.00341	0.00030	0.00193
1459C>T								0.00750	0.00010	0.00000	0.00000	0.00087	0.00001
CAR.540C>T									0.00017	0.00069	0.00069	0.00000	0.00084
329G>T										0.34598	0.34598	0.00188	0.31686
341T>C											**0.98367**	0.00060	**0.90079**
444G>T/C												0.00060	**0.90079**
637T>C													0.00053
835G>C													

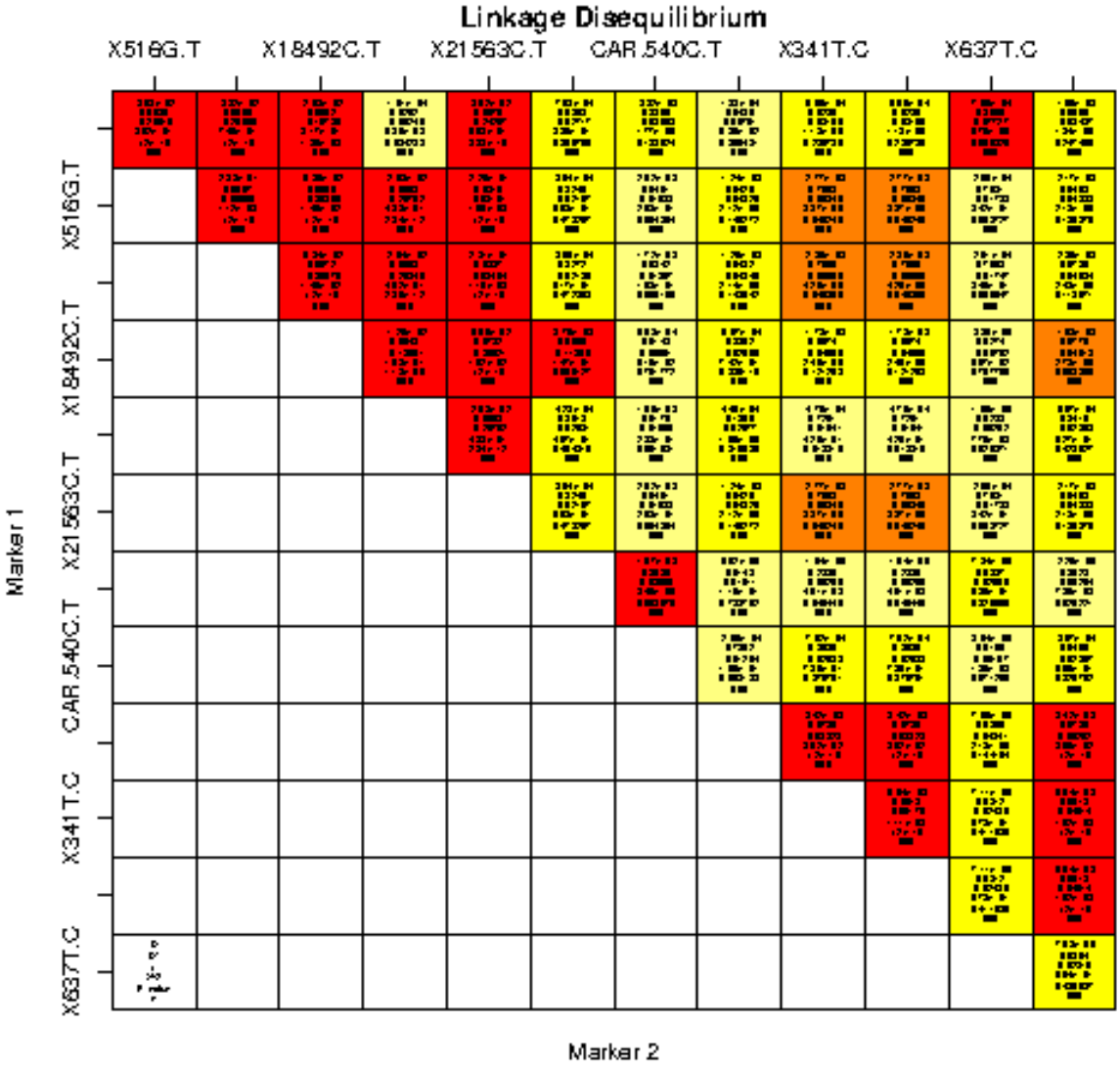
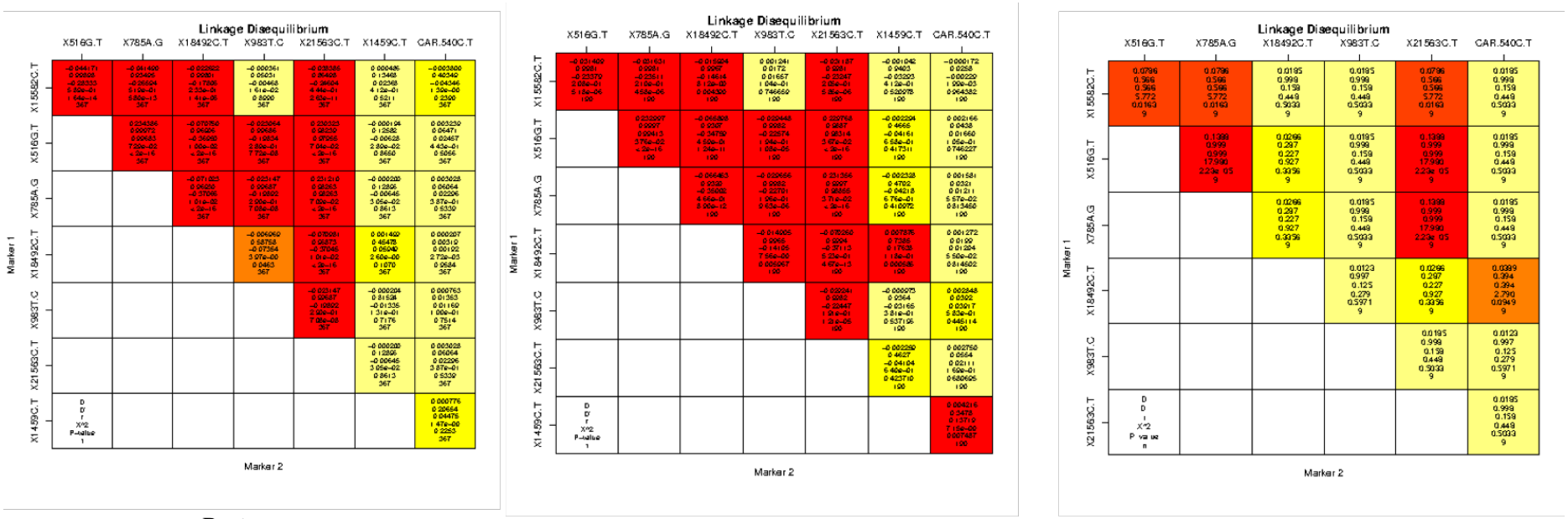


Figure 4.8. Linkage disequilibrium analysis of 6 Single Nucleotide Polymorphisms of CYP2B6 and 1 CAR for patience on NVP and EFV based regimen. Dark red squares: strong evidence of linkage disequilibrium, dark yellow/orange squares: uninformative, light yellow squares: strong evidence of recombination

Evaluating the magnitude of LD among the CYP2B6 and CAR SNP pairs in the three populations studied in Kenya revealed; Strong LD, defined by high values for both D' (≥0.8) and r^2 (≥0.5) parameters, was only observed between SNP pairs CYP2B6 516–785, 516-21563 and 785-2156 (all populations) **(**table 4.32**)**. The 516-18492 pair, 785-18492pair and 18492-21563 pair had high D' (>0.8) and moderate r^2 (0.12–0.351) values in all populations except the Cushitic group. The CAR 540 – 188492 pair, had high D' (≥0.8) and moderate r^2 (0.155) values in the Cushite group. All other SNP pairs had highly variable D' (0–0.8) and low r^2 (<0.1) values in all populations (Figure 4.9)



Bantus **Nilotic** **Cushite**

Figure 4.9. Pairwise linkage disequilibrium in CYP2B6 and CAR SNPs among study populations. Dark red squares: strong evidence of Linkage disequilibrium, dark yellow/orange squares: uninformative, light yellow squares: strong evidence of recombination

4.6.3.5. Variation of NVP and EFV plasma levels across cytochrome P450 2B6 genetic variants

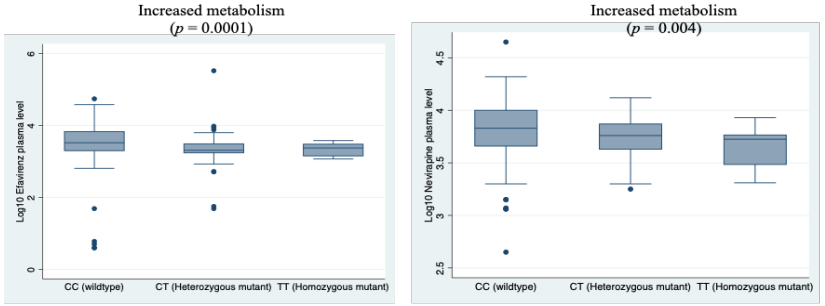
Table 4.30 is a summary of variation in NVP and EFV plasma concentration among CYP2B6 and CAR genetic variants. The following SNPs were associated with high NVP plasma levels (reduced metabolism) including CYP2B6 329 GT; CYP2B6 15582C>T, CYP2B6 516G>T, CYP2B6 785A>G and CYP2B6 21563C>T. On the contrary, SNPs CYP2B6 18492C>T and CYP2B6 983T>C were associated with lower levels of NVP plasma concentration (increased metabolism). The number of SNPs per patient was also associated with high NVP plasma level (reduced metabolism). The higher the number of SNPs per patient, the higher the reduction in NVP metabolism.

For patients on EFV, the following SNPs were associated with high EFV plasma levels including CYP2B6 15582C>T, CYP2B6 516G>T, CYP2B6 785A>G, and CYP2B6 21563C>T. Only SNPs CYP2B6 18492C>T was associated with lowered EFV plasma level. Of interest to note, the SNP CYP2B6 983T>C which was associated with high NVP plasma level was associated also associated high EFV plasma level. Similar to NVP, The higher the number of SNPs per patient, the higher the plasma concentration EFV.

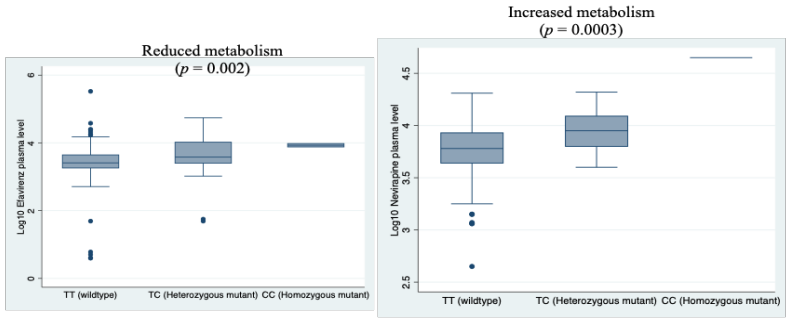
The relationships between NVP and EFV plasma levels and CYP2B6 15582C>T, CYP2B6 516G>T, CYP2B6 18492C>T and CYP2B6 983T>C and CYP2B6 1459C>T genotypes are further summarized in Figures 4.10

Table 4.30 Variation in median NVP and EFV plasma concentration and cytochrome P450 2B6 and CAR genetic variants

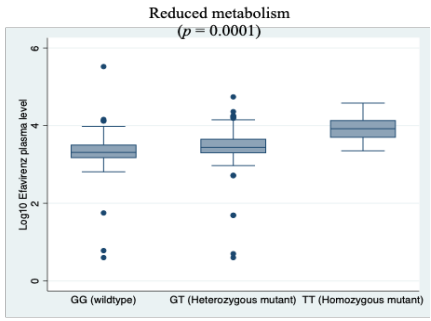
Variable	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
	n	Median	(IQR)		*P*	n	Median	(IQR)		*P*
CYP2B6 329G>T (allele *35)										
GG (wildtype)	250	6191	4503	8875	**0.032**	312	2739.5	1878	4891.5	Omitted
GT (Heterozygous mutant)	4	12328	8627.5	16807.5						
CYP2B6 341T>C (allele *35)										
TT (wildtype)	246	6282	4558	8889	0.368	309	2732	1886	4872	0.837
TC (Heterozygous mutatnt)	8	3675.5	1163.5	12328		3	3680	1652	5308	
CYP2B6 444G>T/C (allele *35)										
GG (wildtype)	246	6282	4558	8889	0.368	309	2732	1886	4872	0.837
GT (Heterozygous mutant)	8	3675.5	1163.5	12328		3	3680	1652	5308	
CYP2B6 15582C>T (allele *1C, *13B, *15)										
CC (wildtype)	204	6170.5	4347.5	8795.5		255	2747	1918	5204	
CT (Heterozygous mutant)	43	6141	4607	8964	**0.033**	50	2402	1633	3519	**0.07**
TT (Homozygous mutant)	7	10858	8034	12278		7	4378	2539	9313	
CYP2B6 516G>T (allele *6,*7,*9,*13A,*13B,*26,*34,*36,*37)										
GG (wildtype)	92	5443.5	4077.5	8017.5		128	2037.5	1500.5	3169.5	
GT (Heterozygous mutant)	131	6390	4673	8875	**0.003**	139	2754	1985	4487	**0.001**
TT (Homozygous mutant)	31	8638	5911	11612		45	8282	5044	13564	
CYP2B6 548T>G (allele *37)										
TT(wildtype)	254	6237.5	4518	8964	Omitted	312	2739.5	1878	4891.5	Omitted
CYP2B6 637T>C										
TT (wildtype)	253	6202	4518	8964	0.526	312	2739.5	1878	4891.5	Omitted
TC (Heterozygous mutatnt)	1	8001	8001	8001						
CYP2B6 785A>G (allele *4,*6,*7,*13A,*13B,*16,*26,*34,*36,*37)										
AA (wildtype)	91	5439	4012	8034		127	2043	1548	3182	
AG (Heterozygous mutatnt)	132	6381	4763	8859	**0.003**	140	2743	1968	4403	**0.001**
GG (Homozygous mutant)	31	8638	5911	11612		45	8282	5044	13564	
CYP2B6 18492C>T										
CC (wildtype)	163	6769	4599	9935		207	3300	2014	6745	
CT (Heterozygous mutant)	79	5692	4247	7396	**0.004**	97	2059	1718	3095	**0.001**
TT (Homozygous mutant)	12	5283.5	3052	5800		8	2391	1434	3033.5	
CYP2B6 835G>C										
GG (wildtype)	246	6282	4558	8889	0.368	308	2722	1878	4891.5	0.541
GC (Heterozygous mutant)	8	3675.5	1163.5	12328		4	4273.5	2666	5087.5	
CYP2B6 983T>C (allele *16,*18)										
TT (wildtype)	224	6042.5	4347.5	8574		268	2579.5	1835.5	4419.5	
TC (Heterozygous mutant)	29	8889	6343	12278	**0.000**	42	3810	2520	10554	**0.002**
CC (Homozygous mutant)	1	44207	44207	44207		2	8522.5	7572	9473	
CYP2B6 21563C>T (allele *9,*13A,*13B,*19,*38)										
CC (wildtype)	91	5439	4012	8001		126	2037.5	1548	3182	
CT (Heterozygous mutant)	134	6402.5	4888	9198	**0.007**	142	2759.5	1985	4487	**0.001**
TT (Homozygous mutant)	29	8575	5911	11085		44	7970	4977.5	13187.5	
CYP2B6 1459C>T/A (allele (CA) *33, *34 (CT) *5)										
CC (wildtype)	250	6237.5	4503	8964		310	2739.5	1870	4872	
CA/CT (Heterozygous mutant)	3	6139	4532	8798	0.478	1	332701	332701	332701	0.19
AA/TT (Homozygous mutant)	1	11046	11046	11046		1	2019	2019	2019	
CAR 540C>T										
CC (wildtype)	216	6308	4510.5	9245.5		264	2687	1878	5025	
CT (Heterozygous mutant	35	6114	4370	7633	0.296	46	3016.5	1833	4872	0.966
TT (Homozygous mutant)	3	7817	7453	9755		2	2640.5	2019	3262	
Number of SNPs per patient										
0	9	3631	3106	4935		24	2047.5	1555.5	2610.5	
1	42	5473.5	4012	8729		51	2007	1375	3067	
2	27	5189	4444	7007		37	2001	1447	3157	
3	52	6524.5	4588	8539		61	2590	1987	3867	
4	70	6393.5	5128	9935	**0.008**	81	2978	1951	4872	**0.001**
5	17	7213	5794	11324		11	4050	2526	13742	
6	30	8478.5	4518	11046		41	7506	4911	11541	
7	7	6291	2630	15831		6	8616.5	3693	17800	

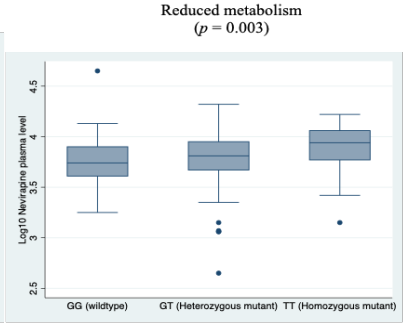


CYP2B6 18492C>T

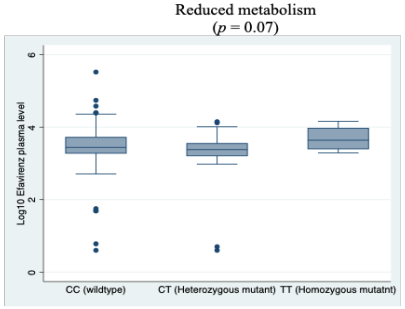


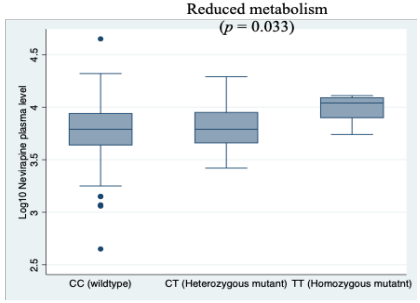
CYP2B6 983T>C





CYP2B6 516G>T





CYP2B6 15582C>T

Figure 4.10. The differences in log_{10}-transformed nevirapine and efavirenz plasma concentrations by genotypes of the following single nucleotide polymorphisms: CYP2B6 329G>T, CYP2B6 444G>T/C, CYP2B6 341T>C and CYP2B6 15582C>T

4.6.3.6. Quantile regression analysis of nevirapine and efavirenz plasma concentration by CYP2B6 genetic variants

Table 4.31 describes quantile regression analysis estimating the relationships between NVP and EFV plasma levels and CYP2B6 genetic variants. In bivariable analysis the SNPs that were associated with increased NVP plasma levels included CYP2B6 516G>T (*p <0.001*), CYP2B6 637T>C (*p <0.001*), CYP2B6 785A>G (*p <0.001*), CYP2B6 983T>C (*p* =0.016), CYP2B6 21563 C>T (*p <0.001*) and number of SNPs per patients (*p* =0. 001). The CYP2B6 18492 C >T was associated with lower NVP plasma level (*p* =0.001). In multivariate analysis SNPs that were independently associated with high NVP plasma level included; CYP2B6 329G>T (*p <0.001*), CYP2B6 516G>T (*p* =0.019) and CYP2B6 983T>C (*p* =0.028) and number of SNPs per patients (*p <0.001*). CYP2B6 341T>C (*p <0.001*), CYP2B6 18492 C >T (*p* =0.004) and CYP2B6 CAR 540 C>T (*p* =0.008 remained significantly associated with lower NVP plasma level.

In bivariable analysis the SNPs that were linked to an increased EFV plasma levels included the presence of CYP2B6 516G>T (*p <0.001*), CYP2B6 785A>G (*p <0.001*), CYP2B6 983T>C (*p* =0.02), CYP2B6 i21563 C>T (*p <0.001*) and number of SNPs per patient). CYP2B6 18492 C >T was associated with lower EFV plasma level (*p* =0.001). In multivariate analysis CYP2B6 516G>T (*p <0.001*), CYP2B6 835 G >C (*p <0.001*) and number of SNPs per patient (*p <0.001*) remained significantly associated with higher EFV plasma level.

Table 4.31. Regression analysis between NVP and EFV plasma concentrations by CYP2B6 genetic variants

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β	(95% CI)		P-value	Unadjusted β	(95% CI)		P-value
CYP2B6 329G>T (allele *35)	7130	-97.3	14357.3	**0.053**		Omitted		
CYP2B6 341T>C (allele *35)	-360	-8375.4	7655.4	0.93	948	-1337.9	3233.9	0.415
CYP2B6 444G>T/C (allele *35)	-360	-8375.4	7655.4	0.93	948	-2103.6	3999.6	0.541
CYP2B6 15582C>T (allele *1C, *13B, *15)	960	-257	2177	0.122	54	-536.4	644.4	0.857
CYP2B6 516G>T (allele *6,*7,*9,*13A,*13B,*26,*34,*36,*37)	1362	938.8	1785.2	**0.0001**	1583	1094.5	2071.5	**0.0001**
CYP2B6 548T>G (allele *37)		Omitted				Omitted		
CYP2B6 637T>C	1799	1436.2	2161.8	**0.0001**		Omitted		
CYP2B6 785A>G (allele *4,*6,*7,*13A,*13B,*16,*26,*34,*36,*37)	1363.5	691.1	2035.9	**0.0001**	1570	1061	2079	**0.0001**
CYP2B6 18492C>T	-923	-1447.6	-398.4	**0.001**	-896	-1398.5	-393.5	**0.001**
CYP2B6 835G>C	-360	-9836.8	9116.8	0.94	948	-1574.4	3470.4	0.46
CYP2B6 983T>C (allele *16,*18)	3872	731.8	7012.2	**0.016**	2068	321.6	3814.4	**0.02**
CYP2B6 21563C>T (allele *9,*13A,*13B,*19,*38)	1363.5	819.2	1907.8	**0.0001**	1577	1028.9	2125.1	**0.0001**
CYP2B6 1459C>T/A (allele (CA) *33, *34 (CT) *5)	2422	-996.4	5840.4	0.164	-364	-267177	266449	0.998
CAR 540C>T	111	-749.7	971.7	0.8	275	-610.3	1160.3	0.542
Number of SNPs per patient	614.5	247.9	981.1	**0.001**	517	364	670	**0.0001**

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β	(95% CI)		P-value	Adjusted β	(95% CI)		P-value
CYP2B6 329G>T (allele *35)	11343.2	5738.8	16947.6	**0.0001**		Omitted		
CYP2B6 341T>C (allele *35)	-8614.2	-9621.7	-7606.7	**0.0001**	-1187	-2711.3	337.3	0.126
CYP2B6 444G>T/C (allele *35)		Omitted			-1187	-2711.3	337.3	0.126
CYP2B6 15582C>T (allele *1C, *13B, *15)	771.2	-735.7	2278.1	0.314	393	-151.8	937.8	0.157
CYP2B6 516G>T (allele *6,*7,*9,*13A,*13B,*26,*34,*36,*37)	3603.4	589.6	6617.2	**0.019**	2414	1876.6	2951.4	**0.0001**
CYP2B6 548T>G (allele *37)		Omitted				Omitted		
CYP2B6 637T>C	786.8	-347.8	1921.4	0.173		Omitted		
CYP2B6 785A>G (allele *4,*6,*7,*13A,*13B,*16,*26,*34,*36,*37)	-3051.6	-7305.8	1202.6	0.159	-1712	-11388.5	7964.5	0.728
CYP2B6 18492C>T	-1301.4	-2195.3	-407.5	**0.004**	-181	-718.3	356.3	0.508
CYP2B6 835G>C		Omitted			1591	1053.6	2128.4	**0.0001**
CYP2B6 983T>C (allele *16,*18)	2948	313.2	5582.8	**0.028**	2877	1498	4256	**0.0001**
CYP2B6 21563C>T (allele *9,*13A,*13B,*19,*38)	-1745	-5138.2	1648.2	0.312	1025	-8636.3	10686.3	0.835
CYP2B6 1459C>T/A (allele (CA) *33, *34 (CT) *5)	-489.8	-1855.4	875.8	0.481	147	-318772.9	319066.9	0.999
CAR 540C>T	-1543.2	-2683.7	-402.7	**0.008**	269	-678.2	1216.2	0.577
Number of SNPs per patient	1129.6	729.9	1529.3	**0.0001**	570	362	778	**0.0001**

4.6.3.7. Quantile regression analysis of NVP and EFV plasma concentration by CYP2B6 and CAR genetic variants controlling for ethnicity

Controlling for ethnicity and ART adherence did not alter the relationships of CYP2B6 and CAR SNPs on both NVP and EFV plasma levels (Table 4.32).

Table 4.32. Regression analysis between NVP and EFV plasma concentrations by CYP2B6 genetic variants adjusting for ethnicity

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Unadjusted β	(95% CI)		P-value	Unadjusted β	(95% CI)		P-value
CYP2B6 329G>T (allele *35)	7130	-1440.6	15700.6	**0.103**		Omitted		
CYP2B6 341T>C (allele *35)	-360	-9104.2	8384.2	0.935	968	-1672.2	3608.2	0.471
CYP2B6 444G>T/C (allele *35)	-360	-8921.9	8201.9	0.934	968	-1916.2	3852.2	0.509
CYP2B6 15582C>T (allele *1C, *13B, *15)	936.5	-693	2566	0.259	77	-434.5	588.5	0.767
CYP2B6 516G>T (allele *6,*7,*9,*13A,*13B,*26,*34,*36,*37)	1442	845.2	2038.8	**0.0001**	1584	1090.8	2077.2	**0.0001**
CYP2B6 548T>G (allele *37)		Omitted				Omitted		
CYP2B6 637T>C	1782	1230.2	2333.8	**0.0001**		Omitted		
CYP2B6 785A>G (allele *4,*6,*7,*13A,*13B,*16,*26,*34,*36,*37)	1442	574.0	2310.0	**0.0001**	1584	912	2256	**0.0001**
CYP2B6 18492C>T	-1006	-1792.0	-220.1	**0.012**	-896	-1345.2	-446.8	**0.001**
CYP2B6 835G>C	-360	-8400.3	7680.3	0.93	971	-1125.5	3067.5	0.363
CYP2B6 983T>C (allele *16,*18)	3855	1039.9	6670.1	**0.007**	2105	666.4	3543.6	**0.004**
CYP2B6 21563C>T (allele *9,*13A,*13B,*19,*38)	1428	750.4	2105.6	**0.0001**	528	1084.0	2025.0	**0.0001**
CYP2B6 1459C>T/A (allele (CA) *33, *34 (CT) *5)	2419.5	-684.9	5523.9	0.126	-373	-267146	266400	0.998
CAR 540C>T	72	-1204.6	1348.6	0.912	300	-270.3	870.3	0.301
Number of SNPs per patient	585	228.8	941.2	**0.001**	528	355	701	**0.0001**

Variable	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
	Adjusted β	(95% CI)		P-value	Adjusted β	(95% CI)		P-value
CYP2B6 329G>T (allele *35)	12655	6429.0	18881.0	**0.0001**		Omitted		
CYP2B6 341T>C (allele *35)	-5190	-6194.0	-4186.0	**0.0001**	-1577	-3774.0	620.0	0.159
CYP2B6 444G>T/C (allele *35)		Omitted				Omitted		
CYP2B6 15582C>T (allele *1C, *13B, *15)	710.4	-433.2	1854.0	0.222	268	-725.9	1261.9	0.596
CYP2B6 516G>T (allele *6,*7,*9,*13A,*13B,*26,*34,*36,*37)	3341	873.4	5808.6	**0.008**	2262	987.7	3536.3	**0.0001**
CYP2B6 548T>G (allele *37)		Omitted				Omitted		
CYP2B6 637T>C	1132	-406.9	2671.7	0.149		Omitted		
CYP2B6 785A>G (allele *4,*6,*7,*13A,*13B,*16,*26,*34,*36,*37)	-2689.2	-5408.2	29.7	0.053	7843	-1920.7	17606.7	0.115
CYP2B6 18492C>T	-1137	-2114.6	-159.4	0.023	-377	-1050.9	296.9	0.272
CYP2B6 835G>C		Omitted			1425	1249.1	4186.9	**0.015**
CYP2B6 983T>C (allele *16,*18)	3474	1389.9	5559.7	**0.001**	2718	1249	4187	**0.0001**
CYP2B6 21563C>T (allele *9,*13A,*13B,*19,*38)	-1460.2	-4473.1	1552.7	0.341	-8922	-18626.6	782.6	0.071
CYP2B6 1459C>T/A (allele (CA) *33, *34 (CT) *5)	-289	-1955.9	1377.9	0.733	26	-318937.3	318989.3	0.999
CAR 540C>T	-1322	-2505.8	-138.2	**0.029**		Omitted		
Number of SNPs per patient	1009.2	407.4	1611.0	**0.001**	195	-528	918	0.596

4.6.3.8. CYP2B6 and CAR haplotype frequency and association with NVP and EFV concentrations and across ethnic communities

Haplotype analysis assess the relationship between manifold SNPs that causing either a decrease or an increase in the metabolic task of CYP2B6 and CAR. Table 4.33 summarizes the haplotype frequencies and their relationship with NVP and EFV plasma concentration. Controlling for ethnicity did not alter the relationships of haplotypes and both NVP and EFV plasma levels (Table 4.33).

Linkage disequilibrium among 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T was detected, giving rise to 8 haplotypes for patient on NVP based regime of which CTGCTTCC was more frequent (0.328), followed by CGACTCCC (0.241), CGATTCCC (0.182), TGACTCCC (0.093), CGACCCCC (0.046), CTGCTTCT (0.040), CGACTCCT (0.018) and CGATTCCT (0.0052).

Out of the 8 different haplotype combinations, compared to CTGCTTCC, five (5) haplotypes (CGACTCCC, CGATTCCC, CTGCTTCT, CGACTCCT and CGATTCCT) were associated with lower NVP plasma levels while 2 (CGACCCCC and TGACTCCC) with higher NVP plasma concentration. Compared to CTGCTTCC haplotypes, the CGACCCCC haplotype was associated with higher NVP plasma concentration mean difference (4293.81; 95%CI = 4293.81 to 4293.81 ng/ml p <0.0001) while CGACTCCC with lower NVP plasma concentration; mean difference (-914.99; 95%CI = -914.99 to -914.99 ng/ml, p <0.0001).

Similarly, for patients on EFV, linkage disequilibrium was observed among 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T, resulting in 8 haplotypes of which CTGCTTCC was most frequent (0.339), followed by CGACTCCC (0.262), CGATTCCC (0.156), TGACTCCC (0.091), CGACCCCC (0.059), CTGCTTCT (0.027), CGACTCCT (0.020) and CGATTCCT (0.019). Contrary to NVP, for EFV compared to CTGCTTCC 3 haplotypes (TGACTCCC, CGATTCCT and CGACTCCT) were linked to reduced

EFV plasma concentration while 4 haplotypes (CGACTCCC, CGACCCCC, CGATTCCC and CTGCTTCT) were related to an increased EFV plasma concentration.

Compared to CTGCTTCC haplotypes, the CGACTCCC haplotype predicted a higher EFV plasma concentration mean difference (1538.13; 95%CI = 1538.13 to 1538.13 ng/ml $p < 0.0001$) while TGACTCCC with lower EFV plasma concentration; mean difference (-1272.06; 95%CI = -1272.06 to -1272.06 ng/ml, $p < 0.0001$). Controlling for ethnicity did not alter the relationships of CYP2B6 and CAR SNPs on both NVP and EFV plasma levels (Table 4.33).

Table 4.33. Relationship between haplotypes and NVP and EFV plasma concentrations

Haplotype association with drug concentration			
Haplotypes	**Frequency**	**Difference (95% CI)**	p value
Nevirapine			
CTGCTTCC	0.3279	0	---
CGACTCCC	0.2409	-914.99 (-914.99 - -914.99)	**<0.0001**
CGATTCCC	0.1822	-501.74 (-501.74 - -501.74)	**<0.0001**
TGACTCCC	0.0925	***376.18 (376.18 - 376.18)***	**<0.0001**
CGACCCCC	0.0461	***4293.81 (4293.81 - 4293.81)***	**<0.0001**
CTGCTTCT	0.0402	-452.34 (-452.34 - -452.34)	**<0.0001**
CGACTCCT	0.0184	-326.92 (-326.92 - -326.92)	**<0.0001**
CGATTCCT	0.0517	-35.94 (-35.94 - -35.94)	**<0.0001**
Efavirenz			
CTGCTTCC	0.3388	0	**---**
CGACTCCC	0.2616	***1538.13 (1538.13 - 1538.13)***	**<0.0001**
CGATTCCC	0.1562	***348.87 (348.87 - 348.87)***	**<0.0001**
TGACTCCC	0.0912	-1272.06 (-1272.06 - -1272.06)	**<0.0001**
CGACCCCC	0.0593	***370.84 (370.84 - 370.84)***	**<0.0001**
CTGCTTCT	0.0266	***317.59 (317.59 - 317.59)***	**<0.0001**
CGACTCCT	0.0204	-67.69 (-67.69 - -67.69)	**<0.0001**
CGATTCCT	0.0185	-1073.47 (-1073.47 - -1073.47)	**<0.0001**

Haplotype association with drug concentration adjusting for ethnicity			
Haplotypes	**Frequency**	**Difference (95% CI)**	**P-value**
Nevirapine adjusted by Ethnicity			
CTGCTTCC	0.3251	0	
CGACTCCC	0.2419	-2078.4 (-2078.4 - -2078.4)	**<0.0001**
CGATTCCC	0.183	-1965.36 (-1965.36 - -1965.36)	**<0.0001**
TGACTCCC	0.0922	***238.85 (238.85 - 238.85)***	**<0.0001**
CGACCCCC	0.0474	***4654.69 (4654.69 - 4654.69)***	**<0.0001**
CTGCTTCT	0.043	-1206.47 (-1206.47 - -1206.47)	**<0.0001**
CGACTCCT	0.0173	-668.35 (-668.35 - -668.35)	**<0.0001**
Efavirenz adjusted by Ethnicity			
CTGCTTCC	0.3392	0	---
CGACTCCC	0.2613	-2131.02 (-2131.02 - -2131.02)	**<0.0001**
CGATTCCC	0.156	-4327.63 (-4327.63 - -4327.63)	**<0.0001**
TGACTCCC	0.0912	-4187.68 (-4187.68 - -4187.68)	**<0.0001**
CGACCCCC	0.0594	***1053.33 (1053.33 - 1053.33)***	**<0.0001**
CTGCTTCT	0.0262	***878.29 (878.29 - 878.29)***	**<0.0001**
CGACTCCT	0.0207	-4154.09 (-4154.09 - -4154.09)	**<0.0001**
CGATTCCT	0.0187	-5709.93 (-5709.93 - -5709.93)	**<0.0001**

4.7.3.7. Metabolic score

The anticipated metabolic scores among the study patients and summarized in table 4.34. Scoring was done as follows: 15582 TT = −2; 15582 CT = −1; 15582 CC = 0; 516 TT = −2; 516 GT = −1; 516 GG = 0; 785 GG = +2; 785 AG = +1; 785 AA = 0; 18492 TT = −2; 18492 CT = −1; 18492 CC = 0; 983 CC = −2; 983 TC = −1; 983 TT = 0; 21563 TT = +2; 21563 CT = +1; 21563 CC = 0; 1459 TT = −2; 1459 TC = −1; 1459 TT = 0; 540 TT = +2; 540 CT = +1; 540 CC = 0. The metabolic score was obtained by summing all the genotypes (15582, 516, 785, 983, 18492, 21563, 1459, and 540) per sample. In general, metabolic scores for NVP and EFV did not significantly deviate from normal distribution by the Kolmogorov-Smirnov test NVP (p = 0.909) and EFV (p = 0.859) and in the three ethnic groups (p = 0.565).

The homogeneity of variances of NVP and EFV based regimens (Bartlett's chi square = 0.1558, df = 1, *P* = 0.693) and the three ethnic groups Bantus, Cushite and Nilotes (Bartlett's chi square = 5.1766, df = 2, *P* = 0.075) were not significantly different (Figure 4.11 and 4.12). Comparing the distribution of the absolute frequencies of the metabolic scores among all population generated (chi-square = 9.607; *P* = 0.65), indicating no statistical difference in the metabolic scores among the three ethnic groups. The CYP2B6 and CAR inferred phenotypes related to a reduced metabolism were identified in 171/566 (30.2%) of the overall population studied; 91/312 (29.2%) patients on EFV and 80/254(31.5%) patients on NVP. The reduction in metabolism was mainly intermediate (62.6%, n = 107/171 of the overall population; 62.6%, n = 57/91 EFV and 62.5%, n = 50/80 NVP) rather than poor metabolizer (37.4%, n = 64/171 overall population; 37.4%, n = 34/91 EFV and 37.5%, n = 30/80 NVP). Phenotypes related to an increased metabolism were found in 39.9% (n = 226/566) of the population studied, (122/312 (39.1%) patients on EFV and 104/254 (40.9%) patients on NVP). The most common was intermediate (57.1%, n = 129/226) metabolizer, with 42% (n = 97/226) individuals presenting a deduced ultra-rapid metabolic phenotype (Table 4.34).

Table 4.34. Frequency distribution of the inferred metabolic phenotype and scores across ethnic group

	Poor metabolizers (PM) (-3)	Poor metabolizers (PM) (-2)	Intermediate metabolizers (IM) (-1)	Extensive metabolizers (EM) 0	Intermediate metabolizers (IM) 1	Ultra-rapid metabolizers (UR) 2	Ultra-rapid metabolizers (UR) 3	Total
Efavirenz	4 (1.3)	30 (9.6)	57 (18.3)	99 (31.7)	66 (21.2)	50 (16.1)	6 (1.9)	**312**
Nevirapine	4 (1.6)	26 (10.2)	50 (19.7)	70 (27.6)	63 (24.8)	34 (13.4)	7 (2.8)	**254**
Total	**8 (1.4)**	**56 (9.9)**	**107 (18.9)**	**169 (29.9)**	**129 (22.8)**	**84 (14.8)**	**13 (2.3)**	**566**
Bantu	5 (1.4)	35 (9.5)	68 (18.5)	115 (31.3)	83 (22.6)	51 (13.9)	10 (2.7)	**367**
Nilotes	3 (1.6)	21 (11.1)	36 (18.9)	49 (25.8)	45 (23.7)	33 (17.4)	3 (1.6)	**190**
Cushites	0	0	3 (33.3)	5 (55.6)	1 (11.1)	0	0	**9**
Total	**8 (1.4)**	**56 (9.9)**	**107 (18.9)**	**169 (29.9)**	**129 (22.8)**	**84 (14.8)**	**13 (2.3)**	**566**

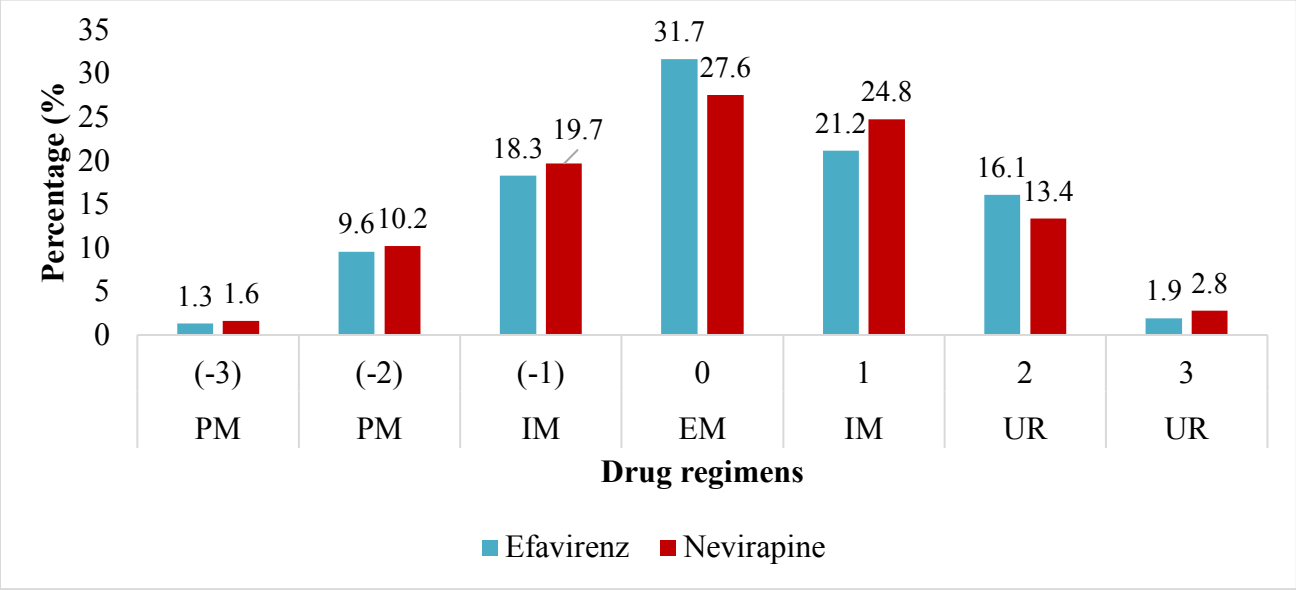


Figure 4.11. Distribution of the inferred metabolic scores by drug regimens

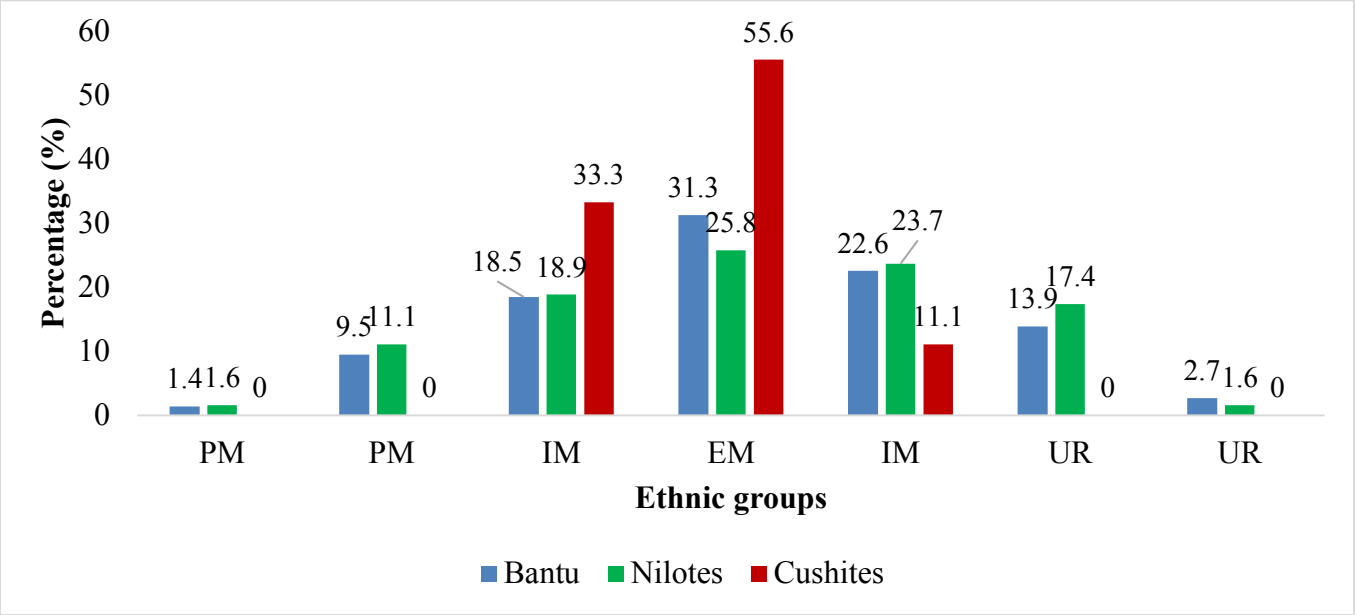


Figure 4.12. Distribution of the inferred metabolic scores by ethnic groups

PM = poor metabolizers; I = intermediate metabolizers (either with delayed or increased metabolism); EM = extensive or 'normal' metabolizers; UR = ultra-rapid metabolizers.

4.6.4. Discussion

While several studies have assessed the contribution of CYP2B6 gene polymorphisms on ARV pharmacokinetics among HIV patients drawn from diverse ethnic groups including Kenya (Kwara et al., 2009; Oluka et al., 2015). The majority of these reports have been limited to either: NVP or EFV based regimen separately, on a small samples size, or a few genetic variants at a time. The information available shows that this report provides the first comprehensive genetic analysis of CYP2B6 and CAR polymorphisms and their role in NVP and EFV plasma levels in one of the leading cosmopolitan ARV treatment centers in Nairobi Kenya. In this although the majority of patients (31.5%) on NVP and 63.8% EFV based regimen had plasma levels within the therapeutic window, there were 14.2% and 4.5% of the patients who had suboptimal NVP and EFV

concentrations respectively. On the other hand, 54.3% and 31.7% of the patients had supra-therapeutic NVP and EFV plasma levels respectively, implying a significant challenge in the management of HIV patients receiving EFV and NVP based regimen in Kenya. This is majorly because NVP and EFV plasma levels in the supra- therapeutic ranges may lead to virologic and immunological failures and occurrence of toxicities including neurotoxicity and serves skin rash.

Studies have shown less substantial contribution of host pharmacoecological factors to the observed wide inter-patient variation in ARV plasma levels (Meng et al., 2015). The most commonly used cART regimens in Kenya and other developing countries at the time of the study, included TDF+3TC+EFVorNVP, 3TC+AZT+EFV or NVP and TDF+3TC+EFV have shown no substantial drug interfaces with NVP and EFV, signifying a reduced influence on metabolism and plasma levels of NVP or EFV (Meng et al., 2015). These findings suggest that host pharmacogenetical factors might contribute significantly to NVP and EFV plasma concentration.

The allele and genotype frequency distributions for 7 of the 13 CYP2B6 SNPs and 1 CAR analyzed were in Hardy–Weinberg equilibrium. The prevalence of variant alleles especially for CYP2B6 516T, CYP2B6 983C, CYP2B6 18492T, CYP2B6 21563T, CYP2B6 1459T, CYP2B6 785G and CYP2B6 835C among the study patients were similar to those reported among other ethnic groupings and populations in African (Matimba et al., 2008; Li et al., 2012; Manosuthi et al., 2014). The frequency of T variant allele of CAR 540T C>T was 14.9% similar to those reported by Wyen, *et al* (2011) which showed 15% variant alleles in black population. The allele frequencies of 516G>T and 785A>G have been consistently higher in the Asian populations, such as Chinese populations (Meng et al., 2015), Japanese and Korean (Cho et al., 2004), but lower in African populations (Oluka et al., 2015). Further, the allele frequencies of CYP2B6 983C was 11.8% and 14.1% among patients on NVP and EFV based regimen respectively which is consist to other studies showing predominant occurrence of this CYP2B6 983C allele in populations of African origin, with allele frequency ranging from 4% to 11% (Mehlotra et al., 2007; Oluka et al., 2015).

Five CYP2B6 SNPs, including 329G>T (exon 2), 341T>C (exon 3), 516G>T (exon 4), 983T>C (exon 7) and 18492C>T (intron 8), and CAR 540C>T were statistically associated with NVP plasma levels. On the other hand, 3 CYP2B6 SNPs 516G>T (exon 4), 835 G>C (exon 6) and 983T>C (exon 7) influenced the EFV plasma levels. These findings reiterate the importance of differences in splicing, coding, and subsequent CYP2B6 enzymatic activity on the effect of NVP and EFV metabolism.

Many studies have demonstrated the role of many CYP2B6 SNPs on enzymatic activity with concomitant effect on NVP or EFV concentrations. In this section we present the association of some of the commonly studied CYP2B6 SNPs as well as others less documented and for the first time CAR. Relationship between 516G>T and NVP or EFV plasma concentrations has received significant attention recently (Meng et al., 2015; Oluka et al., 2015). The allele and genotypes frequencies in the current study of; 36.2% (GG), 51.6% (GT) and 12.2% (TT) for patients on NVP based regimen and 41% (GG), 44.6% (GT) and 14.4% (TT) for patients on EFV based regimen are similar to many previous studies in African, Asian and Caucasian populations. For both patients on NVP and EFV based regimen, those with 516TT and 516GT had higher NVP and EFV concentrations than those with 516GG, re-affirming the significant role of 516G>T to NVP and EFV biotransformation in populations in Kenyan, in agreement with data from prior Kenyan and global populations (Rodriguez-Novoa et al., 2005; Li et al., 2012; Meng et al., 2015; Oluka et al., 2015).

The heterozygous mutant for CYP2B6 983TC was associated with increased NVP plasma (median 8889, IQR 6343 to 12278ng/ml) compared to the wild type TT (median 6042.5; IQR 4347.5 to 8574 ng/ml). There was one patient homozygous for the mutant who had significantly high NVP plasma concentration of 44207 (IQR 44207 to 44207) ng/ml. Both the homozygosity and heterozygosity for mutant were associated with increased EFV plasma concentration than those patients with wild-type 983CC. For both drugs the effect is noteworthy for its magnitude. This outcome is consistent with data from an earlier study in Kenya (Oluka et al., 2015). Reports indicates that 983T>C SNP leads to the formation of variant protein CYP2B6*18 involving a

single amino acid change at I328T. The expression of this variant mutant (CYP2B6*18) does not lead to detectable protein or activity, hence termed as a null allele (Klein et al., 2005; Honda et al., 2012). This null allele accounts for the bigger effect of CYP2B6 983TC for patients on NVP compared to the patients on EFV. In concurrence to this observation, reports from diverse research shown CYP2B6 983TC heterozygosity leading to lowered plasma NVP elimination (Schipani et al., 2011). The effect of this null status is a point of future research for EFV based regimens.

Most of the studies on CYP2B6 18492C>T has been majorly reported on efavirenz based regimen. In this study we compared the role of this SNP on both NVP and EFV based regimens. Patients with heterozygous and homozygous mutant of CYP2B6 18492T>C had markedly lower plasma nevirapine and efavirenz concentrations, which is similar with previous reports (Sukasem et al., 2012; Manosuthi et al., 2014; Soeria-Atmadja et al., 2017). Studies have shown the presence of this CYP2B6 18492T>C SNP co-administered with robust CYP inducer together could lead to patients receiving EFV to have plasma levels in suboptimal ranges (Manosuthi et al., 2014). Most of these studies have been among the Asian population. The role of this SNP on NVP as well as EFV levels in African population needs further assessment.

In both the NVP and EFV based regimens, no homozygous mutation for CYP2B6 835G>C was reported in any patients. About 0.4% of heterozygous mutant for SNP c.835G> C has been reported in Nigerians, Kenyan, Uganda and among African American (Marth et al., 2011). This study showed that the patients with heterozygous mutation for CYP2B6 835G>C had higher EFV plasma concentration than the wildtype. On the other hand, on quartile regression analysis CYP2B6 835G>C was not associated with NVP plasma concentrations, even though patients with heterozygous mutation for CYP2B6 835G>C had lower NVP plasma level than the wildtype. These findings are also consistent among the Rwandese population in which Radloff *et al*., (2013) showed that the heterozygous SNP c.983TC decreased the expression of the CYP2B6 protein by more than 80% compared to the wild protein but the isozymes presented a normal catabolic activity which was associated with high EFV concentrations.

For CYP2B6 329G>T, there were 1.6% patients on the NVP based regimens who had heterozygous mutant for 329 GT. All the 312 patients on EFV had homozygous wild-type CYP2B6 329G>T SNP. A previous study reported SNP c.329G> T of exon 2 was detected in the Luhya tribe of west Kenya with a prevalence of 0.1%. Although there were only 4 patients with heterozygous mutant 329GT, their NVP plasma concentration was 2-fold higher than the wild-type median (IQR) 12328 (8627.5 to 16807.5) ng/ml and 6191 (4503 to 8875) ng/ml respectively. These results are similar with the observation of Marth *et al*., (2011) which reported that most rare genetic variations, even in their heterozygous form, can have a deleterious effect on protein structure and modify the phenotype. Radloff *et al*., (2013) showed four SNPs c.329G> T, c.341T> C, c.548T> G and c.637T> C as coding for CYP2B6 proteins with reduced function or completely inactive these proteins similar to observations of Marth *et al*., (2011).

There were 3.2% and 0.9% patients on NVP and EFV regimen who had heterozygous mutant for CYP2B6 341T>C respectively. Although there were only 8 patients with heterozygous mutant 341TC, their NVP plasma concentration was 2-fold lower than the wild-type median (IQR) 3675.5 (1163.5 to 12328) ng/ml and 6282 (4558 to 8889) ng/ml respectively. On the contrary, for patients on EFV the 8 patients with heterozygous mutant 341TC, their EFV plasma concentration was 1.5-fold higher than the wild-type median (IQR) 3680 (1652 to 5308) ng/ml and 2732 (1886 to 4872) ng/ml respectively. Radloff *et al*., (2013) showed four SNPs c.329G> T, c.341T> C, c.548T> G and c.637T> C as coding for CYP2B6 proteins with reduced function or completely inactive these proteins similar to observations of Marth *et al*., (2011). Thus, explaining the higher concentration of EFV plasma level for the heterozygous mutant 341TC. The role of CYP2B6 341T>C in the NVP concentrations both in-vivo and invitro is an opening for future investigations.

The frequency of the G variant allele for CYP2B6 785A>G, was 64.1% with genotype frequencies of 35.8% (AA), 51.9% (AG) and 12.2% (GG) for patients on NVP based regimen. For patients on EFV based regimen, the G variant allele for CYP2B6 785A>G was 59.3% and genotype frequencies of 40.7% (AA), 44.8% (AG) and 14.4% (GG), respectively. In Indonesia, the CYP2B 6*785A*>G mutant was detected in 29.2% of individuals (Hananta et al., 2018). In other reports

CYP2B6 785A>G allele was shown to occur in about 9 to 63%, depending on the ethnicity of the population studied (Guan et al., 2006). In Caucasian populations, CYP2B6 785G>A was shown to occur in a frequency ranging from 20%–31% and higher (60%) among population of African origin (Rotger et al., 2007; Telenti & Zanger, 2008; Wyen et al., 2011). In this study the homozygous and heterozygous mutations of CYP2B6 785A>G was associated for 1 to 4-fold higher NVP and EFV plasma concentrations respectively than the wild type. This finding is consistent to other studies showing increased EFV plasma levels in clinical settings marked with higher cases of EFV induced neuropsychological toxicity and treatment termination (Rotger et al., 2007; Telenti & Zanger, 2008; Wyen et al., 2011). The association between CYP2B6 785A>G and NVP concentration is an important avenue for future investigation.

The frequency of the T variant allele for CYP2B6 21563C>T was 66.1% while the genotype CC, CT and TT were found in 35.8%, 52.8%, and 11.4%, respectively of patients on NVP based regimens. For patients on EFV based regimen, the T variant allele for CYP2B6 21563C>T was 59.6% and genotype frequencies were 40.4% (CC), 45.5% (CT) and 14.1% (TT), respectively. In Thailand, Manosuthi *et al*., (2013) reported the occurrences of heterozygous and homozygous mutants for 21563C>T as 57% and 5%. Further in Thailand, Sukasem *et al*., (2012) reported CYP2B6 21563C>T heterozygous and homozygous mutant frequencies of 44.2% and 13.5% respectively. In this study patients who had homozygous mutant genotype (TT) and heterozygous mutant (CT) for CYP2B6 21563C>T had 2 to 4-fold higher NVP and EFV plasma concentration respectively, compared to patients with CC wildtype genotype. This is consistent for EFV as reported by Sukasem *et al*., (2012) in Thailand who reported a 4-fold higher EFV plasma concentration for CYP2B6 21563 TT compared to CYP2B6 21563CT and CYP2B6 21563CC genotypes. Manosuthi *et al*., (2013) reported even a 7-fold higher EFV plasma concentration for patients with homozygous mutants for 21563C>T as compared to wild type.

The CYP2B6 637T>C had the frequency of the C variant allele of 0.4% with a frequency of heterozygous mutant of TT (0.4%) for patients on NVP based regimens. All the patients on EFV based regimen, had wildtype genotype for CYP2B6 637T>C SNP. In this study, although there

was one patient with heterozygous mutant 637TC, the median (IQR) (8001, IQR 8001 to 8001 ng/ml) NVP plasma level was about 1.3-fold higher than those for wildtype (6202, IQR 4518 to 8964 ng/ml). The CYP2B6 637T>C SNP was first identified among the Rwandese population in 2013 (Radloff et al., 2013). This SNP codes for CYP2B6 proteins with reduced function or completely inactive these proteins similar to observations of Marth *et al*., (2011). Thus, explaining the higher concentration of EFV plasma level for the heterozygous mutant 341TC. The role of CYP2B6 637T>C SNP in the NVP and EFV concentrations both in-vivo and invitro is an opening for future investigations.

Although there were 3.2% and 0.9% patients on NVP and EFV regimen who had heterozygous mutant for CYP2B6 444G>T, respectively similar to CYP2B6 341T>C, on quartile regression analysis CYP2B6 444G>T was not associated with NVP and EFV plasma levels. No clinical significance of CYP2B6 444G>T has been reported (dbSNP https://www.ncbi.nlm.nih.gov/snp/rs145884402#clinical_significance) which is also supported by Gras (2013) who showed decreased interaction energies for the changes in the CYP2B6 444G>T SNP with no important binding residues, signifying a diminished stability of the ligand–EFV complex. The SNP is also open for future in-vivo and in vitro investigation on ARV concentration.

This is the first report in Kenya to assess the role of constitutive androstane receptor (CAR) 540T C>T in the concentrations for both NVP and EFV. The frequency of the T variant allele for CAR 540T C>T was 14.9% while the genotype CC, CT and TT were found in 85%, 13.8%, and 1.2%, respectively for patients on NVP based regimen. For patients on EFV based regimen, the T variant allele for CAR 540T C>T was 12.2% and genotype frequencies of 84.6% (CC), 14.7% (CT) and 0.6% (TT), respectively. Wyen *et al*., (2011) reported consistent results with ours with the prevalence of T variant in 32.7% among the Caucasians and 15% among the blacks. In Ghana Sarfo *et al*., (2014) reported slightly lower the frequency of variant allele for CAR 540C>T at 7% among patients. In this study, though not significant on linear regression analysis, patients with the homozygous and heterozygous mutation for CAR 540T C>T had higher NVP and EFV plasma concentrations. This was consistent with the two stated reports which observed a trend towards

association between plasma efavirenz concentration and CAR 540C>T (Wyen et al., 2011; Sarfo et al., 2014). This report being the first in Kenya opens the avenue for further rigorous prospective investigations.

This study then evaluated the effects of the number CYP2B6 and CAR genotypes on NVP and EFV plasma levels. There were 203(79.9%) and 237(75.9%) patients on NVP and EFV respectively, with more than two SNPs. Thirty (11.8%) and 41(13.4%) patients on NVP and EFV based regimens respectively had 6 different number of SNPs. There were 7(2.8%) and 6(1.9%) patients on NVP and EFV regimen respectively with 7 different SNPs. This study showed the patients who had larger number of variant alleles, had higher median NVP and EFV plasma levels, with a positive association between the number of SNPs and the levels of NVP (adjusted β 1129.6;95% CI 729.9 to 1529.3) and (adjusted β 570;95% CI 362 to 778) for EFV.

Population of African descent account for the most genetically heterogenous global populaces a fact that jeopardizes the already insufficient treatment approaches developed for several infectious diseases (Prugnolle et al., 2005). Evaluating the function of ethnicity on NVP and EFV plasma which is important and relevant among the Kenyan population for two fundamental reasons; first, currently no elaborate genotyping data are available among the Kenya population and secondly most of the ARVs used for HIV treatment in Kenya are majorly metabolized by CYP2B6 and CAR 540 (Hedrich et al., 2016). The CYP2B6 and CAR genetic data in this population will offer unique understanding of their diversification with regards to ethnicity. Similarities and variation in the frequency of variant alleles were observed across various populations. Example, among Egyptian populations, the frequency of variant alleles for CYP2B6 516G > T, CYP2B6 785A > G and 1459C>T were 29%, 30% and 4% respectively (Ellison et al., 2012). This was also similar to North American population (38%, 0% and 1% for 516G > T, 785A > G and 1459C>T respectively) (Haas et al., 2005)**.** The British reported frequency of 28%, 30% and 12% for 516G > T, 785A > G and 1459C>T respectively (Lang et al., 2001). In German prevalence of 29%, 33% and 14% was reported for 516G > T, 785A > G and 1459C>T respectively (Jacob et al., 2004). In Swiss 24%, 26% and 11% was reported for 516G > T, 785A > G and 1459C>T respectively (Lang et al., 2001;

Jacob et al., 2004; Crettol et al., 2005) . The high frequency of 1459C>T among these three European populations was similar to frequency observed among the Nilotic population in the current study. The Ghanaian and West African populations reported higher frequencies 516G > T and 785A > G and lower frequency of 1459C>T compared to our study (Klein et al., 2005; Mehlotra et al., 2006).

The CYP2B6 983T>C occurs chiefly in African populations, with allele frequencies ranging from 4 to 11% (Dahri & Ensom, 2007; Mehlotra et al., 2007). The current study reported the frequency of variant alleles for 983C>T being within this range among the three ethnic groups (6.1%, 7.9% and 11.1% - Bantu, Nilotic and Cushite respectively). This study reported the presence of homozygosity for the mutant allele CYP2B6 983 CC among the three populations, contrary to a previous study in Kenya by Oluka *et al* (2015) which did not report the presence of CYP2B6 983 CC among Kenyan population. Though not statistically significant, this study reported slightly higher frequencies of variant alleles for 21563C>T among the Bantu populations compared to Nilotes and Cushite population. The 21563C>T SNP has been reported predominantly among the Asian communities than among the African population (Sukasem et al., 2012; Manosuthi et al., 2013). Most of the studies on CYP2B6 18492C>T has shown patients with heterozygous and homozygous mutant of CYP2B6 18492T>C had markedly lower plasma nevirapine and efavirenz concentrations (Sukasem et al., 2012; Manosuthi et al., 2014; Soeria-Atmadja et al., 2017). Most of these studies have been among the Asian population. The observed frequencies of variant alleles for CYP2B6 15582C>T were not significantly different between the Bantu, Nilotes and Cushites (11.7%, 8.4% and 16.7% respectively). Evans *et al*., (2015) showed a similar pattern of the 15582C>T among South Africans (8%) and Cameroonians (6%). Although the *CYP2B6* g.15582C>T SNP allelic and genotypic frequencies are among populations from South Africans, Cameroonians, and African-Americans, differences in minor allele frequencies have been reported (Swart et al., 2013). The frequency of *CYP2B6* g.15582C>T SNP is predominantly higher between 31% to 57% among European, Hispanic and Asian populations significantly lower between 6% to 13% among African population (Dandara et al., 2014). This observation is poised

to significantly affect the individualization of ARV treatment in developing countries. Though not statistically significant, compared to Bantus and Nilotic populations, the Cushite community had higher frequencies of variant alleles for the constitutive androstane receptor (CAR) 540T C>T. In Ghana Sarfo *et al*., (2014) reported the frequency of variant allele for CAR 540C>T was 7% among patients. The study did not identify the ethnicity of the study population. Wyen *et al*., (2011) reported consistent results with ours with the prevalence of T variant of 32.7% among the Caucasians and 15% among the blacks.

Haplotype analysis jointly assesses if the interactions of multiple SNPs, leads to a decrease or increase in the metabolic function of CYP2B6 or CAR. Theoretically, compared to a single SNP, haplotype may be more accurate in forecasting NVP or EFV pharmacokinetics (Carr et al., 2010). The results of haplotype and ARV plasma levels in this study is among the first to be presented in Kenya. For patients on NVP and EFV based regimens, linkage disequilibrium among 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T was observed, resulting in 8 haplotypes among which CTGCTTCC and CGATTCCT had the highest and the lowest frequency, respectively for both patients on NVP and EFV. Compared to CTGCTTCC, five (5) haplotypes (CGACTCCC, CGATTCCC, CTGCTTCT, CGACTCCT and CGATTCCT) were associated with lower NVP plasma levels while 2 (CGACCCCC and TGACTCCC) with higher NVP plasma concentration. Contrary to NVP, for EFV compared to CTGCTTCC 3 haplotypes (TGACTCCC, CGATTCCT and CGACTCCT) were associated with lower EFV plasma concentration while 4 haplotypes (CGACTCCC, CGACCCCC, CGATTCCC and CTGCTTCT) predicted increased EFV plasma concentration.

A significant outcome of this report is that of metabolic deduced phenotypes were comparable among the three ethnic populations. In Botswana, Tawe *et al*., (2018) reported similar outcomes among different bantu populations across the three investigated sites. The outcome of this study might be because of the homeostatic effect combined mutations. Consequently, despite the significant proportion of haplotype diversity in the three communities, a deduced phenotypic merging was observed, showing that, universally, drug metabolism remains steady and similar

among the study populations in agreement to previous studies (Ralph & Coop, 2015). It is vital to point out that CYP2B6 enzyme retains a vital function in metabolism and elimination of several procarcinogens, environmental compounds and many plant chemical defenses consumed in human foods or diets (Malenke et al., 2012; Aung et al., 2014). It is possible therefore that the homogeneous phenotypes among the different ethnic groups of this study are probably due to adaptation to the environmental and/or toxicological conditions of their surrounding areas.

According to the metabolic score, 29.8% of the study population had CYP2B6 and CAR extensive metabolic phenotype, reduced metabolic phenotype reported in 171/566 (30.2%) while 226/566 (39.9%) had increased metabolic phenotype. There were 64/566 (11.3%) study population with poor metabolic phenotype with 97/566 (17.1%) showing ultra-rapid metabolic phenotype. About 107/566 (18.9%) presented an intermediate reduced metabolic phenotype, and 226/566 (39.9%) had an intermediate increased metabolic phenotype. These proportions were higher than those observed among the bantus in Botswana (Tawe et al., 2018) and previously in a Kenyan women population (Oluka et al., 2015). Patients with a CYP2B6 reduced metabolic phenotype faces a higher risk of jeopardized treatment outcomes if taking EFV or NVP-based ART regimen (Russo et al., 2016; Tawe et al., 2018). Of concern is the significantly high number of individuals (17.1%) who had deduced ultra-rapid metabolic phenotype, the effect of their metabolic pattern affects EFV or NVP-based ART regimen by leading to sub-therapeutic drug exposure with a potential increase of selection for viral resistance (Russo et al., 2016). Further studies should focus on this fast metabolizer fraction of the population whose data are unavailable in the scientific literature.

4.6.4. Conclusion

This study identified four SNPs in the *CYP2B6* gene (CYP2B6 785A>G, CYP2B6 18492C>T, CYP2B6 21563C>T and 15582C>T) and one CAR (540T>C) whose polymorphisms could add to stand alone predictors of EFV plasma levels other the known *CYP2B6* c.516G>T and CYP2B6 983T>C polymorphism. Further, six *CYP2B6* SNPs (CYP2B6 329G>T, CYP2B6 637T>C, CYP2B6 785A>G, CYP2B6 18492C>T, CYP2B6 21563C>T and 15582C>T) and one CAR

(540T>C) could also independent predict NVP plasma levels in addition to *CYP2B6* c.516G>T and CYP2B6 983T>C polymorphism. The study reported 1.9% to 13.4% patients on NVP and EFV with 6 to 7 different number of SNPs. Patients who had a larger number of variant alleles, had higher median NVP and EFV plasma levels. The CGACCCCC and TGACTCCC haplotypes were associated with higher plasma NVP concentrations in HIV-infected Kenya adults while CGACTCCC, CGACCCCC, CGATTCCC and CTGCTTCT) were associated with higher EFV plasma concentration in HIV-infected Kenya adults. Though the populations in Kenya are ethnically and genetically different, they demonstrate convergent evolution in their drug metabolism. The occurrence of significant numbers of slow and fast metabolizers can significantly impact the emergence and spread of drug resistance among the HIV patients, either by exposing pathogens to sub-lethal drug doses or inducing non-compliance in patients, with similar consequences. This warrants constant monitoring in the population to identify potential patients with abnormal drug metabolism and adapt treatments accordingly.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of immunological, virologic and adverse clinical outcomes

This study showed that CD4 and HIV RNA viral load responses to ART treatment is dynamic and robust. This is consistent to other studies reported in settings in Africa, Latin America, and Asia (Lok et al., 2010; Nash et al., 2008; Rajasekaran et al., 2009) which also showed the robustness of CD4 responses to ART that are sustained over several years. The positive virological and immunological response to HAART reported in the current study is consistent in other studies (Opravil et al., 2002; Waters et al., 2004).

The ART use was further associated with a drop in the cases of anemia, leucopenia and thrombocytopenia 12 month into ART treatment. Downside of ART use on the other hand, was the occurrence of a number of adverse reactions including hepatotoxicity and renal abnormalities. Liver injuries are the most common non-AIDS cause of death among people with HIV infection reported in several studies (Smith, 2010; Kovari & Weber, 2011). Approximately 18% of deaths occurring among HIV patients during consistent ART treatment has been associated to liver-related complications (Smith, 2010; Kovari & Weber, 2011).

The current CD4 count in this study was associated with duration living with HIV infection, and baseline CD4 remained the single most important factor determining CD4 count in the preceding months. This observation is consistent to other studies (Kaufmann et al., 2003; Kabugo et al., 2005; Nash et al., 2008) that patients with higher CD4 counts at ART initiation achieved a higher CD4 count in the following months and years. The baseline CD4 count, second only to subsequent medication adherence, is the most important predictor of clinical progression and survival after ART initiation (Badri et al., 2006; Etard et al., 2006).

Various immunopathogenesis in patients has been associated with HIV infection (Watkins et al., 1990). Several hematological and biochemical complications have been observed due to HIV infection. These hematological and biochemical abnormalities are shown to occur due to the following actions; HIV infection, sequel of HIV-related opportunistic infections, malignancies and consequence of therapies used for HIV infection and associated conditions (Moyle, 2002). In this study the following hematological abnormalities were thus observed; thrombocytopenia, anemia, leucopenia and lymphocytosis in agreement with other studies (Belperio & Rhew, 2004; Owiredu et al., 2011; Dhurve & Dhurve, 2013).

5.2 Efavirenz and nevirapine plasma levels 12 months into treatment

Therapeutic concentrations of ARV drugs positively predict virologic failure. Unfortunately in many countries therapeutic drug monitoring or pharmacologic measurements into adherence and virologic failure monitoring is not part of HIV treatment programs (Aurpibul et al., 2014). The application of adherence data alone might not be beneficial because even in patients reporting good adherence, some demonstrate subtherapeutic concentrations of drug that confounds interpretation and application of adherence data, often largely due to high interpatient variability (L'Homme et al., 2008). Such variability can be partly due to differences in host genetics and drug metabolism, thus supporting the need for TDM to bolster clinical management of HIV (Holzinger et al., 2012). The long elimination half-life of nevirapine and efavirenz and their low genetic barrier to resistance may limit their long-term therapeutic efficacy (L'Homme et al., 2006; Yilmaz et al., 2012). This aim therefore determined the concentrations and patterns of NVP and EFV among patients in Nairobi

The median (interquartile range – IQR) for NVP plasma concentration was 6237.5 [4518 – 8964] ng/ml with the majority (54.3%) of patients having plasma levels of >6000 ng/ml considered levels for durable viral suppression. There were 80 (31.5%) patients with NVP levels between 3400 to 6000ng/ml considered levels for viral mutant selection windows and the least 36 (14.2%) who had NVP plasma concertation of <3400 ng/ml considered levels for poor viral suppression and the least

(P <0.05). The median (IQR) of EFV plasma concentration was 2739.5 [1878 - 4891.5] ng/ml. The majority (63.8%) of patients on EFV had plasma concentrations between 1000 to 4000ng/ml considered levels for viral mutant selection windows followed by 99(31.7%) who had >4000 ng/ml considered levels for durable viral suppression and the least 14(4.5%) had plasma concentrations of <1000 ng/ml considered levels for poor viral suppression window (P <0.05). The NVP and EFV plasma levels reported in this study was higher compared to other studies both in Kenya and elsewhere (Oluka, 2012; Oluka et al., 2015). In South Africa, Mazanderani *et al*., (2019), United Kingdom, Stöhr *et al*., (2008) and in Italy, Giacomelli *et al*., (2018) reported higher NVP plasma concentration than those reported in this study. On the contrary, In Tanzania Gunda *et al*., (2013) and in India (Gopalan et al., 2017) reported lower NVP plasma concentration.

The EFV plasma concentration in this study was higher than those reported in Liverpool, UK by Stöhr *et al*., (2008) as well as those reported in Tanzania, by Tabb *et al*., (2018). Other studies such as by Marzolini *et al*., (2001) in Swaziland reported higher EFV plasma concentration. The variation in both NVP and EFV plasma levels is observed in various countries and settings. These variations have been attributed to various pharmacoecological and pharmacogenetic factors. In the subsequent objectives, the study evaluated the pharmacoecological and pharmacogenetic etiology of NVP and EFV plasma levels among the study population.

5.3 Relationship between efavirenz and nevirapine plasma concentrations and host pharmacoecological factors

Patients' education level has been cited as one of the drivers determining treatment outcomes in HIV. This study showed that vocational training was associated with a lower durg level especially among patients on NVP regimen. Consistent to this study, Burch *et al*., (2016) showed the non-university education, a measure of lower socioeconomic status, was strongly associated with non-adherence to ART, and with virological non-suppression. Education level affects health literacy and may determine the type of health-seeking behavior necessary to achieve favorable treatment outcomes (Kagee et al., 2011).

This study did not find association between age, gender and efavirenz and nevirapine plasma concentration. Although not significant, the older patients had higher median NVP and EFV plasma level than younger ones. This is consistent with other studies which have indicated that nevirapine metabolism in younger aged is generally more rapid than that observed in older population, and that younger patients required higher doses of nevirapine to achieve therapeutic concentration (Swaminathan et al., 2011; Gopalan et al., 2017). Further, females had slightly higher median NVP and EFV plasma levels than males. These results are comparable to those of Shapaya (2014) done in Kenya as well as by Shiau *et al*., (2014) done in South Africa. These two studies showed females had higher median NVP levels than males. This difference in ARV plasma levels by gender has been attributed to the difference in body size and drug clearance between males and females.

The number of sexual life partners and age of sexual debut in this study influenced NVP and EFV plasma levels. Studies have associated risky sexual behaviors including young age of sexual debut and multiple sexual partner as key drivers for HIV transmission between sexual partners. Some of these transmissions has been with drug resistant viruses. In the US, Goldsamt *et al*., (2011) showed an association between a high prevalence of antiretroviral resistance and risky sexual behaviors. The presence of HIV drug resistance has been demonstrated to affect the NNRTI plasma levels (Vardhanabhuti et al., 2013). Estimates of the prevalence of drug-resistant HIV range from 5% to 17% among newly diagnosed and/or treatment-naïve individuals (Johnson et al., 2008). Higher rates of HIV resistance have been found among individuals with high rates of unprotected anal intercourse and high numbers of concurrent sexual partners (Gorbach et al., 2008). Further, the risk of HIV superinfection which occurs when an individual infected with one strain of HIV acquires a second strain is higher when HIV patients engages in unprotected sexual activities (Piantadosi et al., 2008).

HIV status disclosure in this study was associated with better ART adherence with a concomitant higher median NVP and EFV plasma concentration regardless whether the disclosure was to a spouse, family member, religious leaders and employers. On the contrary, in Thailand, Sirikum *et*

al., (2014) reported no association between HIV disclosure and drug plasma concentrations. Disclosure offers a number of important benefits to the infected individual and to the general public. Disclosure of HIV test results is associated with less anxiety and increased social support among many women (Medley et al., 2004; Johns et al., 2016). In addition, HIV status disclosure may lead to improved access to HIV prevention and treatment programs, increased opportunities for risk reduction and increased opportunities to plan for the future. Disclosure of HIV status also expands the awareness of HIV risk to untested partners, which can lead to greater uptake of voluntary HIV testing and counselling and changes in HIV risk behaviors (Medley et al., 2004; Johns et al., 2016).

HIV self-stigma in this study was associated with higher median NVP plasma levels. This relationship was probably mediated by ART adherence. Stigma and discrimination continue to be the greatest challenge faced by People Living with HIV/AIDS (StigmaIndex, 2013). HIV-related stigma is defined as discounting, discrediting and discriminating against people perceived to have been infected with HIV (Goffman, 2009). Stigma and discrimination negatively affect people living with HIV (Chambers et al., 2015). Studies have shown an association between HIV stigma with poorer physical and mental health outcomes (Rueda et al., 2016). Stigma has also been linked with secondary health-related factors including seeking healthcare and adherence to antiretroviral therapy, and access to and usage of health and social services (Rao et al., 2012; Rueda et al., 2016). Inevitably, these negative outcomes of stigma are bound to affect the overall treatment outcomes in terms of therapeutic monitoring. This is an area that needs more attention especially in longitudinal studies.

Social support to HIV patients in this was associated with higher median NVP and EFV plasma concentration than patients who never had such social support. Accumulating evidence show that community support networks enhances social relationships that demystify HIV-associated stigma (Campbell et al., 2007; Zachariah et al., 2007). A study in Kenya showed that participation in community support networks significantly improved adherence and treatment outcome (Ochieng et al., 2015). Another study in Kenya showed that patients actively involved in community support

networks tended to reach peak NVP plasma concentration early at 4 hours post-dosing, and these cNVP also were substantially higher than seen in patients not actively involved in community support networks. Numerous studies have linked social support to better medication adherence and better clinical outcomes (Gonzalez et al., 2004). Available evidence shows the positive effects of social support and protection on other HIV related outcomes, such as sexual risk behaviors (Handa et al., 2014; Cluver et al., 2016), mental health distress and family relationships (Bhana et al., 2014; Kilburn et al., 2016). Growing evidence of associations between social protection and HIV-risk reduction (Pettifor et al., 2016) is reflected in a number of policy documents by UNICEF, UNAIDS and PEPFAR-USAID that focus on pediatric and adolescent HIV-prevention (UNAIDS, 2014; PEPFAR, 2015).

This study showed that the availability and adequate nutritional uptake was associated with higher NVP and EFV plasma concentration. Further, barrier to nutritional uptake was associated with lower NVP and EFV plasma concentration. Conflicting results have been reported; association of nutritional status has been linked to drug plasma levels of NVP and EFV (Vreeman et al., 2014; Bartelink *et al*., 2015). Other studies have reported no association between nutritional status and NVP and EFV plasma levels (Ellis *et al.,* 2007; Pollock *et al.,* 2009; Fillekes et al., 2011).

5.4 Relationship between efavirenz and nevirapine plasma concentrations and art drug adherence

Studies have shown that optimal adherence to ART is essential and early studies reported that ≥95% adherence to ART was required to achieve and maintain viral suppression (Paterson et al., 2000). Recent studies have shown that virologic suppression may still be achieved with < 95% adherence levels, this is dependent on the ART regimen, duration of treatment and previous ART exposure (Ammassari et al., 2012). Poor adherence may acquisition of HIV resistance to first line NNRTI-based regimens who may require switching to second- and third-line ARV regimens which are costlier, have a higher pill burden, increased dosing frequency and often have less

tolerable side effects (Snedecor et al., 2013). Secondly, resistant HIV strains can be transmitted to others resulting in primary resistance to first line treatment regimens in newly infected ART-naïve patients or acquired resistance in infected patients already on ART (Cambiano et al., 2013). Thirdly, treatment failure due to non-adherence is associated with a greater risk of progression to Acquired Immune Deficiency Syndrome (AIDS) and mortality (Lima et al., 2009).The non-adherence rate in this study for past 30 days was 164 (64.6%) for patients on NVP and 207 (66.3%) for patients on EFV. In a previous study in Kenya, Kimulwo *et al*., (2017) reported 22.4% of patients had poor adherence. In Malawi among patients receiving ART, up to 47% of the subjects were non-adherent to treatment (Van Oosterhout et al., 2005).

This study showed that number of pills taken per day, ARV regimen as important factors affecting NVP and EFV plasma concentration. For beneficial ART clinical outcomes interrogating both previous/initiation ART type and current ART combination is important because it has bearing on the NVP and EFV plasma concentration.

Healthcare access is a known important factor affecting NVP and EFV plasma concentration and if not checked could impose a significant impediment to treatment outcomes. History of hospital admission, time taken to reach the ARV dispensing clinic, number of times visited medical clinic to see a doctor, number of times visited medical clinic due to an acute illness or missing scheduled HIV medical visit are some of the health access variables probed but were not found associated with NVP and EFV plasma levels. Studies however shows the importance of accessing ART clinics or healthcare influencing treatment outcomes. Retention in care and treatment is essential to maintaining good adherence, monitoring of treatment outcomes, and providing ongoing psychosocial support and education to the patient (Nosyk et al., 2015).

Side effects of ART medication have been significantly associated with NVP and EFV plasma concentration. In this study stopping taking medication due to pain in the hands and feet, nausea, weight loss or wasting, fat deposit or weight gain, skin problems such as rash, nervousness or anxiety and depression were shown to influence NVP and EFV plasma level. Consistent to this

study, Mbuagbaw *et al*., (2016) reported association EFV with more people with impaired mental function compared to NVP and fewer people with rash than NVP. These results agreed with those of Kaimal *et al*., (2018). Oluka *et al*., (2015) on the other hand did not show rash and NVP plasma concentration. Studies in Africa shows the nevirapine is associated with hypersensitivity reactions in 6%–10% of patients, including hepatotoxicity, maculopapular exanthema, Stevens–Johnson syndrome and toxic epidermal necrolysis (Carr et al., 2017). Our study also shown the importance of wasting, fat deposit or weight gain and association with NVP and EFV plasma level. Herrmann *et al*., (2013) also reported abnormal fat distribution/lipodystrophy due to ARV effect patients Health-related Quality of life by decrease medication adherence. These results re-affirm the importance of NVP and EFV induced adverse medical outcomes which must always be monitored among HIV patients on these regimens.

Medical outcome to HIV infection and treatment is multifaceted with physical activities being one of the key components. In this component we evaluated the relationship between NVP and EFV plasma concentration and outcomes of physical and daily activities. In general, inability to walk up a hill was associated with higher NVP plasma levels, while inability to bend or lift light objects with lower NVP plasma level. For efavirenz the presence of body pain in the past 30 days was associated with higher EFV plasma levels. Consistent to our study, Herrmann *et al*., (2013) showed the HIV infected persons often experience episodes of tiredness requiring to rest more often and experiencing difficulties in performing day-to-day tasks such as walking short distances or carrying light objects.

5.5 Relationship between efavirenz and nevirapine plasma concentrations and HIV drug resistance and selected side effects

The study provided a comprehensive relationship between NVP and EFV plasma concentrations and clinical outcomes including HIV drug resistance, hepatic, renal, hematological side effects and medical adverse outcomes. Out of the 254 and 312 patients on NVP and EFV based regimen, 9.5% and 3.2% respectively, were infected with HIV virus resistant to one or more NRTI and NNRTI

drugs. This study identified mutations associated with non-nucleoside reverse transcriptase inhibitor (NNRTI) for both patients on NVP and EFV as follows: K103N, Y181C, G190S, V106A/M, Y181C, Y188L and P225H. our results were consistent to the results reported by others (Rhee et al., 2015; De La Cruz et al., 2019). The presence of HIV drug resistant mutation was not associated with NVP plasma level, even though half 12/24 (50%) of the patients with drug resistant mutation had supra-therapeutic NVP plasma levels with some 3/24 (12.5%) having sub-optimal levels.

On the contrary, the presence of HIV resistant mutation was associated with lower EFV plasma levels. About of 30% of the 10 patients with drug resistant mutation had sub-optimal plasma level with some 10% having EFV plasma level considered supra-therapeutic levels. Similar results were reported previously among Kenyan women by Oluka *et al*., (2015) which reported no association between presence of HIV drug resistance and NVP plasma levels. Vardhanabhuti *et al*., (2013) on the other hand reported interesting findings where women of African ancestry with HIV resistance mutations had a lower median time to nevirapine IC_{50} of 412 hours compared to Indian women. Our results and those of others could be due to the fact that during the long-term ART treatment, the frequency of specific HIV drug resistant mutations conferring resistance to NVP and EFV (K103N, Y181C and G190A) are dynamic. Vardhanabhuti *et al*., (2013) showed that during the long-term ART treatment, the frequency of specific HIV drug resistant mutations conferring resistance to NVP and EFV (K103N, Y181C and G190A) were not steady. The study further showed that the frequency of quasi-species with K103N mutation decreases with time, and could even vanish in SGA sequences, while the proportion of Y181C and G190A could increase from 0% to nearly 100%. Further, archival resistance mutations are also key in determining treatment outcomes. Studies have shown that sequencing proviral DNA has demonstrated archival resistance mutations from past treatments (Derache et al., 2015) in cellular reservoirs (Dalai et al., 2009), with phylogenetic transmission clustering (Kassaye et al., 2009), viral evolution (Zaccarelli et al., 2016), and may determine eligibility for drug switching among suppressed patients (Allavena et al., 2017). Inevitably therefore drug resistant mutations whether transmitted, acquired or archived

are crucial in determining the treatment outcomes especially for the NNRTIs which have been shown to have a relatively low genetic barrier to resistance. To develop high-level resistance, one DRM in the case of nevirapine (NVP), one to two DRMs in the case of efavirenz (EFV), and two DRMs in the case of etravirine (ETR), are required (Vingerhoets et al., 2010; Melikian et al., 2014). It is therefore paramount to maintain optimal plasma concentration of NVP and EFV, because a single point mutation at specific position on the *pol* gene on the HIV-1 genome may confer high-level resistance to NVP and EFV (Wang et al., 2014). Studies have indicated that trough plasma nevirapine and efavirenz concentrations (C_{trough}) of ≤3μg/ml and ≤1μg/ml respectively are associated with increased risk of treatment failure by promoting the evolution of drug resistant viral variants (Wang et al., 2011).

This study also reported NRTI viral drug resistance that is caused by mutations referred to as either thymidine analogue mutations (TAMs) – (M41L, D67N and T215T/Y), or nucleoside analogue mutations (NAMs) (M184V/I, K65R and K70E). Viruses already containing multiple TAMs are more likely to develop additional TAMs than K65R when treated with TDF or ABC. Some viruses with multiple TAMs will also develop a double-amino acid insertion at RT position 69 referred to as T69S_SS or simply T69insertion. In combination with multiple TAMs this T69S_SS mutation causes high-level resistance to AZT, ABC, and TDF, and intermediate resistance to 3TC and FTC (Clutter et al., 2016). It is anticipated therefore that patients on first line with NRTI mutations is likely to reduce the concentrations of NNRTI drugs even if there are no NNRTI associated mutations (Clutter et al., 2016).

The concentration of ARV drug found in plasma as well as the amount of drug excreted into breastmilk may affect the rate at which ARVs begin to suppress viral replication, and/or the duration of the effect on viral replication (Davis et al., 2019). Therapeutic drug concentrations are therefore a key to successful ART (Gunda et al., 2013), as any low drug concentrations observed in patients on ART is related to failure to achieve an immediate virological success and a longer-term immunological failure (Boulle et al., 2008). In our study, patients (n=99) on EFV regimen with supra therapeutic EFV plasma level had virologic suppression (mean viral load 49.7; SD

440.6 cells/ml) compared to patients (n=199) with therapeutic EFV plasma level (2385.7; SD 14590.6 cells/ml) and patients (n=14) with sub-optimal EFV plasma level who had significant virologic failure (11,628.5; SD 26,931.6 cells/ml). On the contrary, NVP plasma concentration was not associated with viral load; patients (n=37) with sub-optimal NVP had lower mean (SD) viral load (1763.9; SD 7484.8 cells/ml) followed by patients (n =80) with supra therapeutic NVP plasma level (4024.5; SD 25183.1 cells/ml) and those with therapeutic NVP plasma level (9442.9; SD 48418.1 cells/ml). previously in Kenya, Oluka *et al*., (2015) did not find correlation between NVP plasma level and viral load. Other studies have shown that many HIV patients receiving the efavirenz regimen and have plasma efavirenz concentration of <1.000 mg/L seem to have a higher risk for virological failure and an emergence of selective drug resistance, while patients with efavirenz concentration above 4.000 mg/L appear to experience many neurological adverse effects (Rotger et al., 2007; Gunda et al., 2013). In Tanzania, Gunda *et al*., (2013) observed that the proportion of patients with sub-therapeutic NVP and EFV plasma concentrations significantly increased with increasing viral loads and advancing HIV stage. Further similar observation to ours was reported from previous studies done in Uganda and Italy (Ahoua et al., 2009; Fabbiani et al., 2011). Inevitably, the presence of high rates of sub-therapeutic ARV concentrations among adult patients implies that these patients stand a high risk of inadequate viral suppression and a subsequent potential of developing and accumulating resistant viral strains if these drug concentrations are not corrected timely (Kasang et al., 2011).

The importance of drug levels has also been shown with regards to immunological outcomes. Several studies have demonstrated the importance of $CD4^+$ T-cell count in accurately predicting progression to AIDS and death (Perrone et al., 2014). Although we did not find association between CD4 count and both NVP and EFV plasma levels, there was a trend towards having higher CD4 count among patients with sub- therapeutic NVP plasma level and higher CD4 count for patient with supra-therapeutic EFV plasma level. The mean (SD) current CD4 count for sub-therapeutic NVP level was 600.9 (1617.2) cells/μL compared to 313.3(217.9) cells/μL for patients with supra-therapeutic NVP levels. The mean (SD) current CD4 count for sub- therapeutic EFV level was 335.8 (266.4) cells/μL compared to 421.4(190.5) cells/μL for patients with supra-

therapeutic EFV levels. Conflicting results have been reported regarding ARV drugs and CD4 count, Oluka *et al*., (2015) reported plasma nevirapine levels predicted greater change in CD4 cell count after ART initiation while Perrone *et al*., (2014) reported no differences between NNRTI drug levels with regard to whole $CD4^+$ T-cell count or the percentage of patients with a $CD4^+$ T-cell count within the therapeutic range. Regardless of our results, the importance of ART initiation earlier in the disease course, when CD4+ T-cell lymphocyte cell (CD4) counts are higher, has expanded (Lima et al., 2015). Further, mathematical modelling has demonstrated how earlier treatment initiation can reduce community-level viral load and curb the incidence of new infections (Granich et al., 2009). These evidence led to the new guideline by WHO that all individuals living with HIV should begin ART immediately after diagnosis, regardless of CD4 cell count (Cohen et al., 2011; Lundgren et al., 2015).

Though this study reported no association between NVP and EFV with hepatotoxicity (ALT and AST), there was evidence towards increased ALT and AST levels among patients with supra-therapeutic NVP and EFV plasma concentrations. Studies show patients with supra-therapeutic plasma NNRTI have a high risk of developing drug toxicity (Gunda et al., 2013). Patients with supra-therapeutic NVP plasma concentrations tended to have anemia, leucopenia, and thrombocytopenia but not lymphocytosis. Patients on EFV had varied relationship with hematologic abnormalities. Patients with sub-therapeutic EFV plasma concentrations tended to have anemia, thrombocytopenia and lymphocytosis while patients with supra-therapeutic EFV plasma concentrations had leucopenia. Moyle (2002) has reported significant hematological and biochemical complications due to HIV. These abnormalities have been attributed to either; HIV infection, sequel of HIV-related opportunistic infections, malignancies and consequence of therapies used for HIV infection and associated conditions. This area still remains a grey area of research globally and many patients would benefit significant on the outcome of this monitoring.

5.6 Role of CY2B6 and constitutive androstane receptor (CAR) on nevirapine and efavirenz plasma concentrations

Studies have assessed the relationship between CYP2B6 gene polymorphisms and EFV and NVP pharmacokinetics in HIV-infected patients of different ethnicities including Kenya (Kwara et al., 2009; Oluka et al., 2015), these studies have been limited to either: NVP or EFV based regimen separately, on a small samples size, or a few genetic variants at a time. This study provided a comprehensive genetic analysis of CYP2B6 and CAR polymorphisms and their association with NVP and EFV plasma concentrations in one of the largest cosmopolitan ARV treatment centers in Nairobi Kenya. Despite the finding that NVP and EFV plasma concentrations of the majority of patients (31.5% and 63.8% NVP and EFV respectively) were within the therapeutic window, there were 14.2% and 4.5% of the patients who had suboptimal NVP and EFV concentrations respectively. On the other hand, 54.3% and 31.7% of the patients had supra- therapeutic NVP and EFV plasma concentrations respectively, implying a significant challenge in the management of NVP and EFV based regimens in HIV-infected Kenyan patients, since EFV concentrations are associated with risk of treatment failure and toxicity, particularly neurotoxicity while rash for NVP.

Five CYP2B6 SNPs, including 329G>T (exon 2), 341T>C (exon 3), 516G>T (exon 4), 983T>C (exon 7) and 18492C>T (intron 8), and CAR 540C>T were significantly associated with NVP concentrations. On the other hand, 3 CYP2B6 SNPs 516G>T (exon 4), 835 G>C (exon 6) and 983T>C (exon 7) were significantly associated with EFV plasma concentrations. These findings reiterate the importance of variations in splicing, coding, and subsequent CYP2B6 enzymatic activity on the effect of NVP and EFV metabolism.

Many studies have demonstrated the role of many CYP2B6 SNPs on enzymatic activity with concomitant effect on NVP or EFV concentrations. Associations between 516G>T and NVP or EFV plasma concentrations has been documented in a number of recent studies (Meng et al., 2015; Oluka et al., 2015). In this study CYP2B6 516G>T was associated higher NVP and EFV concentrations, re-affirming the significant role of 516G>T to NVP and EFV metabolism among

the Kenyan population, consistent with previous findings in Kenya and other global populations (Rodriguez-Novoa et al., 2005; Li et al., 2012; Meng et al., 2015; Oluka et al., 2015)

The CYP2B6 983T>C SNP was associated with higher NVP and EFV plasma level. For both drugs the effect is noteworthy for its magnitude. This finding is consistent with previous report in African populations (Oluka et al., 2015). Reports indicates that CYP2B6 983T>C SNP results in the variant protein CYP2B6*18 with an I328T as the only amino acid change. Its expression in vitro results in no detectable protein or activity, hence termed as a null allele (Klein et al., 2005; Honda et al., 2012). This null status explains the greater impact of CYP2B6 983TC for patients on NVP compared to the patients on EFV. In agreement with this results, other studies have reported that CYP2B6 983TC heterozygosity leads to reduction in nevirapine clearance (Schipani et al., 2011).

Patients with heterozygous and homozygous mutant of CYP2B6 18492T>C was associated with markedly lower plasma nevirapine and efavirenz concentrations, which is consistent with previous studies (Sukasem et al., 2012; Manosuthi et al., 2014; Soeria-Atmadja et al., 2017). Studies have shown the presence of this CYP2B6 18492T>C SNP together with coadministration of a strong CYP inducer may increase the likelihood of subtherapeutic plasma efavirenz concentrations (Manosuthi et al., 2014).

This study showed an association of CYP2B6 835G>C had higher EFV plasma concentration than the wildtype. On the other hand, in linear regression analysis CYP2B6 835G>C was not associated with NVP plasma concentration, even though patients with heterozygous mutation for CYP2B6 835G>C had lower NVP plasma level than the wildtype. These findings are also consistent with a study among the Rwandese population (Radloff *et al*., 2013).

For CYP2B6 329G>T, there were 1.6% patients on NVP based regimen who had heterozygous mutant for 329 GT. All the 312 patients on EFV had homozygous wild-type for CYP2B6 329G>T SNP. A previous study reported 0.1% frequency of SNP c.329G> T of exon 2 was detected in the Luhya tribe of west Kenya. Although there were only 4 patients with heterozygous mutant 329GT, their NVP plasma concentration was 2-fold higher than the wild-type. This observation is

consistent with the observation of Marth *et al*., (2011) which reported that most rare genetic variations, even in their heterozygous form, can have a deleterious effect on protein structure and modify the phenotype. Radloff *et al*., (2013) showed four SNPs c.329G> T, c.341T> C, c.548T> G and c.637T> C as coding for CYP2B6 proteins with reduced function or completely inactive these proteins similar to observations of Marth *et al*., (2011).

There were 3.2% and 0.9% patients on NVP and EFV regimen who had heterozygous mutant for CYP2B6 341T>C respectively. Although there were only 8 patients with heterozygous mutant 341TC, their NVP plasma concentration was 2-fold lower than the wild-type median (IQR) 3675.5 (1163.5 to 12328) ng/ml and 6282 (4558 to 8889) ng/ml respectively. On the contrary, for patients on EFV the 8 patients with heterozygous mutant 341TC, their EFV plasma concentration was 1.5-fold higher than the wild-type median (IQR) 3680 (1652 to 5308) ng/ml and 2732 (1886 to 4872) ng/ml respectively. Radloff *et al*., (2013) showed four SNPs c.329G> T, c.341T> C, c.548T> G and c.637T> C as coding for CYP2B6 proteins with reduced function or completely inactive these proteins similar to observations of Marth *et al*., (2011). Thus, explaining the higher concentration of EFV plasma level for the heterozygous mutant 341TC. The role of CYP2B6 341T>C in the NVP concentrations both in-vivo and invitro is an opening for future investigations.

This is the first report in Kenya to assess the role of constitutive androstane receptor (CAR) 540T C>T in the concentrations for both NVP and EFV. In this study, though not significant in linear regression analysis, the homozygous and heterozygous for mutation for CAR 540T C>T had higher NVP and EFV plasma concentrations. This was consistent with the two stated reports which observed a trend towards association between plasma efavirenz concentration and CAR 540C>T (Wyen et al., 2011; Sarfo et al., 2014). This report being the first in Kenya opens the avenue for further rigorous prospective investigations.

We then evaluated the effects of the total number of CYP2B6 and CAR genotypes per patient on NVP and EFV plasma levels. There were 203(79.9%) and 237(75.9%) patients on NVP and EFV respectively, with more than two SNPs. The study reported 30 (11.8%) and 41(13.4%) patients on

NVP and EFV based regimen respectively with 6 different number of SNPs. There were 7(2.8%) and 6(1.9%) patients on NVP and EFV regimen respectively with 7 different SNPs. This study showed the patients who had larger number of variant alleles, had higher NVP and EFV plasma levels, with a positive association between the number of SNPs and the levels of NVP and EFV.

In this study we evaluated the role of ethnicity on NVP and EFV plasma which is important and relevant among the Kenyan population. The important for this evaluation was that currently there are no elaborate genotyping data for Kenya population and the second that this population is currently using ARVs, which are metabolized by CYP2B6 and CAR 540 (Hedrich et al., 2016). Similarities and variation in the frequency of variant alleles were observed across various populations across the globe (Haas et al., 2005; Ellison et al., 2012). Of importance to note is that there was no significant difference between genotype and allele frequencies across the three ethnic blocks in this study (Bantus, Cushite and Nilotes). Further controlling for ethnicity did not change the relationship between the CYP2B6 and CAR 540 SPNs among this population.

Haplotype analysis collectively assesses the interactions of multiple SNPs, leading to a decrease or increase in the metabolic function of CYP2B6 or CAR. In theory, haplotype accuracy may be higher, compared with single SNPs in predicting NVP or EFV pharmacokinetics (Carr et al., 2010). To our knowledge, this study represents the first report of relationship between haplotype and NVP and EFV concentrations in Kenya. For both NVP and EFV based patients, linkage disequilibrium among 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T was observed, resulting in 8 haplotypes among which CTGCTTCC and CGATTCCT had the highest and the lowest frequency, respectively for both patients on NVP and EFV. Compared to CTGCTTCC, five (5) haplotypes (CGACTCCC, CGATTCCC, CTGCTTCT, CGACTCCT and CGATTCCT) were associated with lower NVP plasma levels while 2 (CGACCCCC and TGACTCCC) with higher NVP plasma concentration. Contrary to NVP, for EFV compared to CTGCTTCC 3 haplotypes (TGACTCCC, CGATTCCT and CGACTCCT) were

associated with lower EFV plasma concentration while 4 haplotypes (CGACTCCC, CGACCCCC, CGATTCCC and CTGCTTCT) were associated with higher EFV plasma concentration.

According to the metabolic score 29.8% of the population study showed a CYP2B6 and CAR extensive metabolic phenotype, whereas 171/566 (30.2%) and 226/566 (39.9%) had a reduced and increased metabolic phenotype respectively. The poor and the ultra-rapid metabolic phenotype were observed in 64/566 (11.3%) and 97/566 (17.1%) of individuals respectively, whereas 107/566 (18.9%) showed an intermediate reduced metabolic phenotype, and 226/566 (39.9%) had an intermediate increased metabolic phenotype. These proportions were higher than those observed among the bantus in Botswana (Tawe et al., 2018) and previously in Kenyan women population (Oluka et al., 2015). Individuals with a CYP2B6 reduced metabolic phenotype may have an increased risk of an impaired outcome of EFV or NVP-based ART regimen (Russo et al., 2016; Tawe et al., 2018). Of concern is the significantly high number of individuals (17.1%) having an inferred ultra-rapid metabolic phenotype, the effect of their metabolic pattern affects EFV or NVP-based ART regimen by leading to sub-therapeutic drug exposure with a potential increase of risk of selection for viral resistance (Russo et al., 2016). Further studies should focus on this fast metabolizer fraction of the population whose data are unavailable in the scientific literature.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In conclusion these data establish prolonged immunologic/virologic response to ART among patients continuing on therapy. The prevalence of anemia, thrombocytopenia and leucopenia was low with minimal renal and hepatotoxicity impairment observed. Wide interindividual variability were observed in NVP and EFV plasma concentrations with a large proportion of patients falling outside of therapeutic window, proposing potentially an increased risk of treatment failure or toxicity. This study demonstrates the importance of therapeutic drug monitoring in determining the ARV treatment outcome. Six SNPs of the *CYP2B6* gene (CYP2B6 329G>T, CYP2B6 637T>C, CYP2B6 785A>G, CYP2B6 18492C>T, CYP2B6 21563C>T and 15582C>T) and one CAR (540T>C) could potentially act as additional independent predictors of NVP and EFV plasma concentrations beyond that provided by the *CYP2B6* c.516G>T and CYP2B6 983T>C polymorphism. Host pharmacoecological factors influence ART drug adherence impacting on the NVP and EFV plasma concentrations.

The current data established prolonged immunologic/virologic response to ART among patients continuing on therapy. Patients who had immunologic/virologic failure were more likely to be on treatment with NVP based regimen. ART use was associated with improvement of all hematological indices resulting in a remarkable reduction in the prevalence of adverse effects such (hepatic and renal impairment, thrombocytopenia, anemia, leucopenia and lymphocytosis) after 12 months.

Wide interindividual variability were observed in NVP and EFV plasma concentrations with a large proportion of patients falling outside of therapeutic window, proposing potentially an increased risk of treatment failure or toxicity. This study demonstrates the importance of therapeutic drug monitoring in determining the ARV treatment outcome

Demographic characteristics such as age, gender, education levels are important specific pharmacoecological variables influencing NNRTI plasma levels by affection adherence to ART. Sexual behavior such as sexual life partners, age of sexual debut influences ART adherence which in turn is associated to NNRTI plasma levels. The risky sexual practices are key to infection and transmission of HIV drug resistance virus which has been demonstrated to affect the NNRTI plasma levels. HIV stigma, disclosure, social support which are interlinked with each other influences medication adherence and better clinical outcome**.** Nutritional status was important determinant affecting NNRTI exposures.

Various mutations conferring resistant to ART were identified. These mutations were associated with treatment outcomes in terms of virologic failure and altered drug plasma levels. Side effect of ARV lead to treatment discontinuation leading to altered drug plasma levels

This study identified six SNPs in the *CYP2B6* gene (CYP2B6 329G>T, CYP2B6 637T>C, CYP2B6 785A>G, CYP2B6 18492C>T, CYP2B6 21563C>T and 15582C>T) and one CAR (540T>C) which could potentially act as additional independent predictors of nevirapine and efavirenz plasma concentrations beyond that provided by the *CYP2B6* c.516G>T and CYP2B6 983T>C polymorphism. A significant number of the study patients 1.9% on NVP and 13.4% on EFV had 6 to 7 different number of SNPs. Patients who had larger number of variant alleles, had higher median NVP and EFV plasma levels. Two types of haplotype CGACCCCC and TGACTCCC haplotypes were associated with higher plasma NVP concentrations while four haplotypes CGACTCCC, CGACCCCC, CGATTCCC and CTGCTTCT) were associated with higher EFV plasma.

6.2 Recommendation

Although the study showed the use of ART was associated with improvement of all hematological indices, a substantial proportion of patients still had liver enzymes elevation and hematological abnormalities after 12 months of ART, raising the need for routine screening of biochemical and

hematological outcomes, investigating the underlying causes and instituting appropriate treatment and management strategies to mitigate the adverse effects of ART.

This study showed wide inter-individual variability in NVP and EFV plasma levels. This study established the importance of incorporating therapeutic drug monitoring in determining the ARV treatment outcome.

In this geographical defined region of Africa, large prospective studies are proposed to confirm further the association between pharmacogenetic and pharmacoecological parameters on NNRTI therapeutic drug levels on toxicities in order to guide their application in the individualization ART treatment in Kenya

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Appendix 1: Informed Consent Document

Description: Nevirapine (NVP) or Efavirenz (EFV) are NNRTI antiretroviral agents used in combination with other antiretroviral drugs to manage HIV/AIDS infection. Reports show that patients have varied drug blood level with some sustaining high while others low levels. Different factors contribute to this difference in blood levels including adherence to drugs, presence of drug resistant HIV or different enzyme breaking these drugs either faster or slower. We would wish to conduct a study to find out what are the reasons why blood levels of EFV and NVP vary from person to person. You are asked to participate in a research study to determine the causes of why you are not responding to your ARV treatment. This information would help us to design accurate methods to prevent your inability to respond treatment. If you agree to participate we will ask to engage you on a face to face interview to gather information that is related to ARV treatment. We will also take the blood samples during your normal visit to the FACES program which will be stored for specialized testing. At a later date, part of your samples will be transported to Laboratory of Retrovirology, Public Research Centre for Health in Luxembourg for confirmation and specialized testing.

Inclusion and exclusion Criteria

In order to qualify to participate in the study, you should meet the following criteria

1. You must be HIV/AID infected males and females
2. Aged between 18 – 55 years
3. Attending FACES HIV treatment program
4. Able to voluntarily given written informed consent prior to the commencement of the study
5. Must have been taking either EFV/NVP containing HAART regimen for at least 8 weeks

Exclusion Criteria

You will not be able to participate in this study if you are;

1. Pregnant women
2. History of any psychiatric illness or any other condition which may impair the ability to provide written informed consent
3. Aged below 18 years and above 55 years
4. Not able to provide informed consent
5. Patients on either EFV/NVP containing HAART regimen for less than less than 8 weeks

Risks and discomfort: One potential risk of being in the study is the loss of privacy. However, we will do our best to make sure that the personal information gathered during this study is kept private. Also, you might feel a little discomfort at the time we will be taking your blood sample. There is no monetary benefit for your participation in this study.

Benefits: The benefit which may reasonably be expected to result from this study includes your contributions to efforts to prevent ARV treatment failures by guiding correct treatments for your disease. If you are found harboring drug resistant virus, or other factors hindering your good response to treatment the information will be communicated to your health care provider for better management.

Confidentiality: The study personnel will take all the precaution to keep your participation in the study confidential. Your samples will be identified only by coded number. In any reports generated from this study none will use your names.

Voluntary participation: The decision to participate in this study is purely your choice. Your decision whether or not to participate in this study will not affect your current enrollment in the FACES program.

Time involvement: This study will be part of your routine visit to FACES-KEMRI. The general time involved in your program at FACES – KEMRI will not be extended**.**

Questions: You are free to ask any questions at any time about the study as well as regarding your rights as a research volunteer. You will not be giving up any of your rights by signing this consent form

Further information: Please contact the following

Musa Otieno Ngayo

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If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish the Chairperson of the University of Nairobi/Kenyatta National Hospital Research and Ethics Committee (UoN/KNH-ERC), PO Box 19676, Nairobi, Kenya; Tel: +254 020 2726300 ext 44102.

Statement of consent: I have read this form or had it read to me in a language that I understand. I have discussed the information with study staff. My questions have been answered. My decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time. By signing this form, I do not give up any rights that I have as a research participant.

I have read or have had the document read to me: YES___ NO____

I agree to participate in this research study: YES___ NO____

I agree to have my blood collected/stored and analyzed for study assays: YES___NO___

_____ _____

Participant Signature/ Thumbprint Date

I, the undersigned have fully explained the relevant details of this research study to the participant named above and believed that the participant has understood and has knowingly given his consent.

_____ _____ _____

Study Staff Conducting Study Staff Signature Date

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KEMRI/RES/7/3/1 **December 16, 2021**

TO: **MUSA OTIENO NGAYO,**
PRINCIPAL INVESTIGATOR.

Forwarded 20/12/2021

THROUGH: **THE DEPUTY DIRECTOR, CMR,**
NAIROBI.

Dear Sir,

RE: **SSC PROTOTOCOL NO 2539 (REQUEST FOR ANNUAL RENEWAL & DEVIATION): ETIOLOGY OF SUB-OPTIMAL RESPONSES TO NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NNRTI) AMONG HIV PATIENTS ON ANTIRETROVIRAL TREATMENT IN NAIROBI KENYA**

Thank you for the continuing review report for the period November 6, 2020 to December 7, 2021. This is to inform you that at the 318th joint committee A, B, and C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **December 14, 2021** noted that a protocol deviation form has been submitted as the request for annual renewal was done after the expiration date of the last approval.

This is to inform you that the Review team of the SERU was of the informed opinion that the progress made during the report period is satisfactory. The study has therefore been granted **approval.**

This approval is valid from December 16, 2021 through to December 15, 2022. Please note that authorization to conduct this study will automatically expire on **December 14, 2022**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuing approval to SERU by **November 3, 2022.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to SERU for review prior to initiation. You may continue with the study.

Yours faithfully,

PROF. CHARLESS OBONYO,
THE ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT.

APPENDIX 3: DATA EXTRACTION FORM

Section A: socio-demographic characteristics

Date of Birth: _____
Gender: Male ____ Female____
Marital status: Married ____Single _____Divorced____
Highest education level: Degree____Diploma ____ Secondary ____ Primary ____
Occupation: Employed____ Unemployed ____ Self employed____
Ethnicity: _____
Smoker: Yes____ No____
Alcohol use: Never ____ Occasionally____ Regularly____
Date diagnosed with HIV: _____
Date commenced HAART: _____
Concurrent medical conditions at the time of data collection

1.
2.

Allergies _____

Section B: Selected Laboratory Values at Baseline

Parameter	Date	Value	Normal value
ALT			
AST			
Bilirubin			
Creatinine			
CD4 count			
Viral load			

Section C: Medical History

(Only list medication that the patient has been taking at least 2 weeks before and after the date of sample collection)

	Drug Name	Dose	Frequency
	Antiretrovirals		
1.			
2.			
	Others		
5.			
6.			

Section D: Laboratory Values During Treatment

Date						
Parameter	1st	2nd	3rd	4th	5th	6th
ALT						
AST						
Bilirubin						
Creatinine						
CD4 counts						
Viral load						

Date						
Parameter	7th	8th	9th	10th	11th	12th
ALT						
AST						
Bilirubin						
creatinine						
CD4 counts						
Viral load						

Section E: Clinical notes during treatment

(Examine the clinical notes for the following)

Did the patient complain of rash? Yes ___ No ____

If yes to the above, what was the severity? Mild ____ Severe ____

Is the patient noted to be non-adherent? Yes ____ No ____

A. HEALTHCARE ACCESS

Date of Interview: ☐☐/☐☐/☐☐☐☐ (Day / Month / Year)

Participant ID: ☐☐☐☐☐

Interviewer Initials: ☐☐

1	What is your age?	☐☐ years		999○Don't know
2	What is your date of birth?	☐☐/☐☐/☐☐☐☐ (Day / Month / Year)		999○Don't know
3	What is your religion?	1○Protestant	4○Catholic	7○None
		2○Other Christian	5○Saved/Pentecostal	8○Other _____
		3○Seventh Day Adventist	6○Muslim	
4	What is the highest level of school you completed?	1○none	5○secondary	8○diploma
		2○some primary	6○some college	9○degree
		3○primary	7○certificate	10○postgraduate
		4○some secondary		
5	Have you completed any other type of vocational school?	1○Yes		
		2○No		
6	Have you ever been married?		1○Yes	2○No
7	Are you currently married or living together with a partner?		1○Yes	2○No
8	Beside you do you have other partner have other wives or does he live with any other women as if married?		1○Yes	2○No
9	Do you have a previous husband or partner who died?		1○Yes	2○No
11	Did you attend the clinic for antenatal care during your last pregnancy?	1○Yes	2○No	
12	How many times did you attend antenatal care?	1○1 time 2○2-3 times 3○4 or more times		
Now I am going to ask you some questions about transportation.				
13	How long does it normally take to travel from your house to this clinic – a one way trip?	☐☐☐ **Minutes** Convert all time to minutes. 1 hour = 60 minutes		
14	How much does it cost for you to travel from your house to this clinic and then back – in other words, round-trip? Include the cost of any overnight stays.	☐,☐☐☐ Shillings		
Now I am going to ask you about your health and health care in the past 3 months.				
15	In the **past 3 months**, were you admitted in a	1○yes	2○no	

	hospital for any reason?

A. HEALTHCARE ACCESS

16	How many different **times** were you admitted to a hospital?	☐☐ times	
17	In the **past 3 months**, how many days did you spend most of the day lying down because of an illness?	☐☐ times →If 00,SKIP TO E.7	
18	How about in the **past 30 days**? How many days did you spend most of the day lying down because of an illness?	☐☐ times	
19	How many times in the **past 3 months**, did you visit a medical clinic to see a doctor or nurse?	☐☐ times	
20	How many different **times** in the past 3 months did you visit a medical clinic because you were acutely ill?	☐☐ times	
21	In this past 3 months, have you missed a scheduled HIV visit for any reason?	1○yes	2○no → SKIP TO E.11
22	What was the reason you missed a scheduled visit?	1○Not having money 2 ○Not having childcare 3 ○Working on field 4○Forgetting	5○Not enough food 6○No transport 7○Other
23	In the past **3 months**, did you visit, or were you visited by any of the following? Mark all that apply. Do not include visits solely for research.		
23.1	Public health nurse	1○yes	2○no
23.2	Pharmacist	1○yes	2○no
23.3	Community health worker	1○yes	2○no
23.4	Traditional healer	1○yes	2○no

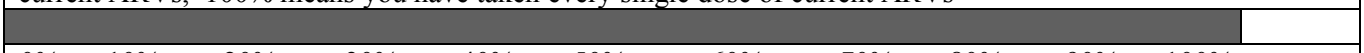
B. ARV HISTORY AND ADHERENCE

24	What ARVs are you taking now?	[1] ○ LAMIVUDINE, NEVIRAPINE, STAVUDINE [2] ○ LAMIVUDINE, NEVIRAPINE, ZIDOVUDINE [3] ○ LAMIVUDINE, NEVIRAPINE, TENOFOVIR [4] ○ LAMIVUDINE, EFAVIRENZ, TENOFOVIR [5] ○ LAMIVUDINE, EFAVIRENZ, ZIDOVUDINE [6] ○ LAMIVUDINE, LOPINAVIR, TENOFOVIR, RITONAVIR [7] ○ OTHER, Specify_____
24	*Ascertain from the best possible source the following information regarding how ARVs are supposed to be used by participant (i.e., prescribed by health care provider)*	
24.1	Prescribed # of times used per day	☐
24.2	Prescribed # of pills used at each time	☐
24.3	Data obtained from (mark all that apply)	○ Pill bottle ○ Medical document ○ Subject self-report ○ Other, specify_____
25	We consider any situation where you stopped taking current ARVs for 30 days or more and when you then re-started it to count as a new episode of use. With this in mind, when did you begin to use *current ARVs* during your most current episode of use? *Probe participant to ensure that prior lapses of 30 days or more are counted as beginning of new episode.*	☐☐/☐☐/☐☐☐☐ Day Month Year *Leave day or month blank if unknown*
26	When was the last time you took *current ARVs*? *Date should be within 3 days of today's date*	☐☐/☐☐/☐☐☐☐ Day Month Year *Leave day or month blank if unknown*
27.1	Did you ever miss taking current ARVs for a whole day or more?	[1] ○ Yes [2] ○ No
27.2	How many different days did you miss taking current ARVs?	☐☐ days
27.3	What was the longest number of days continuously or without a break that you missed taking *current ARVs?*	☐☐ days
27.4	What was the smallest number of days continuously or without a break that you missed taking *current ARVs?*	☐☐ days
28	How many times did you miss taking *current ARVs*:	
28.1	For at least 1 day but no more than 3 days?	☐☐ times

28.2	For at least 4 days but no more than 13 days?	☐☐ times
28.3	For 14 days or more?	☐☐ times
29	Now I want to ask you about the past 3 days. How many doses of current ARVs did you miss:	

B. ARV HISTORY AND ADHERENCE

	Days	0	1	2	3
29.1	Yesterday?	○	○	○	○
29.2	2 days ago?	○	○	○	○
29.3	3 days ago?	○	○	○	○

	Refer to the date given in question 3 and record if subject been taking current ARVs for at least the past 30 days.	[1]○ Yes [2]○ No
31	In the past 30 days, how many times do you think you missed a dose of *current ARVs*?	☐☐ times
32	*Give instrument and pen to participant* Please place an "X" on the line below at the point showing your best guess about how much current ARVs you have taken in the past 30 days 0% means you have taken no current ARVs, 50% means you have taken half your current ARVs, 100% means you have taken every single dose of current ARVs  0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%	
32.1	Other than your current use of *current ARVs*, was there ever a time in the past when you used this drug? Consider breaks of 30 or more days to constitute discrete episodes	[1]○ Yes [2]○ No
32.2	*Ask subject and record answers for each episode below. Record the most recent episode first:* Consider breaks of 30 days or more to constitute discrete episodes.	

		When did you first start taking this drug?	When did you stop taking this drug?	*Calculate length of each episode in months or days*.	Did you take this drug as part of preventing spread of HIV to your baby?
	Prior episode	Start Date mm/yyyy	Stop Date mm/yyyy	Length of Use	MTCT
C.11.2.1	1	☐☐/☐☐☐☐	☐☐/☐☐☐☐	☐☐ 1 ○ days 2 ○ months	[1]○ Yes [2]○ No [3]○ N/A

C.12.1	People stop or interrupt taking medications for different reasons. Did you stop/interrupt taking your current ARVs?	[1]○ Yes [2]○ No ~~If no~~, skip to end [3]○ N/A
C.12.2	Did you stop or interrupt taking your current ARVs because your doctor wanted you to, you decided to on your own without your doctor's knowledge or you and your doctor came to the decision together?	[1]○ doctor [2]○ self [3]○ together
C.13	I will read a list of possible reasons that caused you to stop/interrupt taking ARVs. Please answer yes or no for each one.	

C.13.1	You didn't have enough money	[1]O yes	[2]O no	O primary
C.13.2	You were experiencing side effects	[1]O yes	[2]O no	O primary
C.13.3	You had no transportation to the pharmacy	[1]O yes	[2]O no	O primary
C.13.4	You felt well or better and felt you didn't need the medications	[1]O yes	[2]O no	O primary
C.13.5	You felt the medication was not helping you	[1]O yes	[2]O no	O primary
C.13.6	Your doctor felt the medication was not working	[1]O yes	[2]O no	O primary

B. ARV HISTORY AND ADHERENCE

C.13.7	You gave your medications away to someone else	[1]O yes	[2]O no	O primary
C.13.8	You needed to start medication to treat your TB and therefore could not take your HIV medications	[1]O yes	[2]O no	O primary
C.13.9	Lapse of drug supply by payment source	[1]O yes	[2]O no	O primary
C.13.10	You did not have enough food to eat	[1]O yes	[2]O no	O primary
C.13.11	Were there any other reasons which caused you to stop/interrupt?	[1]O yes Specify_____	[2]O no	O primary
	If only one reason is cited, then mark that as the primary reason. If more than one reason is cited, ask: Please tell me what is the main reason which caused you to stop/interrupt. Mark this as primary reason.			
C.14	If side effects are listed as "yes", ask: I will read a list of possible side effects that may have caused you to stop/interrupt taking ARVs. Please answer yes or no for each one.			
C.14.1	Fatigue or loss of energy	[1]O yes	[2]O no	
C.14.2	Dizziness or lightheadedness	[1]O yes	[2]O no	
C.14.3	Headache	[1]O yes	[2]O no	
C.14.4	Pain, numbness or tingling in the hands or feet	[1]O yes	[2]O no	
C.14.5	Nausea or vomiting	[1]O yes	[2]O no	
C.14.6	Diarrhea or loose bowel movements	[1]O yes	[2]O no	
C.14.7	Bloating, pain or gas in your stomach	[1]O yes	[2]O no	
C.14.8	Problems with weight loss or wasting	[1]O yes	[2]O no	
C.14.9	Problems with fat deposits or weight gain	[1]O yes	[2]O no	
C.14.10	Skin problems, such as rash, dryness or itching	[1]O yes	[2]O no	
C.14.11	Felt nervous or anxious	[1]O yes	[2]O no	
C.14.12	Felt sad, down or depressed	[1]O yes	[2]O no	
C.14.13	Was there another side effect you experienced?	[1]O yes Specify_____	[2]O no	

C. STIGMA

Now, please tell me if you either Agree or Disagree with each of these statements.			
D.1	It is difficult to tell other people about my HIV infection	1 ○ Agree	2 ○ Disagree
D.2	Being HIV positive makes me feel immoral.	1 ○ Agree	2 ○ Disagree
D.3	I feel guilty that I am HIV positive.	1 ○ Agree	2 ○ Disagree
D.4	I am ashamed that I am HIV positive.	1 ○ Agree	2 ○ Disagree
D.5	I sometimes feel worthless because I am HIV positive.	1 ○ Agree	2 ○ Disagree
D.6	It is my own fault that I am HIV positive	1 ○ Agree	2 ○ Disagree
D.7	I hide my HIV status from others	1 ○ Agree	2 ○ Disagree
D.8	I feel certain that I can tell my primary sex partner that I have HIV.	1 ○ Agree	2 ○ Disagree

D. MORBIDITY

Now I am going to task you about HIV, your health and health care in the past 3 months.

E.1	When was the first time you tested positive for HIV?	Month ☐☐	Year ☐☐☐☐
		○ Do not know month	○ Do not know year
E.2	Before you tested HIV-positive, when was the last time you remember testing HIV-negative?	Month ☐☐	Year ☐☐☐☐
		○ Do not know month	○ Do not know year ○ Never tested negative
E.3	Have you had any serious condition or illness that you felt was caused by your HIV/AIDS infection?	○ Yes	○ No
	Refer to disease codes	What year?	
E.3.a	Other code ☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.b	Other code ☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.c	Other code ☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.d	Other code ☐☐☐☐	☐☐☐☐	○ Do not know year

Disease/condition	***Code***
Candidiasis of bronchi, trachea or lungs	*CAND*
Cervical cancer, invasive	*CECA*
Coccidioidomycosis, disseminated or pulmonary	*COCC*
Cytomegalovirus disease (other than liver, spleen or nodes)	*CMVD*
Encephalopathy, HIV-related	*ENCE*
Herpes simplex: chronic ulcers (greater than 1 month's duration; or bronchitis, pneumonitis, or esophagitis)	*HERP*
Histoplasmosis, disseminated or extrapulmonary	*HIST*
Isosporiasis, chronic intestinal (greater than 1 month's duration)	*ISOS*
Mycobacterium avium complex (MAC) or M. kansasii, disseminated or extrapulmonary	*MACK*
Mycobacterium species other than Mycobacterium tuberculosis, avium complex, or kansasii, or unidentified mycobacterial species; disseminated or extrapulmonary	*MYCO*
Pneumonia, recurrent	*PNAR*
Progressive multifocal leukoencephalopathy	*PMLE*
Salmonella septicemia, recurrent (other than Salmonella typhi)	*SALM*

For conditions that do not have disease codes, complete the following

		What year?	
E.3.e	Other condition ☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.f	Other condition ☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.g	Other condition ☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.h	Other condition ☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐	○ Do not know year
E.3.I	Other condition ☐☐☐☐☐☐☐☐☐☐☐☐	☐☐☐☐	○ Do not know year

E. SOCIAL SUPPORT

Now I am going to ask you some questions about your social supports. For each of these questions, you may answer 'as much as I would like,' "less than I would like", 'much less than I would like', or never.

Statement		As much as I would like	Less than I would like	Much less than I would like	Never
F.1	I get useful advice about important things in my life	1O	2O	3O	4O
F.2	I get chances to talk to someone about problems at work or with my housework	1O	2O	3O	4O
F.3	I get chances to talk to someone I trust about my personal and family problems	1O	2O	3O	4O
F.4	I have people who care what happens to me	1O	2O	3O	4O
F.5	I get love and affection	1O	2O	3O	4O
F.6	I get help with household-related work	1O	2O	3O	4O
F.7	I get help with money in an emergency	1O	2O	3O	4O
F.8	I get help when I need transportation	1O	2O	3O	4O
F.9	I get help when I am sick	1O	2O	3O	4O

F. MEDICAL OUTCOME OF HIV

Now, I would like to ask you a few questions about your health.			
J.1	In general would you say your health is excellent, very good, good, fair or poor?	1 ○ Poor 2 ○ Fair 3 ○ Good	4 ○ Very good 5 ○ Excellent
J.2	How much bodily pain have you generally had during the past 30 days? Would you say none, very mild, mild, moderate, severe or very severe?	1 ○ none→skip to Q4 2 ○ very mild 3 ○ mild	4 ○ moderate 5 ○ severe 6 ○ very severe
J.3	During the past 30 days, how much did pain interfere with your normal work, including both work outside the home and housework? Would you say not at all, some, or a lot?	1 ○ Not at all 2 ○ Some 3 ○ A lot	
	The following questions are about activities that a person might do during a typical day. Does your health now limit you in the following activities? If so, does your health limit you a lot or a little bit?		
		Not Limited at all	Limited a Little / Limited a Lot
J.4	Vigorous activities you can do like digging, carrying water from the river to home, or splitting firewood?	1 ○	2 ○ / 3 ○
J.5	Moderate activities you can do like washing clothes, moving a jerrican of water, cooking, sweeping the yard, carrying children or moving a bundle of fire wood from one place to another.	1 ○	2 ○ / 3 ○
J.6	Walking up a hill	1 ○	2 ○ / 3 ○
J.7	Bending, lifting light objects or kneeling.	1 ○	2 ○ / 3 ○
J.8	Walking a distance, like the length of a football pitch, about 100 meters.	1 ○	2 ○ / 3 ○
J.9	Eating, dressing, bathing or using the latrine.	1 ○	2 ○ / 3 ○
J.10	Does your health keep you from working at a job, doing work around the house or attending school?	1 ○	2 ○ / 3 ○
J.11	Have you been unable to do certain kinds or amounts of work, housework or schoolwork, because of your health?	1 ○	2 ○ / 3 ○

J. ALCHOHOL USE

Now I have some questions about alcohol. Because alcohol use can affect many areas of health, it is important for us to know how much you drink and whether you have experienced any problems with your drinking. Please try to be as honest and accurate as possible. It is important in assessing your health for us to know what you actually do.

Alcohol includes **wine, beer, or hard liquor, [insert other local terms for alcoholic beverage]**, or **any beverage that contains alcohol.** Please **do not include** communion wine or wine that you received at church or a religious ceremony when answering these questions.

For the purposes of this study, we consider that one drink is equal to two-thirds of a 500 milliliter bottle of beer, or a drink with 40 milliliters (about 1.3 ounces) of hard liquor,or one 140 milliliter (or 4.5 ounce) glass of wine.

K.1	How often did you have a drink containing alcohol? Was it never, 1 time, 2 to 4 times, 2 to 3 times each week, or 4 or more times a week?	1 ○ Never → Skip to next section 2 ○ 1 time 3 ○ 2 to 4 times	4 ○ 2 to 3 times a week 5 ○ 4 or more times a week
K.2	How many drinks of any kind containing alcohol did you have on a typical day when you were drinking?	1 ○ 1 or 2 2 ○ 3 or 4 3 ○ 5 or 6	4 ○ 7, 8, or 9 5 ○ 10 or more 6 ○ Cannot estimate because of use of non-standardized non-bottled home-brewed beverages
K.3	How often did you have six or more drinks of any kind on one occasion? Never, less than monthly, monthly, weekly, or daily or almost daily?	1 ○ Never 2 ○ Less than monthly 3 ○ Monthly	4 ○ Weekly 5 ○ Daily or almost daily 6 ○ Cannot estimate because of use of non-standardized non-bottled home-brewed beverages

H. SEXUAL BEHAVIOUR

As you may know, a person may get the AIDS virus through sexual activity. To help prevent the spread of AIDS, we need to know more about all the different types of sexual practices that people have. Some of these questions need to be rather detailed and personal. Since this survey is confidential, no one will know your answers. We would appreciate your participation in answering these questions as openly as possible.

L.1	How old were you when you had sex for the very first time? By sex, I mean when a man puts his penis in a woman's vagina.	☐☐ years 999 ○ Don't know
L.2	How many different people have you had sex with in your life?	☐☐☐ lifetime sexual partners 999 ○ Don't know
L.3	In the past 3 months, with how many different men have you had sex? By sex, I mean when a man puts his penis in a woman's vagina.	☐☐ partners 999 ○ Don't know
L.4	Of those with whom you had sex in the past 3 months, how many of these people were your spouses?	☐☐ spousal partners
L.5	Of those with whom you had sex in the past 3 months, how many of these people were steady partners?	☐☐ steady partners
L.6	Of those with whom you had sex in the past 3 months, how many of these people were casual partners? NB: A casual partner is someone who is not your spouse and with whom you have only had sex with once or only a couple of times a year or with whom you do not plan on having a long-term relationship.	☐☐ casual partners

Check if the number of partners in questions L.4 + L.5 + L.6 is equal to number of partners in question L.3. Probe and correct if necessary.

|__|__| + |__|__| + |__|__| = |__|__|
L.4 L.5 L.6 L.3

I. DISCLOUSER AND NUTRITIONAL PROFILE

M.1	Have you told anyone about your HIV status?	$_1$O yes	$_2$O no →Skip to next secion	
M.2	Have you told any of the following people about your HIV status:			
M.2.1	Husband/Wife/Partner	$_1$O yes	$_2$O no	$_3$O Not applicable
M.2.2	Other family member(s)	$_1$O yes	$_2$O no	$_3$O Not applicable
M2.3	Friend	$_1$O yes	$_2$O no	$_3$O Not applicable
M.2.4	Neighbor	$_1$O yes	$_2$O no	$_3$O Not applicable
M2.5	Employer(s)	$_1$O yes	$_2$O no	$_3$O Not applicable
M.2.6	Religious leader	$_1$O yes	$_2$O no	$_3$O Not applicable
M2.7	Public	$_1$O yes	$_2$O no	$_3$O Not applicable
M.2.8	Other (specify)	$_1$O yes	$_2$O no	$_3$O Not applicable
M.3	How many people know about your HIV status in your home where you usually live? Would you say that it is no one, a few of the people, half of the people, most of the people, or everyone?	$_1$O No one $_2$O A few of the people $_3$O Half of the people $_4$O Most of the people $_5$O Everyone		

I. DISCLOUSER AND NUTRITIONAL PROFILE

	Nutritional Profile			
N.1.1	Height (CM) _____ Weight (Kg) _____	BMI (Kg/m2)_____	MUAC (CM)_____ Oedema 1+? _____	
N.1.2	Have you regular access to staple food: _____; Other groups of food_____			
N.1.3	Ability to perform normal duties	$_1$O yes	$_2$O no	$_3$O Not applicable
N.1.4	Meals prepared by?	$_1$O Self	$_2$O family members	$_3$O Others
N.1.5	Do you regularly consume uji	$_1$O yes	$_2$O no	$_3$O Not applicable
N.1.6	On other food supplements	$_1$O yes	$_2$O no	$_3$O Not applicable
N.1.5	Food intake in the last 5-10 days	$_1$O None	$_2$O Poor	$_3$O Moderate / Adequate
N.1.6	Barriers to food intakes	OPoor appetite anorexia O Nausea / Vomiting	ODiarrhoea OConstipation	OPain OFever ODepressions
N.2.1	Weight gain for revisiting patients	OGaining weight	OLosing weight	ONo change
N.2.2	Adherence to FBP	$_1$OCups/bowls of porideg taken per day___	$_2$O Number of RUTF sachets used per day___	

I. DISCLOUSER AND NUTRITIONAL PROFILE

Diagnosis				
N.3.1	Wasting	1 O Mild	2 O Moderate	3 O Severe
N.3.2	Presence of complications?	1 O yes	2 O No	
N.3.3	Inferred cause of Wasting Poor dietary practices/Food insecurity	1 O yes	2 O no	3 O Not applicable
N.3.4	Uncontrolled barriers to food intake	O Poor appetite anorexia O Nausea / Vomiting	O Diarrhoea O Constipation	O Pain O Fever O Depressions
N.3.5	Feeding syndrome risk	1 O Low	2 O High	
N.1.6	Barriers to food intakes	O Poor appetite anorexia O Nausea / Vomiting	O Diarrhoea O Constipation	O Pain O Fever O Depressions

SSC 2539 (SHIPMENT)

22nd August, 2013

The Ag. Secretary

KEMRI ERC

Thro'

The Ag. Director CMR,

NAIROBI

KEMRI/ERC RECEIVED 22 AUG 2013 P.O. Box 54840-00200, NAIROBI

Forwaded on 22/08/2013
[illegible]
Dr B.-. Nzuki
& DCMR.

Dear Madam,

REF: SSC No. 2539 - Request for permission to export biological samples for research

Please find enclosed a request for permission to export biological samples for research for protocol SSC No. 2539 "Etiology of sub-optimal responses to Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) among HIV patients on antiretroviral treatment in Nairobi Kenya ".

We intend to export plasma, whole blood and serum samples to Laboratory of Retrivirology Centre de Recherche in Luxembourg for further analysis and storage.

Copies of the informed consent forms (English and Swahili), SSC and ERC approval letters are also enclosed. Please do not hesitate to contact me if any questions arise.

Sincerely,

Musa Otieno Ng'ayo
Principal Investigator

[illegible] 23 AUG 2013

Form SSC 1/91d

KENYA MEDICAL RESEARCH INSTITUTE

SCIENTIFIC STEERING COMMITTEE (SSC)

REQUEST FOR EXPORTATION OR STORAGE OF HUMANBLOOD AND OTHER BIOLOGICAL MATERIALS FOR RESEARCH

PART A: Project Information

i. **Project Title:** Ethology of Sub-optimal responses to non-nucleoside Reverse transcriptase Inhibitor (NNRTI) among HIV patients in Nairobi **SSC No.** 2539

ii. **KEMRI Centre of Affiliation:** CMR

iii. **Principal Investigator(s):**

1. Musa Otieno Ngoyo
2.
3.

iv. **Other Investigators:**

1. Dr. Christina Mwachari
2. Dr. Margaret A. Oluka
3. Dr. Faith Apolot okolebo
4. Dr. Carole Devaux
5.

PART B: Specimen Details:

i. **Is the request for specimen exportation or storage or both?**

Both

(Request for storage is necessary if the samples are to be stored beyond the duration of the present study)

ii. **Description of specimen(s) to be exported/stored:**

1. 600 300 µl plasma samples
2. 600 300 µl whole blood
3. 600 300 µl sperm samples

iii. **Reason(s) for exportation/storage of samples:**

1. Determination of Nevirapine (NVP) & Efavirenze (EFV) plasma level
2. Detection of NVP & EFV resistant mutations
3. Determination of Cytochrome P450 2B6 polymorphism

iv. **Duration of specimen storage:** One year

v. **For samples originating from human subjects, state whether or not written consent for specimens exportation or storage:**

Written consent for specimen exportation and storage were obtained

vi. **Name and address of recipient institution/department responsible for the specimens:**

Laboratory of Retrovirology Centre de Recherche Public de la Sante 1A-B rue Thomas Edison L-1445 Strassen

(If samples are to be sent to more than one institution/department, a separate request form should be completed for each recipient)

vii. **Name(s) and address of person(s) responsible for the specimens in the recipient institution:**

Dr. Carole Devaux; Associate head Laboratory of Retrovirology 84, Val Fleuri, L-1526 Luxembourg
Tel +352 26970 224 Fax +352 26970 215

vi. **Name and role in the project of the Kenyan investigator(s) expected to carry out investigations on the specimens in the overseas institution:**

Musa Otieno Ngoje principal investigator

PART C: Declarations: (To be completed at the time of shipping samples)

i. Declaration by the person requesting exportation/storage of research specimens:

I certify that the information provided in this request form is true and correct to the best of my knowledge, and I hereby declare that the specimens referred to herein will be utilized for the stated purpose only

Name: Musa Ofieno Ngayo **Role in the Project:** PI

Signature: Musa **Date:** 19/8/2013

ii. Declaration by Recipient Institution:

This is to certify that the specimens referred to herein being sent to Centre de Recherche Public de la Santé (Name of Institution) for further analyses/experimentation will be in the custody of the Department of Laboratory of Retrovirology, and I hereby confirm that they will be utilized for the purpose stated in this request form, and I accept full responsibility and control over the usage of these samples.

CRP-Santé
1A-B, rue Thomas Edison
L-1445 STRASSEN

Name of Department/Institution Head: Mr Thomas LENTZ
Directeur administratif et financier
Centre de Recherche Public de la Santé

Signature: **Date:** 2 0 AOUT 2013

iii. Declaration by Centre Director:

I certify that the protocol SSC No 2539 referred to in this request was approved by the Centre's Scientific Committee on October 2012 and that the request to export the biological specimens referred to in this request was found to be valid and justifiable. I further confirm that the study participants in this project have consented in writing to the exportation/ storage of samples taken from them, for use in further research.

Name: Dr. Wilhelm K. Sang **Signature:**

Centre: CMR **Date:** 19/08/2013

PART D: (For SSC and ERC Use Only)

i. Request Considered and Approved by the SSC during its Meeting Held on

---

ii. Request Forwarded to the Ethical Review Committee (ERC) for consideration on

---

iii. Request Approved by the ERC on ----------

iv. Request Considered and Deferred Due to the Following Reasons:

1) ----------

2) ----------

3) ----------

4) ----------

5) ----------

PART E: Approvals

Request Approved By:

i. Chairman, SSC: ---------- **Date:** 29/8/2013

ii. Chairman, ERC: ---------- **Date:** 29/8/2013

iii. Director, KEMRI: ---------- **Date:** 10/9/2013

DIRECTOR
KENYA MEDICAL RESEARCH INSTITUTE

KENYA MEDICAL RESEARCH INSTITUTE

SCIENTIFIC STEERING COMMITTEE (SSC)

AUTHORITY TO EXPORT BIOMEDICAL RESEARCH MATERIALS*

This is to certify that MUSA OTIENO NGATO

Principal Investigator/Co-Principal Investigator/Investigator in the research project titled:

Ethology of Sub-optimal responses to non-nucleoside Reverse Transcriptase Inhibitor (NNRTI) among HIV patients in Nairobi

and referenced SSC No: 2539 being undertaken in collaboration with the

Kenya Medical Research Institute (KEMRI) has been granted permission to send out

600 of 300µl each Whole blood, serum and plasma samples

(Number and Description of the Samples)

to Laboratory of Retrovirology, Centre de Recherche public de la Santa

(Name of Department and/or Institution)

in Luxembourg

(Country of Destination)

for the purpose of (i) Determination of Nevirapine (NVP) and Efavirenze (EFV) plasma levels (ii) Detection of NVP & EFV resistant mutations and (iii) Determination of Cytochrome P450 polymorphism

(Description of the type(s) of investigations or analysis to be conducted on the samples)

This certificate is issued with the understanding that the investigator will not use the samples for purposes other than those stated above. The investigator will submit a copy of the results of the investigations/analyses undertaken on these samples to the Director, KEMRI; and will ensure that KEMRI's intellectual property rights arising from work on the stated samples will be protected and safeguarded, and the findings thereof are published with the approval of the Director, KEMRI.

Recommended by: Dr. Willie K. Sang 19/09/2013

Name and Signature of Centre Director **Date**

Authorized by: Dr. Solomon Mpoke 19/9/2013

Name and Signature of Director, KEMRI **Date**

DIRECTOR
KENYA MEDICAL RESEARCH INSTITUTE

***This certificate is valid for a period of 30 (thirty) days with effect from the date of authorization. Please direct any queries to the Director, KEMRI, PO Box 54840-00200 Nairobi, Kenya; Phone: (254-20) – 2722541; Fax: (254-20) – 2720030; E-mail: director@kemri.org**

Endorsed by Faith Apolot Okalebo
22-08-2022

Endorsed
Dr. E.M. Guantai - Chairman, Dept. of Pharr
22nd August 2022

Thesis - PHARMACOGENETIC AND PHARMACOECOLOGICAL DETERMINANTS OF THERAPEUTIC RESPONSE TO NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AMONG HIV PATIENTS IN KENYA

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GRADEMARK REPORT

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Research Article Open Access

Impact of First Line Antiretroviral Therapy on Clinical Outcomes Among HIV-1 Infected Adults Attending One of the Largest HIV Care and Treatment Program in Nairobi Kenya

Musa Otieno Ngayo[1,2*], Faith Apolot Okalebo[2], Wallace Dimbuson Bulimo[3], Christina Mwachari[3], Anastasia Nkatha Guantai[2] and Margaret Oluka[2]

[1]*Centre of Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya*
[2]*Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, Kenya*
[3]*Department of Biochemistry, School of Biological and Physical Sciences, University of Nairobi, Kenya*
[4]*Centre of Respiratory Disease Research - Kenya Medical Research Institute, Nairobi, Kenya*

Abstract

Objective: This study evaluated the immunologic (CD4 cell count), virological (HIV RNA viral load), hepatic (alanine and aspartate aminotransferase - ALT and AST), renal (creatinine) and hematological (hemoglobin -HB, White Blood Cell - WBC, Lymphocytes - LYM and platelets - PLT) response to a six months ART treatment among HIV participants in Nairobi Kenya.

Methods: Blood samples were obtained from 599 consenting HIV infected participants receiving HIV treatment in Nairobi. CD4 cell counts were measured using flow cytometer and viral load determined using real-time polymerase chain reaction. The blood hematology, liver and kidney function tests were also measured. One-way ANOVA and Linear regression analysis were conducted.

Results: The median age at ART initiation was 41 years (IQR 35-47 years). The majority of participants (60.3%) were female and 56.1% started on regimens with 2 NRTIs and efavirenz based NNRTI. About 40% of the participants were failing treatment 6 month post ART initiation. The CD4 count significantly increased at the 6-month post ART initiation (301.7 ± 199.4 to 329.4 ± 305.8; $P<0.05$). Hepatotoxicity (ALT and AST levels >5 times the upper limit of normal - ULN) and renal abnormalities (elevated serum creatinine levels) were all high at month 6 compared to baseline; ALT (2.5 to 10.5%), AST (5.3 to 23.4%) and creatinine (63.4 to 68.84%). Fewer participants at month 6 had anemia (29.4% verses 56.4%), leucopenia (42.4% vs. 46.9%) and thrombocytopenia (6.5% vs. 84.1%) compared to baseline. In multivariable models, baseline levels of this parameter, ART regimen and duration with HIV at ART initiation were the most important determinant of month 6 levels.

Conclusion: These data demonstrate sustained immunologic/virologic response to ART among participants remaining on therapy. Anemia, leucopenia and thrombocytopenia were minimized with marginal hepatotoxicity and renal impairment seen. Interventions leading to earlier HIV diagnosis and initiation of ART could substantially improve patient outcomes in Kenya.

Keywords: First line antiretroviral therapy; Clinical outcomes; HIV-1 infected adults; Largest HIV treatment program; Nairobi; Kenya

Introduction

Combination antiretroviral therapy (ART) use has slowed disease progression, decreased mortality and improved the quality of life for many persons with HIV [1-3]. The CD4 cell count and HIV RNA viral load are important measures of the efficacy and effectiveness of antiretroviral therapy (ART) among HIV participants enrolled in HIV care and treatment programs. Robust improvements in CD4 cell counts following ART initiation have been documented [2-4]. The CD4 count at ART initiation determines the degree of immunologic and virologic ART response [5,6] as well as subsequent risk of morbidity and mortality [1,7]. Increasingly however, adverse effects due to combination ART are being reported and are emerging as a major safety concern limiting the clinical benefits of these drugs. In particular, hepatotoxicity [8,9] and hematologic abnormalities [10], are common affecting the quality of life and are associated with HIV/AIDS progression and decreased survival.

Incidence of severe hepatotoxicity has been reported as 5 to 10 per 100 person-years during ART [11]. Almost every licensed ARV has been associated with liver enzyme elevations, with severe hepatic outcomes being frequent during treatment with NNRTI-based regimens than NRTI- or PI-based regimes [12,13]. Hematologic abnormalities such as peripheral blood cytopenia, anaemia, neutropenia, and thrombocytopenia have also been reported among HIV participants receiving ART [14].

As a buildup to reports documenting clinical and immunologic outcomes in sub-Saharan Africa [2] of ART comparable to those observed in resource-rich settings, we provide data on the immunological and virological responses as well as hepatotoxicity and hematological abnormalities due to ART among a cohort population on lifelong ART treatment in Nairobi Kenya.

***Corresponding author:** Musa Otieno Ngayo, Center for Microbiology Research, Kenya Medical Research Institute: (CMR-KEMRI) Kenyatta National Hospital Complex, off Ngong Road, P.O. Box 19464-00202, Nairobi, Kenya; Tel: 254720607890; E-mail: musaotieno@yahoo.com

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Methods

Study design and setting

This was a prospective observational cohort study. Data presenting in this study was part of a study that aimed at evaluating the etiology of sub-optimal responses to non-nucleoside reverse transcriptase inhibitor (NNRTI) among HIV participants receiving HIV care and treatment at the Family AIDS Care and Educational Services (FACES) based at Kenya Medical Research Institute (KEMRI) in Nairobi Kenya. FACES is a collaboration between the KEMRI and the University of California, San Francisco (UCSF), funded through the US President's Emergency Plan for AIDS Relief (PEPFAR). The program is a family-focused, comprehensive HIV prevention, care, and treatment program that initially launched with one HIV site in Nairobi in September 2004 administered under CRDR-KEMRI. Cumulatively to date, this program has enrolled close to 4000 participants, nearly 90% being adults and 10% pediatrics. FACES program also offers counseling services, HIV testing and counseling, TB diagnosis, reproductive health, and nutritional support for the clinically malnourished and other laboratory services including CD4, blood chemistry, full blood count and liver function tests to improve the quality of care.

Sampling and enrollment

Using the population proportion estimation with specified relative precision sample size formula [15] and setting alpha (α) at 0.05, relative precision (ε) at 0.07 and proportion of HIV-1 infected individuals experiencing virological failure with NNRTI sub-optimal plasma levels during a 6 month ARV was not expected to be below 60% [16]; a total of 599 participants were recruited to achieve 0.95 power.

Participants meeting the recruitment criteria (receiving first line ARV (Zidovudine (AZT) or Abacavir (ABC), 3TC, and EFV/NVP for six months, were consented and enrolled into the study using two sampling techniques; first, random sampling was used to intentionally enroll eligible participants. To increase the participants flow and shorten recruitment period the second technique the snowballing or word-of-mouth technique was used. In this case, the participants already enrolled were used as referral sources. These participants were given cards in order to recommend other eligible participants. Face to face interviews using structured questionnaires were used to collected both qualitative and quantitative data.

This study was carried out between 2014 and 2016 and was approved by Ethical Review Committee of Kenya Medical Research Institute (SSC No. 2539 on 21st May, 2013).

Data collection

A detailed structured interview was used to gathered information on demographic data, clinical history, adherence, stigma and medical history. The interviews were conducted six months after treatment initiation. Blood specimens were also collected for CD4 cell count, HIV RNA viral load, AST, ALT and full blood count testing. The blood samples were collected at the time of ART initiation and six-month post ART initiation.

Participants records

The medical files of recruited subjects were retrogressively retrieved and assessed for the following information: liver function tests, renal tests, CD4 count, and HIV RNA viral load and full blood count.

Hematological, blood chemistry, immunological and virological analyses

CD4 cell counts were measured using a FACSCount TM flow cytometer (BD Biosciences, San Jose, USA), which gives the results in absolute numbers and percentages. The plasma HIV-1 RNA were measured using Generic HIV Viral Load® (Biocentric, Bandol, France), a real-time polymerase chain reaction assay based on long-terminal repeat with a detection limit of 300 RNA cp/ml in 0.2 ml of plasma.

The full blood cells were measured using the SYSMEX KX21N hematology instrument (Sysmex Corporation, Kobe, Japan). Blood chemistry were conducted with a Lisa 300 Plus analyzer (Hycel Diagnostics, Massy, France).

Definition

Biochemical and hematological abnormalities were defined as follows: Liver hepatotoxicity was defined as ALT greater than 56 U/L or AST greater than 40 U/L. Renal abnormalities was considered when creatinine was greater than 0.8 mg/dL. Anemia was defined as hemoglobin <13 g/dl (men) and <12 g/dl (women) while leucopenia as total WBC count less than 4.3×10^9 cells per liter. Total platelet count $<150 \times 10^3$/µl was considered as thrombocytopenia. Lymphocytosis, an increase in the number of lymphocytes was considered when the absolute lymphocyte count was greater than 4000 cells/µl.

Immunological failure was defined as CD4 count falls to the baseline (or below) or Persistent CD4 levels below 100 cells/mm. Virologic failure was defined as plasma viral load above 1000 copies/ml based after 6 months of treatment, with adherence support.

Statistical analysis

All data analysis was performed using SPSS version 21 package. Data was presented as means ± standard deviation (SD) and calculations carried out using the Student's t-test and one-way ANOVA. Linear regression models were used to assess associations between predictor variables and laboratory outcomes following ART initiation. The level of significance was set at $p<0.05$ and confidence level at 95%.

Results

Month 6 characteristics of study participants

A total of 599 HIV infected participants on first line ARV d4T or AZT, 3TC and EFV/NVP were enrolled and their month 6 characteristics are summarized in Table 1. There were 357 (59.6%) participants responding versus 242 (40.4%) failing treatment. Among the 242 failures 21/242 (8.7%) had virologic failure, 14/242 (5.8%) both virologic and immunologic failure while 207/242 (85.5%) had immunologic failures.

The majority (60.3%) of participants were female. Amongst these, 60.8% and 59.5% in the overall population were responders and in the failing cases respectively. The median age at ART initiation was 41 years (IQR 35-47 years). The ages of participants were generally similar between responding and failing participants (P=0.862). More than 64% of the participants were Bantus with no significant difference between responders and failures (P=0.524). The median duration with HIV disease for all participants was 6 years (IQR 4–8 years). Responders had HIV infection longer than the failures (P=0.017).

The overall median baseline CD4 cell count was 288 cells/ml (IQR 138-410 cells/ml). The responders had higher median CD4 cell count than failures (305 cells/ml [IQR 210-409 cells/ml] versus 206

Parameters	Unit	All Patients	Treatment Response		P Value
		N=599 n (%)	Responder N=357 n (%)	Failure N=242 n (%)	
Gender	Male Female	238 (39.7) 361 (60.3)	140 (39.2) 217 (60.8)	98 (40.5) 144 (59.5)	0.799
Age (Years)	Median (IQR) 21-25 26-30 31-40 >40	41 (35-47) 19 (3.2) 53 (8.8) 225 (37.6) 302 (50.4)	41 (35-47) 11 (3.1) 29 (8.1) 137 (38.4) 180 (50.4)	40 (33.7-47) 8 (3.3) 24 (9.9) 88 (36.4) 122 (50.4)	0.862
Education	None College Primary Secondary	7 (1.2) 193 (32.2) 180 (30.1) 219 (36.6)	6 (1.7) 117 (32.8) 108 (30.3) 126 (35.3)	1 (0.4) 76 (31.4) 72 (29.8) 93 (38.4)	0.524
Ethnicity	Bantu Cushite Nilot	387 (64.6) 9 (1.5) 203 (33.9)	231 (64.7) 6 (1.7) 120 (33.6)	156 (64.5) 3 (1.2) 83 (34.3)	0.929
Years HIV positive	Mean (± SD) Median (IQR) <5 6-10 <10	6.4 (3.1) 6 (4-8) 255 (42.6) 291 (48.6) 53 (8.8)	6.7 (3.1) 7 (4-8) 135 (37.8) 188 (52.7) 34 (9.5)	6.1 (3) 6 (4-8) 120 (49.6) 103 (42.6) 19 (7.9)	0.017
Current ARV regimen	ABC/3TC/NVP TDF/3TC/NVP AZT/3TC/NVP D4T/3TC/NVP ABC/3TC/EFV TDF/3TC/EFV AZT/3TC/EFV	1 (0.2) 159 (26.5) 102 (7.1) 1 (0.2) 1 (0.2) 210 (35.1) 125 (20.9)	0 81 (22.7) 53 (14.8) 1 (0.3) 1 (0.3) 133 (37.3) 88 (24.6)	1 (0.4) 78 (32.2) 49 (20.2) 0 0 77 (31.8) 37 (15.3)	0.002
CD4 (cells/ml)	Median (IQR) <500 >501	288 (138-410) 509 (85) 90 (15)	305 (210-409) 307 (85.9) 50 (14.1)	206 (94-419) 202 (83.5) 40 (16.5)	0.001
VL (copies/ml)	Mean (Range) <1000 >1000	2644.7 (1-367728) 509 (85) 90 (15)	41.8 (1-160) 307 (85.9) 50 (14.1)	6484 (1-367728) 202 (83.5) 40 (16.5)	0.001
ALT (U/L)	Median (IQR) <56 >56	16.9 (11-23.9) 585 (97.7) 14 (2.3)	18 (10.7-24) 350 (98) 7 (2)	15.7 (11.5-22.2) 235 (97.1) 7 (2.9)	0.583
AST (U/L)	Median (IQR) <40 >40	17 (11-24) 567 (94.7) 32 (5.3)	18 (11-24) 336 (94.1) 21 (5.9)	15.5 (11-21.6) 231 (95.5) 11 (4.5)	0.58
Creatinine (mg/dL)	Median (IQR) <0.8 >0.8	0.9 (0.6-1.2) 219 (36.6) 380 (63.4)	0.9 (0.6-1.2) 137 (38.4) 220 (61.6)	0.9 (0.7-1.1) 82 (33.9) 160 (66.1)	0.3
HB (g/dL)	Median (IQR) <13 >13	12.6 (11-14.3) 338 (56.4) 261 (43.6)	12.8 (11.3-14.6) 190 (53.2) 167 (46.8)	12.2 (10.7-13.9) 148 (61.2) 94 (38.8)	0.198
WBC ($10^3/mm^3$)	Median (IQR) × 10^3 ≤ 4.3 × 10^3 >4.3 × 10^3	4.6 (3.6-5.8) 281 (46.9) 318 (53.1)	4.6 (3.6-5.8) 166 (46.5) 191 (53.5)	4.6 (3.6-5.9) 115 (47.5) 127 (52.5)	0.868
Platelets (10^9/L)	Median (IQR) × 10^9/L <150 × 10^9 >150 × 10^9	289 (219-350) 504 (84.1) 95 (15.9)	288 (214-339) 300 (84) 57 (16)	290.5 (216.5-360) 204 (84.3) 38 (15.7)	0.98
Lymphocytes (10^9/L)	Median (IQR) × 10^9 <4 × 10^9 ≥ 4 × 10^9	2.2 (1.8-2.8) 584 (97.5) 15 (2.5)	2.2 (1.8-2.9) 347 (97.2) 10 (2.8)	2.1 (1.8-2.7) 237 (37.9) 5 (2.1)	0.791

Comparison of data was done at P<0.05. Data on gender, education, ethnicity, marital status, occupation and ARV regimen was presented as absolute numbers (n) and percentages (%) while age and during living with HIV infection was shown as mean ± standard deviation (SD) in years. All the laboratory data were presented in median (interquartile range-IQR). The categories showing the normal ranges are shown as absolute numbers (n) and percentages (%). Where: TDF: Tenofovir; 3TC: Lamivudine; EFV: Efavirenz; ABC: Abacavir; d4T: Stavudine; NVP: Nevirapine; kg-kilogram; ml: Milliliter; U: Units; L: Liter; mg-milligrams; Dl: Deciliters; g: Grams; mm: Millimeter

Table 1: Demographic, clinical and laboratory characteristics of the study participants.

cells/ml [IQR 94-419 cells/ml]) (P=0.001). The overall mean VL was 2644.7 (range 1-367728) copies/ml. The failures had higher mean VL than responders (6484 copies/ml (range 1-367728) copies/ml versus 41.8 copies/ml (range 1-160 copies/ml) (P=0.001). The overall median baseline ALT level was 16.9 U/L (IQR 11–23.9 U/L) with no significant difference between responders and failures (P=0.583). The overall month 6 median hemoglobin (HB) level was 12.6 g/dl (IQR 11–14.3 g/dl) with no significant difference between responders and failures (P=0.065). There was no significant difference between responders and failures in the median WBC level (P=0.868), PLT (P=0.98) and LYMP (P=0.791) (Table 1).

Changes in the clinical outcomes 6 month post ART initiation

Immunological, hematological and biochemical changes 6

Parameters	Patient Type	No	Baseline	Month 12	P-Value
Non ART Adherence Yes No	All	599		 134 (22.4%) 465 (77.6%)	
HIV-1 RNA (copies/ml) Mean (± SD) <1000 >1000.1	All	599		 2644.7 (21680.9) 34 (5.7%) 565 (94.3%)	
CD4+ (cell/µL) Mean (± SD)	All Responders Failure	599 357 242	301.7 ± 199.4 329.4 ± 185.6 261.2 ± 212.3	329.4 ± 305.8 468.8 ± 312.2 123.6 ± 129.8	0.001 0.001 0.028
ALT (U/L) Mean (± SD)	All Responders Failure	599 357 242	20.8 ± 17.2 20.9 ± 16.4 20.7 ± 18.3	31 ± 20.5 31.4 ± 20.8 30.4 ± 19.9	0.495 0.361 0.017
AST (U/L) Mean (±SD)	All Responders Failure	599 357 242	19.5 ± 11.9 19.9 ± 12.3 18.9 ± 11.4	31.5 ± 22.7 31.9 ± 25.3 30.9 ± 18.3	0.001 0.001 0.001
Creatinine (mg/dL) Mean (± SD)	All Responders Failure	599 357 242	0.97 ± 0.55 0.96 ± 0.53 0.99 ± 0.57	0.95 ± 0.47 0.95 ± 0.51 0.94 ± 0.41	0.092 0.816 0.602
Hb (g/dL) Mean (± SD)	All Responders Failure	599 357 242	12.4 ± 2.7 12.6 ± 2.6 12.1 ± 2.7	14.1 ± 2.5 14.1 ± 2.6 14 ± 2.4	0.001 0.206 0.095
WBC ($10^3/mm^3$) Mean (± SD)	All Responders Failure	599 357 242	4.8 ± 1.9 4.9 ± 1.9 4.9 ± 1.9	5.1 ± 1.8 5.1 ± 1.8 5.1 ± 1.8	0.001 0.001 0.001
Lymphocytes ($10^9/L$) Mean (± SD)	All Responders Failure	599 357 242	2.2 ± 0.89 2.3 ± 0.89 2.2 ± 0.88	2.4 ± 0.84 2.4 ± 0.84 2.4 ± 0.82	0.001 0.001 0.001
Platelets ($10^9/L$) Mean (± SD)	All Responders Failure	599 357 242	293.1 ± 109.5 289.9 ± 107.9 297.6 ± 111.9	297.6 ± 100.6 295.3 ± 99.7 301 ± 102	0.001 0.001 0.001

Laboratory data are presented using mean ± standard deviation. Month 6 data were compared against the baseline values using ANOVA at a 5% level of significance. Data with P>0.05 indicates significant difference

Table 2: Changes in the levels of laboratory parameters at baseline and month 6 among HIV infected participants undergoing ART treatment.

months post ART initiation is summarized in Table 2. The CD4 count significantly increased at the month 6 post ART (301.7 ± 199.4 to 329.4 ± 305.8; p<0.05). The levels of AST (19.5 ± 11.9 to 31.5 ± 22.7; p<0.05) was significantly increased (upper range) at the 6 month post ART. There was no significant increase in the mean ALT levels 6 months into ART; ALT (20.8 ± 17.2 to 31 ± 20.5 U/L; p=0.495). Serum creatinine levels (0.97 ± 0.55 to 0.95 ± 0.47; P=0.092) decreased non-significantly at the month 6 of ART.

For all the participants, a consistent increase was noted in the hemoglobin levels (12.4 ± 2.7 to 14.1 ± 2.5g/dL) (P<0.001), total white blood cell count (WBC) (4.8 ± 1.9 to 5.1 ± 1.8 $10^3/mm^3$) (p<0.001), absolute lymphocyte (2.2 ± 0.89 to 2.4 ± 0.84 $10^9/L$) (p<0.001) and absolute platelets (293.1 ± 109.5 to 297.6 ± 100.6 $10^9/L$) (P<0.001) from the baseline to month 6 post ART initiation.

Prevalence of hepatotoxicity, nephrotoxicity and blood abnormalities

Various biochemical and hematological abnormalities that could have been associated with administration of antiretroviral drugs were observed (Figure 1). Hepatotoxicity due to elevated ALT and AST were much higher at month 6 compared to baseline; ALT (2.5% to 10.5%) and AST (5.3% to 23.4%). Participants with renal abnormalities due to elevated serum creatinine levels were higher at month 6 (68.8%) compared to baseline (63.4%). The study revealed higher cases of anemia (56.4%), leucopenia (46.9%) and thrombocytopenia (84.1%) among all the HIV infected participants at baseline. Conversely, these decreased (anemia 29.4%, leucopenia 42.4% and thrombocytopenia to 6.5%) at month 6 post ART initiation. Lymphocytosis (2.5%) was low at the first visit but showed higher values at 5.3% at month 6.

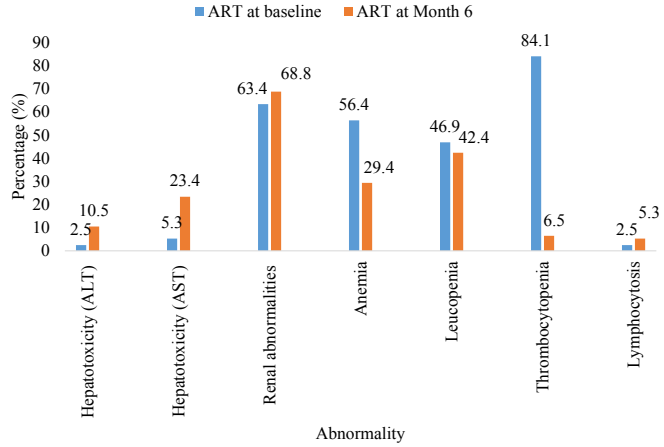

Figure 1: Distribution of biochemical and hematological abnormalities observed among HIV infected adults on ART. The bars compare the abnormality (in percentages %) at baseline and at 6th month of ART administration. Abnormalities presented include hepatotoxicity, renal anemia, leucopenia, thrombocytopenia and lymphopenia.

Factors associated with clinical outcomes 6 month post ART

The independent variables associated with laboratory outcomes in multivariable regression models are summarized in Table 3. The CD4 cell counts (beta 0.441 cells/µL, 95% CI, 0.308 to 0.574 cells/µL, P=0.001) and duration with HIV disease (years) (beta 10.1 cells/µL, 95% CI, 1.707 to 18.484 cells/µL, P=0.018) at baseline significantly predicted month 6 CD4 cell count. Baseline AST (beta 0.711 U/L, 95%

Model	Unstandardized	Coefficients	95%Confidence	Interval for B	Standardized Coefficients	t	P-value
	B	Std. Error	Lower Bound	Upper Bound	Beta		
CD4 cell count							
Baseline CD4	0.44	0.07	0.31	0.57	0.27	6.50	0.001
Duration with HIV	10.10	4.27	1.71	18.48	0.10	2.36	0.018
AST (U/L)							
Baseline AST	0.711	0.08	0.56	0.86	0.37	9.12	0.001
Creatinine (mg/dL)							
ART regimen	0.088	0.04	0.00	0.17	0.09	1.98	0.048
Hb (g/dL)							
Baseline HB	0.122	0.04	0.04	0.20	0.13	3.02	0.003
WBC (10^3/mm^3)							
Baseline WBC	0.851	0.02	0.82	0.89	0.91	49.50	0.001
Lymphocytes (10^9/L)							
Baseline LYMP	0.885	0.02	0.85	0.92	0.93	51.24	0.001
Duration with HIV	-0.011	0.00	-0.02	0.00	-0.04	-2.39	0.017
Platelets (10^9/L)							
Baseline PLT	0.833	0.02	0.80	0.87	0.91	48.28	0.001
Baseline weight	0.377	0.17	0.05	0.70	0.04	2.28	0.023

Table 3: Regression models showing independent variables predicting changes in month 6 laboratory parameters.

CI, 0.558 to 0.864 U/L; P=0.001) significantly predicted month 6 AST levels. ART regimen predicted the month 6 creatinine level (beta 0.088 mg/dl, 95% CI, 0.818 to 0.885 10^3/mm^3; P=0.048).

In other models, baseline levels played a key role in the changes in the month 6 outcomes as shown in Table 3. None of the independent variables (ART regimen, duration of ART, duration with HIV disease, adherence, gender, age, baseline CD4, Log10-transformed viral load, baseline ALT) were predictors of month 6 ALT levels.

Discussion

This study provides data on ART treatment outcomes among one of the largest cohort of HIV positive population receiving HIV care and treatment in Nairobi Kenya. The study demonstrates specific robust CD4 and HIV RNA viral load responses to ART treatment. The ART adverse reactions particularly hepatotoxicity and renal abnormalities were sustained at month 6 compared to baseline. Cases of anemia, leucopenia and thrombocytopenia dropped at the 6th month of ART treatment. Our results are thus encouraging and are a pointer to a long-term effectiveness of ART particularly in HIV Infected individuals who are able to adhere and remain on ART for extended periods.

Studies in resource-limited settings in Africa, Latin America, and Asia [17-19] demonstrate robust CD4 responses to ART that are sustained over several years. In this study, at month 6 the mean CD4 had increased by 27.7 cell/µl above that of baseline with some 43.7% of the participants having CD4 cell count above 350 cell/µl. Further, only 5.7% of the participants had HIV RNA viral load >1000 copies/ml while 94.3% attained viral suppression 6-month post ART treatment. This study reaffirms the positive virological and immunological response to HAART seen in other studies [20,21]. Some reports suggest that suppression of viraemia and maintenance of CD4 cell counts continues after several years sometimes beyond 7 years of therapy in participants who achieve ongoing viral suppression [17].

This study showed that apart from the duration with HIV infection, patient's baseline CD4 remained the single most important factor determining CD4 count in month 6. This observation has been documented by other investigators [17,22] that participants with higher CD4 cell counts at ART initiation achieve a higher CD4 cell count in the following months and years. The importance of this observation cannot therefore be overstated. The baseline CD4 cell count, second only to subsequent medication adherence, is the most important predictor of clinical progression and survival after ART initiation [23,24].

Due to the increased ART scale up programs, increased focus on the toxicities and adverse reactions of combination ART, such as drug-induced liver injury, neuropathy, and pancreatitis continue to attract attention. Several studies show that liver injuries are the most common non-AIDS cause of death among people with HIV infection [25]. Further, during this era of effective ART, about 18% of deaths among HIV participants are due to liver-related complications [26,27]. Our study showed a significant elevation in the mean liver transaminase enzymes (AST and ALT) from baseline to month 6. Cases of hepatotoxicity due to elevated ALT and AST (>5 times ULN) were much higher at month 6 compared to baseline at 2.5% to 10.5% and 5.3% to 23.4%, respectively. Other clinical studies have indicated that grade 3 (ALT and/or AST levels >5 times the ULN) and grade 4 (ALT and/or AST levels >10 times the ULN) hepatotoxicity is observed in 5%–10% of HIV-positive participants treated with combination ART for >6 months [14,28].

Baseline hepatic parameters (AST and ALT) were great pointers to the month 6 levels. Studies have shown that HIV patient's susceptibility to the hepatotoxic effects of ART is due to the interplay of the effects of the ART and the associated risk factors, such as alcohol use, underlying diseases, and concomitant drugs [29]. It is therefore generally recommended that long term administration of ART should be carefully monitored to avoid possible drug induced injuries.

Creatinine level, an excellent indicator of kidney function, in this study was marked by a non-significant decrease at the month 6 of ART. The month 6 creatinine levels were dependent upon the ART regimen used by the patent. This lack of significant increase in the creatinine levels between baseline and month 6 may indicate normal, functional and intact kidneys.

HIV infection contributes significantly in various degrees to immunopathogenesis in man [30]. Significant hematological and biochemical complications have been observed due to HIV. Abnormalities may occur in individuals as a result of the following actions; HIV infection, sequel of HIV-related opportunistic infections, malignancies and consequence of therapies used for HIV infection and associated conditions [31]. In this study, cases of thrombocytopenia, anemia, leucopenia and lymphocytosis were observed. Other studies

have reported significant variation in the prevalence of hematological abnormalities in HIV participants, with anemia shown to range from 1.3% to 95% [32,33]. At the 6th month post ART initiation, thrombocytopenia, anemia, and leucopenia were reduced to 6.5%, 29.4% and 42.4% respectively while lymphocytosis increased marginally (2.5 to 5.3%). Our findings are similar to those of [14,34] who showed significant reduction in some hematological abnormalities due to ART. Although several research has shown that administration of ART especially Zidovudine (AZT) therapy causes anemia with a significant reduction in hemoglobin in HIV participants [35], our study was marked by increase in the mean hemoglobin levels at month 6 marked by reduction of anemia. Our results indicate that ART administration reverses the HIV associated anemia, a fact confirmed by Johannessen et al. [36]. The reduction in opportunistic infections including TB, as well as the reduction of inflammatory cytokines such as tumor necrosis factor (TNF) that are implicated in the suppression of erythropoiesis could be mechanisms that may account for the improvement of anemia after initiation of ART [37].

Our study showed that baseline hemoglobin level predicted the hemoglobin level at month 6. In other studies, stage of HIV, age and gender of the participants predicted the prevalence of anemia in HIV participants [38]. Despite a significant reduction in the prevalence of anemia at 6 months, close to 29.4% of the participants had anemia implying the need for routine screening of anemia and subsequent investigation of its causes.

As a practice, it is important to monitor the overall White Blood Cell (WBC) count because elevation of WBC may indicate infection, lack of response to treatment or an abnormality. In our study the mean WBC count was increased at month 6 during ART treatment. The progressive increase observed in absolute lymphocyte and total WBC may indicate a concerted suppressive activity of both immune system and the antiretroviral drug on the virus with the resultant decrease in leucopenia and lymphocytopenia. Leucopenia (a decrease in the number of white blood cells) and Lymphopenia (decrease in lymphocyts) are important hallmarks of HIV infection, and are also caused by certain medications such as ART [39,40] and certain infections [41]. Further investigation is needed to ascertain the mechanism responsible for leucopenia and lymphopenia among this cohort.

One of the major strength of this study was its design as a prospective study in a well-characterized cohort in which clinical outcomes were carefully measured and recorded. Some of the limitations of this study include; the short duration of follow up (6 months) and the relatively small sample size may have failed to detect smaller differences in some outcomes. The lack accurate ART adherence monitoring as well as the lack of other information such as nutritional status, use of herbal remedies during the study, or use of medications obtained outside the research clinic that may have influenced the observations of this study. The lack of plasma drug levels, ART drug potency, host pharmacodynamics and HIV drug resistance information which are important when evaluating the effectiveness of any ART call for a cautious interpretation of our results.

However, these limitations notwithstanding, the study has demonstrated that a robust immunological and virological response is achievable among participants receiving HIV treatment in the largest cohort of HIV positive population receiving HIV care and treatment in Nairobi Kenya. Further, it has shown that ART resulted in a remarkable reduction in the prevalence of adverse effects such (hepatic and renal impairment, thrombocytopenia, anemia, leucopenia and lymphocytosis) after 6 months. However, a substantial proportion of participants still had liver enzymes elevation and hematological abnormalities after 6 months of ART, raising the need for routine screening of biochemical and hematological outcomes, investigating theirs causes and instituting appropriate treatment and management strategies to mitigate the adverse effects of ART.

The most important determinant of the effectiveness of ART (improvement in CD4 count, achievement of viral suppression and reduction in adverse effects) were the baseline laboratory parameters at ART initiation. This data therefore suggests that earlier HIV diagnosis and initiation of ART in Kenya should be adopted in order to achieve optimal treatment outcomes.

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Author's Contribution

MON, FAO, WDB, CM, ANG, MO conceived the study. CM supervised sample collections while MON, FAO, WDB and MO supervised laboratory analysis. MON, FAO and MO analyzed the data and prepared the draft manuscript. FAO, WDB, ANG and MO provided guidance and mentorship during the implementation of the study. All authors reviewed and approved the final manuscript.

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Association between social psychological status and efavirenz and nevirapine plasma concentration among HIV patients in Kenya

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HIV-related stigma, lack of disclosure and social support are still hindrances to HIV testing, care, and prevention. We assessed the association of these social-psychological statuses with nevirapine (NVP) and efavirenz (EFV) plasma concentrations among HIV patients in Kenya. Blood samples were obtained from 254 and 312 consenting HIV patients on NVP- and EFV-based first-line antiretroviral therapy (ART), respectively, and a detailed structured questionnaire was administered. The ARV plasma concentration was measured by liquid chromatography-tandem mass spectrometry (LC–MS/MS). There were 68.1% and 65.4% of the patients on NVP and EFV, respectively, who did not feel guilty for being HIV positive. The disclosure rates were approximately 96.1% and 94.6% of patients on NVP and EFV, respectively. Approximately 85% and 78.2% of patients on NVP and EFV, respectively, received social support as much as needed. There were 54.3% and 14.2% compared to 31.7% and 4.5% patients on NVP and EFV, respectively, with supratherapeutic and suboptimal plasma concentrations. Multivariate quantile regression analysis showed that feeling guilty for being HIV positive was associated with increased 954 ng/mL NVP plasma concentrations (95% CI 192.7 to 2156.6; $p = 0.014$) but not associated with EFV plasma concentrations (adjusted $\beta = 347.7$, 95% CI = − 153.4 to 848.7; $p = 0.173$). Feeling worthless for being HIV positive was associated with increased NVP plasma concentrations (adjusted $\beta = 852$, 95% CI = 64.3 to 1639.7; $p = 0.034$) and not with EFV plasma concentrations (adjusted $\beta = -143.3$, 95% CI = − 759.2 to 472.5; $p = 0.647$). Being certain of telling the primary sexual partner about HIV-positive status was associated with increased EFV plasma concentrations (adjusted β 363, 95% CI, 97.9 to 628.1; $p = 0.007$) but not with NVP plasma concentrations (adjusted $\beta = 341.5$, 95% CI = − 1357 to 2040; $p = 0.692$). Disclosing HIV status to neighbors was associated with increased NVP plasma concentrations (adjusted $\beta = 1731$, 95% CI = 376 to 3086; $p = 0.012$) but not with EFV plasma concentrations (adjusted $\beta = -251$, 95% CI = − 1714.1 to 1212.1; $p = 0.736$). Obtaining transportation to the hospital whenever needed was associated with a reduction in NVP plasma concentrations (adjusted $\beta = -1143.3$, 95% CI = − 1914.3 to − 372.4; $p = 0.004$) but not with EFV plasma concentrations (adjusted $\beta = -6.6$, 95% CI = − 377.8 to 364.7; $p = 0.972$). HIV stigma, lack disclosure and inadequate social support are still experienced by HIV-infected patients in Kenya. A significant proportion of patients receiving the NVP-based regimen had supra- and subtherapeutic plasma concentrations compared to EFV. Social-psychological factors negatively impact adherence and are associated with increased NVP plasma concentration compared to EFV.

Although the current trend in the global HIV epidemic has stabilized, data still imply disappointingly high levels of infection, an indictment of irregular control progress in countless countries[1]. The HIV pandemic continues to be the leading cause of death in sub-Saharan Africa, with Kenya having the joint third-largest HIV epidemic in

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the world (alongside Tanzania), with 1.6 million people living with HIV[1]. HIV infection affects every breadth of life, including physical, psychological, social and spiritual dimensions[2,3]. In as much as HIV infection has been reported in Kenya for the last four decades, this infection is still dreaded by many, mainly due to misinformation about the disease and consequently the stigma and exclusion associated with the infection[4]. People living with HIV (PLWHA) are burdened with both medical and social problems associated with the disease[5]. HIV infection among a large population results in stigma for both infected and affected individuals[6,7]. Furthermore, infection consistently results in loss of socioeconomic status, employment, income, housing, health care and mobility[5]. The outcome of stigma includes but is not limited to increased secrecy (lack of disclosure) and denial, which is not only a stimulus for HIV transmission but also a cause for poor disclosure and subsequent lack or inadequate social support[5,7].

Antiretroviral therapy (ART) is an integral component in reducing the burden of HIV. Globally, at the end of 2020, 67% of 38 million PLWHA were on ART[1]. A remarkable scale-up of ART has put Kenya on track to reach the target for AIDS-related deaths. At the end of 2020, approximately 74% of adults and 73% of children in Kenya needing ART were essentially receiving it[1]. A remarkable fraction of these patients (68%) had attained viral suppression (UNAIDS, 2020). At the time of this study, the first-line ART guidelines for children, youth and adults in Kenya typically contained a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs; zidovudine [AZT], or tenofovir [TDF] with lamivudine [3TC]), plus one nonnucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine (NVP) or efavirenz (EFV)[8].

Therapeutic drug exposure is a major requirement for ART management[9]. Suboptimal exposure to ART, especially NNRTIs (NVP and EFV), jeopardizes ART treatment success[10]. Generally, efavirenz and nevirapine plasma concentrations are associated with several factors, including host pharmacogenetics, as well as pharmacoecological factors, such as social-psychological status and adherence[11]. Although pharmacoecological factors are those that primarily affect adherence, social psychological status could independently affect ARV plasma concentration[11]. HIV stigma negatively affects ART utilization and the quality of care[5]. Social support and disclosure have been shown to significantly affect treatment outcomes in many settings[4]. Counselling and social support for both infected and affected people is associated with effective coping with each stage of the infection and enriches the quality of life and hence adherence to ART[2]. This study assessed the association between HIV stigma, disclosure and social support on ART adherence and the steady-state plasma concentrations of NVP and EFV among HIV patients receiving ART in one of the largest and oldest cosmopolitan care and treatment centers in Kenya.

Methods

Study design and setting. This was a cross-sectional study conducted between August 2016 and January 2020. The data presented in this study were part of a study that aimed to assess the pharmacogenetic and pharmacoecological etiology of suboptimal responses to nonnucleoside reverse transcriptase inhibitors (NNRTIs) among HIV patients in Nairobi, Kenya. Patients were recruited in this study if they were HIV-infected adults (aged above 18 years); receiving first-line ART comprising zidovudine (AZT) or abacavir (ABC) or tenofovir (TDF) or stavudine (d4T), lamivudine (3TC), and efavirenz (EFV) or nevirapine (NVP) for at least 12 months; and willing to give voluntary written informed consent. This study was based at the Family AIDS Care and Educational Services (FACES) based at Kenya Medical Research Institute (KEMRI) in Nairobi Kenya. The ART regimen formulation and dosing used in this study were performed according to the guidelines of the Ministry of Health, National AIDS & STI Control Program[8]. The EFV-based ART regimen comprised the following: ABC 300 mg/3TC 150 mg combination taken twice daily plus EFV 600 mg once daily, TDF 300 mg/3TC 300 mg/EFV 600 mg one fixed dose combination taken once daily, or AZT 300 mg/3TC 150 mg combination taken twice daily plus EFV 600 mg once daily. The NVP based regimen comprised the following: ABC 600 mg/3TC 300 mg combination taken once daily plus NVP 200 mg twice daily, or TDF 300 mg/3TC 300 mg combination taken once daily plus NVP 200 mg twice daily, or AZT 300 mg /3TC 150 mg NVP 200 mg one fixed dose combination taken twice daily and D4T 30 mg/3TC 150 mg/NVP 200 mg one fixed dose combination taken twice daily. The study population and site have been described in detail in our previous publication Ngayo et al.[12]. This research was carried out in accordance with the basic principles defined in the Guidance for Good Clinical Practice and the Principles enunciated in the Declaration of Helsinki (Edinburg, October 2000). This protocol and the corresponding informed consent forms used in this study were reviewed, and permission was obtained from the Kenya Medical Research Institute Scientific Review Unit (SERU) (Protocol No SSC 2539). Written informed consent was obtained from all patients before enrollment.

Sample size. Sample size calculation used the formula described by Lemashow[13] based on population proportion estimation with specified relative precision. The alpha (α) was set at 0.05, the relative precision (ε) was set at 0.20 and the proportion of HIV-infected individuals with suboptimal NVP/EFV plasma concentrations during a 12-month ART was set at 15%[14,15]. A total of 599 patients were recruited to achieve 0.95 power, where recruitment of patients per treatment arm was done proportionate to size, yielding 269 and 330 patients on NVP- and EFV-based regimens, respectively.

Data collection. *ART drug adherence assessment.* Screening for adherence to ART in this study was conducted by review of pharmacy refill data or medical records as described by Ochieng et al.[16]. Adherence was measured based on dose compliance during the 30 days preceding the latest refill. The quantity of dose pills at refill was counted and reconciled against the dose counts dispensed at last refill. Furthermore, pill count data were obtained from patient cards for the four months preceding the study period. Nonadherence was determined as

the percentage of overdue dose at refill, averaged over a four-month period and used to assign adherence as good (< = 5% dose skipped), fair (6–15% dose skipped) or poor (> 15% dose skipped).

Structured interviews. Structured interviews (Supplementary file) were used to collect patient-related information from all the study patients. The data collected included demographic characteristics, clinical history, HIV stigma, HIV disclosure and social support and adherence. A pilot study was conducted to test the questionnaire and other key points in the interviews. Some of the key points explored in the structured questionnaire included stigma and segregation of people living with HIV (self-worth, guilt, emotional feeling); challenges of living with HIV, such as access to health services and community life; experiences/issues with HIV disclosure and adherence to medications. The interviews were conducted by a clinician in a separated private room. The second part of the questionnaire was filled out by retrospective review of patient medical records to abstract data on the occurrence of any adverse drug reactions, evidence of treatment failures and adherence to ART.

Whole blood samples (5 mL) at 12–16 h post ARV drug dose were collected using EDTA anticoagulant tubes to determine the concentration of NVP and EFV plasma concentrations.

Determination of nevirapine and efavirenz plasma concentrations. The nevirapine and efavirenz plasma concentrations were measured using a tandem quadrupole mass spectrometer (LC/MS/MS) designed for ultrahigh performance: Xevo TQ-S (Waters Corporation, U.S. A) as described by Reddy et al.[17]. Plasma samples were first subjected to a thorough in-house method for the inactivation of the HIV virus. Plasma samples were extracted using Bond Elut C18 cartridges according to the manufacturer's instructions (Agilent Technologies, USA). The eluents were then completely evaporated using Thermo Scientific™ Reacti-Vap™ Evaporators (Thermo Fisher Scientific Inc., USA) at 37 °C for 30 min. This was then reconstituted using 100µl100 ul of equal parts 1:1 acetonitrile and water, vortexed briefly and transferred into 50 ml capped vials and placed into Xevo TQ-S (Waters Corporation, U.S. A) for quantification. Approximately 1 µl of the samples was injected automatically into the LC/MS/MS instrument and quantified within 5 min.

Data analysis. All data were subjected to descriptive data analysis. Frequencies and percentages were used to present the sociodemographic data. The relationship between HIV stigma, disclosure and social support-related variables and ART drug adherence was first evaluated using the chi-square test or Fisher's exact test. The social-psychological variables were then analyzed for association with NVP and EFV plasma concentrations. Steady-state NVP and EFV plasma concentrations were not normally distributed by the Shapiro–Wilk test; hence, the Kruskal–Wallis test and Dunn's test and quantile regression analysis were used to evaluate variations and associations with NVP and EFV plasma concentrations at the 5% significance level. All statistical analyses were performed using STATA v 13 (StataCorp LP, Texas, USA). The NVP plasma concentrations were categorized as < 3400 ng/mL (below the therapeutic range), 3400–6000 ng/mL (therapeutic range) and > 6000 ng/mL (above the therapeutic range). For EFV, concentrations of < 1000 ng/ml were considered below the therapeutic range, 1000 to 4000 ng/ml considered the therapeutic range and > 4000 ng/ml considered supratherapeutic concentrations[18,19].

Ethics approval. Ethical approval for this study was obtained from the KEMRI Scientific Review Unit (SERU). The protocol number is SSC No. 2539.

Consent to participate. Written informed consent was obtained from all subjects before the study.

Results

Baseline characteristics of study patients. Table 1 summarizes the baseline characteristics of the study population. The results from the 254/269 (94.4%) and 312/330 (94.5%) response rates of patients on NVP and EFV, respectively, with all the relevant data were analyzed. The median age of the patients was 41 years (IQR = 35–47 years), with a median duration of living with HIV infection of five years (IQR = 1–11 years) and a median duration since ART initiation of three years (IQR = 1–8 years). Among these patients, 342 (60.4%) were female, 379 (67%) were married, 367 (64.8%) were Bantus, and 106 (18.2%) had a previous partner who died. Only 3.5% and 5.8% and 19.7% and 17.3% (on NVP and EFV, respectively) were currently smoking and taking alcohol, respectively.

Out of 254 patients on NVP and 312 on EFV, the majority 74.4% and 73.3% stated difficulties disclosing their HIV status. In contrast, the majority (79.1% and 75.9%; 68.1% and 65.4% on NVP and EFV, respectively) did not feel immoral or guilty for being HIV positive, respectively. For patients on either NVP or EFV, the majority did not feel ashamed or worthless for being HIV positive and were very ready to tell their primary sexual partner of their HIV status. The majority, 85% (NVP) and 78.2% (EFV), were satisfied with advice received about important things in life (p = 0.022). Similarly, the majority of these patients had adequate psychosocial support in finding someone to talk to about work/household problems, about personal/family problems and had people who cared about their situations and received much love and affection. The majority of the patients also received emergency financial and transportation support, but there was no significant difference between the ART regimens.

ART adherence. Among all the study patients, 371 (n = 566; 65.6%), 164 (n = 254; 64.6%) on NVP and 207 (n = 312; 66.3%) on EFV were categorized as poor adherence to ART (Fig. 1).

Variable		All patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		*p value*
		n	(%)	n	(%)	n	(%)	
Age (years)	**Median (IQR)**	41	(35–47)	42	(36–48)	40	(34–47)	0.046
	20–30	66	11.7	25	9.8	41	13.1	
	31–40	210	37.1	84	33	126	40.4	
	41–50	202	35.7	106	41.7	96	30.8	
	> 51	88	15.5	39	15.4	49	15.7	
Gender	Female	342	60.4	163	64.2	179	57.4	0.102
	Male	224	39.6	91	35.8	133	42.6	
Marrital status	Married	379	67	165	65.0	214	68.6	0.703
	Single	154	27.2	72	28.4	82	26.3	
	Divorced	26	4.6	14	5.5	12	3.9	
	Widow	7	1.2	3	1.2	4	1.3	
Occupation	Employed	193	34.1	80	31.5	113	36.2	0.354
	Unemployed	102	18	44	17.3	58	18.9	
	Self employed	271	47.9	130	51.2	141	45.2	
Ethnicity	Bantu	367	64.8	161	63.4	206	66.0	0.256
	Nilotes	190	33.6	91	35.8	99	31.7	
	Cushites	9	1.7	2	0.8	7	2.2	
Education level	Primary	174	30.7	69	27.2	105	33.4	0.17
	Secondary	203	35.9	102	40.2	101	32.4	
	Tertiary	182	32.2	81	31.9	101	32.4	
	Non-formal	7	1.2	2	0.8	5	1.6	
Cigarette smoking	Yes	27	4.8	9	3.5	18	5.8	0.24
	No	539	95.2	245	96.5	294	94.3	
Alcohol consumption	Yes	104	18.4	50	19.7	54	17.3	0.099
	No	462	81.6	204	80.3	258	82.7	
Age of sexual debut (Years)	**Median (IQR)**	18	(17–20)	18	(17–19)	18	(17–20)	0.929
	< 18	371	65.6	166	65.4	205	65.7	
	> 18	195	34.5	88	34.7	107	34.3	
Lifetime sexual partners	**Median (IQR)**	2	(1–5)	2	(1–4)	3	(1–5)	0.019
	None	3	0.5	2	0.8	1	0.3	
	1	214	37.8	110	43.3	104	33.3	
	> 1	349	61.7	142	55.9	207	66.4	
Current ART regimen	3TC, ABC, EFV	1	0.2	0	0	1	0.3	0.0001
	3TC, TDF, EFV	187	33.1	0	0	187	59.9	
	3TC, ZDV, EFV	124	21.9	0	0	124	39.7	
	3TC, ABC, NVP	1	0.2	1	0.4	0	0	
	3TC, TDF, NVP	159	28.1	159	62.6	0	0	
	3TC, ZDV, NVP	93	16.4	93	36.6	0	0	
	3TC, d4T, NVP	1	0.2	1	0.4	0	0	
Difficult to tell others about my HIV infection	Agree	418	73.8	189	74.4	229	73.4	0.848
	Disagree	148	26.2	65	25.6	883	26.6	
Feeling guilty for being HIV positive	Agree	189	33.4	81	31.9	108	34.6	0.531
	Disagree	377	66.6	173	68.1	204	65.4	
Feeling worthless for being HIV positive	Agree	137	24.2	55	21.7	82	26.3	0.236
	Disagree	429	75.8	199	78.4	230	73.7	
Hide HIV status from others	Agree	403	71.2	186	73.2	217	69.5	9.352
	Disagree	163	28.8	68	26.8	95	30.5	
Disclose HIV status to anyone	Yes	539	95.2	244	96.1	295	94.6	0.435
	No	27	4.7	10	3.9	17	5.4	
Disclosed HIV status to partner or spouse	Yes	446	78.8	204	80.3	242	77.8	0.665
	No	63	11.1	25	9.8	38	12.2	
	Not applicable	57	10.1	25	9.8	32	10.3	
Disclosed HIV status to family members	Yes	349	61.7	166	65.4	183	58.7	0.178
	No	212	37.5	87	34.4	125	40.1	
	Not applicable	5	0.9	1	0.4	4	1.3	

Continued

Variable		All patients (n = 566)		Nevirapine (n = 254)		Efavirenze (n = 312)		p value
		n	(%)	n	(%)	n	(%)	
Disclosed HIV status to the public	Yes	12	2.1	5	1.9	7	2.2	0.965
	No	513	90.6	231	90.4	282	90.4	
	Not applicable	41	7.2	18	7.1	23	7.4	
Get useful advice about important things in life	As much as I wouild like	460	81.3	216	85.0	244	78.2	0.022
	Less than I wouild like	79	13.9	33	12.9	46	14.7	
	Much less than I wouild like	11	1.9	1	0.4	10	3.2	
	Never	16	2.8	4	1.6	12	3.9	
Get financial help during emergency	As much as I wouild like	337	59.5	162	63.8	175	56.1	0.066
	Less than I wouild like	92	16.3	40	15.8	52	16.7	
	Much less than I wouild like	44	7.8	12	4.7	32	10.3	
	Never	93	16.4	40	15.8	53	16.9	
Get transportation help when needed	As much as I wouild like	357	63.1	169	66.5	188	60.3	0.19
	Less than I wouild like	81	14.3	38	14.9	43	13.8	
	Much less than I wouild like	45	7.9	15	5.9	30	9.6	
	Never	83	14.7	32	12.6	51	16.4	
Get general help when sick	As much as I wouild like	456	80.6	212	83.5	244	78.2	0.437
	Less than I wouild like	67	11.8	27	10.6	40	12.8	
	Much less than I wouild like	18	3.2	6	2.4	12	3.9	
	Never	25	4.4	9	3.5	16	5.2	

Table 1. Baseline characteristics of the study patients.

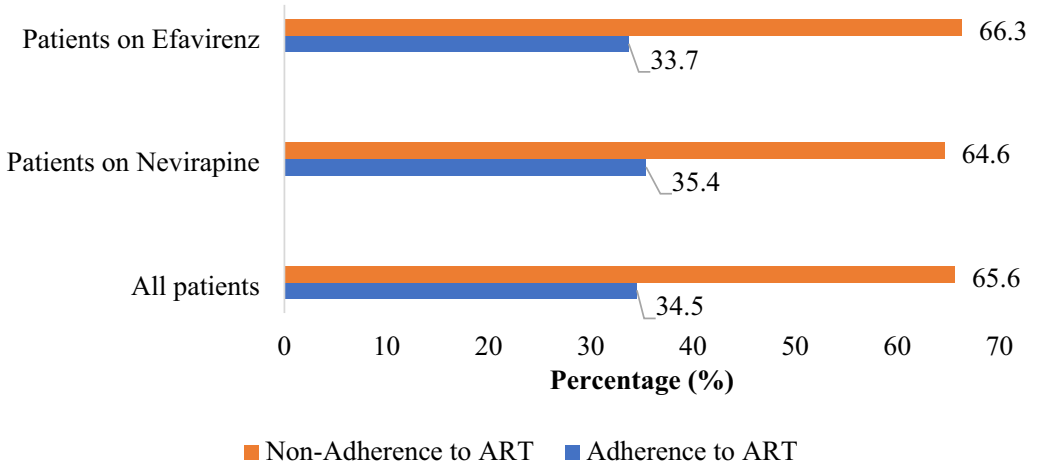

Figure 1. Distribution of patients with ART adherence in the past 30 days.

Efavirenz and Nevirapine plasma concentration. Among the patients on the nevirapine-based ART regimen, the majority 138 (n = 254; 54.3%) had plasma concentrations of > 6000 ng/ml, which are considered levels for durable viral suppression. There were 80 (n = 254; 31.5%) patients with NVP concentrations between 3400 and 6000 ng/ml considered levels for viral mutant selection windows and a few 3 (n = 254; 14.2%) who had NVP plasma concentrations of < 3400 ng/ml considered levels for poor viral suppression (p = 0.0001). For patients on the efavirenz-based ART regimen, the majority 199 (n = 312; 63.8%) had plasma concentrations between 1000 and 4000 ng/ml considered levels for viral mutant selection windows followed by 99 (n = 312; 31.7%) with EFV plasma concentrations of > 4000 ng/ml considered levels for durable viral suppression. Fourteen (n = 312; 4.5%) patients had EFV plasma concentrations of < 1000 ng/ml, which are considered concentrations for a poor viral suppression window (p < 0.05).

There was no significant difference in NVP plasma concentrations across dosing formulations (p = 0.248) or among EFV dosing formulations (p = 0.352) (Table 2).

Variable	FISHER'S EXACT TEST	
	ART drug Adherence	
	Nevirapine	Efavirenz
	P value	*P value*
Socio-demographic variables		
Gender	0.318	0.253
Age	0.393	0.129
Marital status	0.06	0.368
Occupation	0.952	0.565
Religion	0.785	0.689
Education	0.611	0.124
Vacational schooling	0.482	0.209
Living with partner	0.363	0.871
Had more than one partner	0.97	0.533
Previous partner died	0.919	0.953
Smoking	0.725	0.798
HIV stigma related factors		
Difficult to tell others about my HIV infection	0.234	0.281
Being HIV positive makes me feel immoral	0.260	0.005
Being HIV positive makes me feel guilty	0.035	0.314
Being HIV positive makes me feel ashamed	0.570	0.794
Being HIV positive makes me feel it worthless	0.750	0.344
Being HIV positive makes me feel it is my own fault	0.111	0.318
Hide HIV status from others	0.005	0.605
Feel certain to tell primary sexual partner being HIV positive	0.0001	0.0001
HIV disclosure related factors		
Disclose HIV status to anyone	0.332	0.033
Disclosed HIV status to partner or spouse	0.197	0.578
Disclosed HIV status to family members	0.570	0.730
Disclosed HIV status to friends	0.908	0.383
Disclosed HIV status to neighbor	0.306	0.202
Disclosed HIV status to employers	0.217	0.579
Disclosed HIV status to religious leaders	0.362	0.582
Disclosed HIV status to the public	0.748	0.331
Number disclosed about HIV status in the family	0.185	0.055
HIV social support		
Get useful advice about important things in life	0.022	0.005
Get chance to talk to someone about work or household problems	0.005	0.001
Get chance to talk to someone about personal or family problems	0.071	0.002
I have people who cares about what happens to me	0.256	0.038
I get love and affection	0.0001	0.008
Help with household duties	0.007	0.001
Get financial help during emergency	0.005	0.045
Get transportation help when needed	0.001	0.014
Get general help when sick	0.138	0.009

Table 2. Relationship between HIV stigma, disclosure and social support and ART drug adherence.

Relationship between HIV-related stigma, disclosure and social support and ART adherence. The HIV stigma-related factors associated with adherence to NVP-based regimens included feeling guilty for being HIV positive, hiding HIV status from others and feeling certain to tell primary sexual partners about HIV status. Feeling immoral for being HIV positive and feeling certain to tell primary sexual partners about HIV status was associated with adherence to EFV-based regimens.

Being able to disclose HIV status to anyone and to family members was associated with adherence to EFV-based regimens. The majority of HIV social support-related factors, including getting useful advice about important things in life, getting a chance to talk to someone about work or household problems, getting love and affection, was associated with ART adherence to both NVP- and EFV-based regimens (Table 2).

Variation in median nevirapine and efavirenz plasma concentrations and HIV stigma, disclosure and social support-related factors. Table 3 summarizes the variation in the median NVP and EFV plasma concentrations and sociodemographic, sexual behavior, HIV stigma and disclosure characteristics. Patients who disclosed their HIV status to their employer had higher median (IQR) EFV plasma concentrations (3157, IQR = 2001–5976 ng/mL) than those who did not (2173.5, IQR = 1655.5–3208.5 ng/mL; $p = 0.041$). Patients who did not disclose their HIV status to religious leaders had higher median (IQR) EFV plasma concentrations (2821.5, IQR = 1945–5270 ng/mL) than those who did (1998.5, IQR = 1548–2520 ng/mL; $p = 0.0031$). Furthermore, patients who disclosed their HIV status to the public had higher median (IQR) EFV plasma concentrations (3097, IQR = 2872–5976 ng/mL) than patients who did not (1965, IQR = 1639–2763 ng/mL; $p = 0.0117$).

Patients with higher median (IQR) EFV plasma concentrations were those who did not feel guilty for being HIV positive (6511, IQR = 4607–9863 ng/mL) compared to patients who felt guilty (5557, IQR = 4247–7633 ng/mL; $p = 0.0163$). Patients who disclosed their HIV status to their spouse (6402.5, IQR = 4564.5–9180.5 ng/mL) had higher median (IQR) NVP plasma concentrations than those who did not (4853, IQR = 3450–6202 ng/mL; $p = 0.0362$).

Factors associated with drug plasma concentrations. *Stigma.* In multivariate quantile regression analysis, feeling guilty for being HIV positive (adjusted $\beta = 954$, 95% CI = 192.7 to 2156.6; $p = 0.014$) or feeling worthless for being HIV positive (adjusted $\beta = 852$, 95% CI = 64.3 to 1639.7; $p = 0.034$) were independent factors associated with increased NVP plasma concentrations. For patients on EFV, being certain of telling the primary sexual partner about HIV-positive status was associated with increased EFV plasma concentrations (adjusted β 363, 95% CI, 97.9 to 628.1; $p = 0.007$) (Table 4).

Disclosure. In multivariate quantile regression analysis, disclosing patients' HIV status to neighbors (adjusted $\beta = 1731$, 95% CI = 376 to 3086; $p = 0.012$) was associated with increased NVP plasma concentrations. None of the HIV disclosure-related factors were associated with EFV plasma concentrations (Table 4).

Social support. In multivariate quantile regression analysis, transportation to the hospital whenever needed (adjusted $\beta = -1143.3$, 95% CI = −1914.3 to −372.4; $p = 0.004$) was associated with lower NVP plasma concentrations. None of the HIV social support-related factors were found to be associated with EFV plasma concentrations (Table 4).

Discussion

Every blueprint and policies geared towards individualization of ART treatment aimed at prolonging the life of HIV patients contributes significantly to the components of HIV treatment programs in many countries, including Kenya. The recommendation by the World Health Organization (WHO) requiring testing and treatment of all HIV-positive patients regardless of their CD4 or viral load[20] must also appreciate that optimal ART outcomes require an in-depth understanding of the individual's variation in response to ART, both efficacy and toxicity. The concentration of ARV drug found in plasma has been shown to affect the rate at which ARVs begin to suppress viral replication and/or the duration of the effect on viral replication[21]. Therapeutic drug concentrations are therefore a key to successful ART[14]. Low drug concentrations observed in patients on ART are related to failure to achieve immediate virologic success and longer-term immunological failure[22]. ARV drug plasma concentrations are associated not only with patients' pharmacogenetic and pharmacoecological factors[23] but also to social psychological (defined as human behavior as a result of the relation between mental state and social situation) well-being of patients. Stigma, disclosure and social support are social psychological—mental representations are important influence of our interactions with others and environment. This is among the first studies to assess the association between HIV stigma (a mark of disgrace, discounting, discrediting and discriminating associated with HIV infection and ARV use)[24], HIV disclosure (action of making new or secret of being HIV positive known) and HIV social support (the perception and actuality that one is cared for or having assistance available from other people) on the steady-state plasma concentrations of nevirapine and efavirenz among HIV patients receiving treatment in Nairobi Kenya.

HIV stigma, disclosure and availability of social support are key determinants of patients' behavior and are associated with adherence to HIV care, treatment and prevention. Previously, in Kenya, involvement in community support networks considerably enriched adherence and treatment outcome[25]. Furthermore, patients vigorously partaking in community support networks tended to attain peak NVP plasma concentrations early hours postdosing, which were markedly higher than those seen in patients not actively involved in community support networks. Countless studies have interconnected social support to better medication adherence and better clinical outcomes[26].

The association of patients' social psychological status with ARV plasma concentration and treatment outcomes might be multifactorial. Social psychological status could indirectly be associated with ARV plasma concentration and treatment outcomes by affecting adherence to ART[25,27,28]. In this study, social psychological factors were significantly associated with adherence among patients on EFV compared to those on NVP. The EFV-based regimen is prescribed as a fixed-dose, single-tablet regimen, while NVP is prescribed as two or more pills per day[8]. It is possible that the higher pill burden among patients on NVP could be associated with the patient's social psychological status and adherence and hence NVP plasma concentration. Studies have related a lower pill burden with both better adherence and virological suppression[29,30] as well as patients' emotional satisfaction[31]. Although not investigated in this study, studies have reported a common cause between social psychological status and non-adherence, both of which could independently be associated with ARV plasma

Variable	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
	n	Median	(IQR)		P	n	Median	(IQR)		P
Age group (Years)										
20–30	25	6034	4448	7817		41	2961	1679	4603	
31–40	84	6207	4558	8946.5	0.667	126	2698.5	1918	5976	0.476
41–50	106	6368	4599	9784		96	2685.5	1950.5	4282.5	
> 51	39	6011	4518	8843		49	2754	1833	4074	
Gender										
Male	91	5917	4449	8638	0.387	133	2747	1918	5336	0.728
Female	163	6364	4558	9293		179	2712	1868	4647	
HIV drug resistant mutation										
Yes	24	6062	4119	8786	0.519	10	1373.5	49	2807	0.006
No	230	6237.5	4532	9163		302	2758.5	1918	5139	
HIV viral load (Cells/mls)										
< 1000	230	6237.5	4532	9095	0.609	300	2764	1919.5	5171.5	0.002
≥ 1001	24	6062	4119	8866		12	1373.5	52.5	2714	
Current ART regimen										
3TC, ABC	1	8798	8798	8798		1	1434	1434	1434	
3TC, TDF	159	6698	4599	9755	0.2481	187	2621	1838	5139	0.3519
3TC, ZDV	93	5729	4448	8323		124	2796.5	1968	4726.5	
3TC, d4T	1	3552	3552	3552						
Being HIV positive makes me feel guilty										
Agree	81	5557	4247	7633	0.016	108	2645.5	1895	5171.5	0.927
Disagree	173	6511	4607	9863		204	2854	1869	4839.5	
Being HIV positive makes me feel it worthless										
Agree	55	5243	3975	7311	0.054	82	2756	1951	4319	0.837
Disagree	199	6511	4599	9755		230	2720.5	1838	5204	
Disclosed HIV status to partner or spouse										
Yes	204	6402.5	4564.5	9180.5		242	2759.5	1886	5204	
No	25	4853	3450	6202	0.036	38	2991	1918	5336	0.565
Not applicable	25	6273	4577	9909		32	2556	1750	3488	
Disclosed HIV status to family members										
Yes	166	5967.5	4444	7966		183	2592	1917	5044	
No	87	6868	4951	10,635	0.064	125	2867	1870	4911	0.312
Not applicable	1	8034	8034	8034		4	1699.5	456.5	3118.5	
Disclosed HIV status to neighbor										
Yes	13	5239	3631	7009		22	3079	1917	7572	
No	234	6237.5	4558	9095	0.210	280	2739.5	1902	4837	0.088
Not applicable	7	7966	6372	9909		10	2027.5	857	2961	
Disclosed HIV status to religious leaders										
Yes	18	4479	2960	7009		26	2440	1633	5909	
No	222	6317	4607	9293	0.055	264	2821.5	1945	5270	0.003
Not applicable	14	6371.5	4211	8034		22	1998.5	1548	2520	
Disclosed HIV status to public										
Yes	5	5736	5239	7009		7	3097	2872	5976	
No	231	6202	4503	9163	0.869	282	2766.5	1918	5139	0.012
Not applicable	18	6595.5	4558	8034		23	1965	1639	2763	
Get financial help during emergency										
As much as I would like	162	6365.5	4558	8964		175	2836	1918	4911	
Less than I would like	40	5468.5	4275	8191.5	0.492	52	2309.5	1789.5	5038	0.797
Much less than I would like	12	7275.5	6056.5	9583		32	2747.5	1615.5	8797.5	
Never	40	5710.5	4046	9867.5		53	2872	2043	4241	
Get transportation help when needed										
As much as I would like	169	6538	4571	9198		188	2821.5	1895	5223.5	
Less than I would like	38	5527.5	4336	8382	0.550	43	2462	1818	4872	0.917
Much less than I would like	15	6202	4180	6868		30	2670	1679	6875	
Never	32	5635.5	3955.5	8750		51	2786	1942	3875	

Continued

Variable	NEVIRAPINE (N = 254)					EFAVIRENZ (N = 312)				
	n	Median	(IQR)		P	n	Median	(IQR)		P
Get general help when sick										
As much as I wouild like	212	6351	4448.5	9129		244	2796.5	1895	4977.5	
Less than I wouild like	27	5911	4990	9411	0.970	40	2569	1999.5	5589.5	0.534
Much less than I wouild like	6	7039	5729	8405		12	2447.5	911	4693	
Never	9	5692	5457	7009		16	2931.5	1613.5	4168	

Table 3. Variation in median nevirapine and efavirenz plasma concentration and HIV stigma, disclosure and social support-related variables.

concentration[27,28]. Reverse causality is also possible; efavirenz is associated with high rates of neuropsychiatric side effects, including vivid dreams, insomnia and mood changes, which could impact internal feelings of shame and interest in seeking social support[32]. It is presumed that this neuropsychiatric effect of EFV could affect treatment outcomes, including ARV plasma concentration.

HIV-associated stigma-related factors such as feeling guilty and worthless for being HIV positive were associated with higher median NVP plasma concentrations. For patients on an EFV-based regimen, those who were certain to reveal their HIV status to their primary sexual partner had better ART adherence accompanied by higher median EFV plasma concentrations. Stigma and discrimination remain the paramount challenges confronted by people living with HIV/AIDS[33]. Although data are skewed on the association between HIV stigma and NNRTI plasma concentrations, stigma and discrimination negatively affect people living with HIV[34]. HIV-related stigma is a wide-ranging and worldwide social phenomenon that is exhibited within multiple social spheres, including healthcare encompassing denial of care or treatment, HIV testing without consent, confidentiality breaches, negative attitudes and humiliating practices by health workers[35]. Studies have shown an association between HIV stigma and poorer physical and mental health outcomes[27]. Stigma has also been linked with secondary health-related factors, including seeking healthcare and adherence to antiretroviral therapy and access to and usage of health and social services[27,28]. Inevitably, these negative outcomes of stigma are bound to affect the overall treatment outcomes in terms of therapeutic monitoring.

HIV status disclosure to anyone and family members in this study was associated with ART adherence to an EFV-based regimen and not NVP. In multivariate analysis, disclosure of HIV status to neighbors was associated with increased median NVP plasma concentration. Patients on EFV with lower pill count are more likely to disclose HIV status compared to those on NVP-based regimens, hence better adherence and better treatment outcomes[29,30]. Contrary to our study, in Thailand, Sirikum et al.[36] reported no significant difference in the median ART adherence by pill count, CD4 count, or HIV viral load between HIV patients who disclosed their status compared to those who did not. Studies have shown that HIV disclosure has two possible treatment outcomes[37]. On the one hand, HIV status disclosure to sexual partners is a vital prevention target underlined by both the WHO and the Centers for Disease Control and Prevention (CDC)[38]. At an individual level and to the general public, HIV disclosure is accompanied by numeral benefits[36]. HIV infection disclosure to sexual partners is associated with less anxiety and increased social support, especially among women[37,38]. Further, HIV status disclosure is accompanied by improved access to HIV prevention and treatment programs, increased opportunities for risk reduction and increased opportunities to plan for the future. Disclosure of HIV status also expands the awareness of HIV risk to untested partners, leading to better acceptance and utilization of voluntary HIV testing and counselling and changes in HIV risk behaviors[37,38]. In addition, disclosure of HIV status to sexual partners empowers couples to make educated reproductive health choices that may eventually lower the number of unintended pregnancies among HIV-positive women[37]. Along with these benefits, however, there are a number of potential risks from disclosure for HIV-infected women, including loss of economic support, blame, abandonment, physical and emotional abuse, discrimination and disruption of family relationships[37,38]. These risks may lead women to choose not to share their HIV test results with their friends, family and sexual partners. This, in turn, leads to lost opportunities for the prevention of new infections and for the ability of patients, especially women, to access appropriate treatment, care and support services where they are available[37,38].

In our study, patients who had adequate social support, such as getting useful advice about important things in life, having a chance to talk to someone about work, household, personal or family problems, getting love and affection, had higher median NVP and EFZ plasma concentrations. In South Africa, Brittain et al.[39] showed a correlation between social support and stigma influencing the development of depressive symptoms. The importance of community support networks in enhancing social relationships demystifying HIV-associated stigma is well documented[40,41]. Evidence shows the positive effects of social support and protection on other HIV-related outcomes, such as sexual risk behaviors[42,43], mental health distress and family relationships[44,45]. Growing evidence of associations between social protection and HIV risk reduction[46] is reflected in a number of policy documents by UNICEF, UNAIDS and PEPFAR-USAID that focus on pediatric and adolescent HIV prevention[47,48].

Some of the important limitations worth mentioning in this study included. First, the use of NVP-based ART regimens in Kenya and other countries, especially developed countries, has been considerably reduced in the recent past, meaning that this study could be relevant to a restricted number of patients. Second, standardized tools for measuring stigma, disclosure and social support were not used in this study, limiting the generalizability of this study outcomes. Third, this was a cross-sectional study, which only permitted the description of

	NEVIRAPINE (N = 254)				EFAVIRENZ (N = 312)			
Variable	**Unadjusted β**	**(95% CI)**		***p value***	**Unadjusted β**	**(95% CI)**		***p value***
Age	− 14	− 56.2	28.2	0.307	− 13.7	− 38.7	11.4	0.284
Gender	447	− 545.5	1439.5	0.376	− 35	− 536.5	466.5	0.891
Alcohol use number of times	− 198	− 680.7	284.7	0.42	330	− 534.4	1194.4	0.453
Age of sexual debut	− 364	− 1385.8	657.8	0.484	54	− 459.7	567.7	0.836
Number of sexual life partners	− 600	− 1285.7	85.7	0.086	− 557	− 918.0	− 196.0	0.003
Number of sexual acts in the past 3 months	− 46.5	− 748.9	655.9	0.896	− 106	− 648.9	436.9	0.701
Presence of HIV drug resistant mutation	− 117	− 2064.4	1830.4	0.906	1388	484.1	2291.9	0.003
Viral load (Cells/mls)	117	− 2474	2708	0.929	− 1390	− 2642.7	− 137.3	0.03
Difficult to tell others about my HIV infection	141	− 958.8	1240.8	0.801	− 126	− 703.9	451.9	0.668
Being HIV positive makes me feel guilty	954	26.7	1881.3	0.044	210	− 281.3	701.3	0.401
Being HIV positive makes me feel it worthless	1268	379.4	2156.6	0.005	− 33	-744.7	678.7	0.927
Feel certain to tell primary sexual partner being HIV positive	372	− 453.2	1197.2	0.376	426	24.3	827.7	0.038
Disclose HIV status to anyone	− 539	− 1578.8	500.8	0.308	983	− 1058.0	3024.0	0.344
Disclosed HIV status to family members	1051.5	− 541.5	2644.5	0.195	134	− 381.8	649.8	0.61
Disclosed HIV status to neighbor	1675	137.5	3212.5	0.033	− 445	− 1441.0	551.0	0.38
Disclosed HIV status to employers	− 112	− 1203.3	979.3	0.84	− 489	− 1037.2	59.2	0.08
Disclosed HIV status to religious leaders	1609	− 98.7	3316.7	0.065	− 410	− 907.9	87.9	0.106
Get useful advice about importat things in life	− 539	− 1778.4	1303.7	0.762	− 134.3	− 483.8	215.1	0.45
Get financial help during emergency	− 124.7	− 541.9	292.6	0.557	18.7	− 189.8	227.1	0.86
Get transportation help when needed	− 300	− 512.1	− 87.9	0.006	− 5	− 177.6	167.6	0.955
Get general help when sick	− 217	− 599.7	165.7	0.265	− 158	− 562.3	246.3	0.442
	NEVIRAPINE (N = 254)				**EFAVIRENZ (N = 312)**			
Variable	**Adjusted β**	**(95% CI)**		***p value***	**Adjusted β**	**(95% CI)**		***p value***
Age	0.421	− 71.7	72.5	0.991	− 15.5	− 52.5	21.6	0.412
Gender	172	− 1010.5	1354.5	0.775	− 40.4	− 832.7	751.9	0.92
Alcohol use number of times	− 162.5	− 811	486	0.622	398	− 431.2	1227.2	0.346
Age of sexual debut	− 1008.1	− 2745.4	729.1	0.254	563.5	− 424.6	1551.6	0.263
Number of sexual life partners	− 988	− 2156.8	180.8	0.097	− 845.7	− 1315.0	− 376.4	0.0001
Number of sexual acts in the past 3 months	− 2180.8	− 5358.2	996.6	0.178	487.3	− 3224.2	4198.8	0.796
Presence of HIV drug resistant mutation	226.1	− 7513.4	7965.6	0.954	− 1192.0	− 5251.2	2867.2	0.564
Viral load (Cells/mls)	559.9	− 6645.1	7764.9	0.878	0.0	0.0	0.1	0.339
Difficult to tell others about my HIV infection	− 528.5	− 1633.9	576.9	0.347	− 177	− 1021.3	667.3	0.68
Being HIV positve makes me feel guilty	954	192.7	1715.3	0.014	347.7	− 153.4	848.7	0.173
Being HIV positve makes me feel it worthless	852	64.3	1639.7	0.034	− 143.3	− 759.2	472.5	0.647
Feel certain to tell primary sexual partner being HIV positive	341.5	− 1357.0	2040.0	0.692	363	97.9	628.1	0.007
Disclose HIV status to anyone	− 1042.9	− 2597.4	511.6	0.188	1342	1653.6	4337.6	0.379
Disclosed HIV status to family members	812.9	− 483.3	2109.1	0.218	245	− 365.8	855.8	0.431
Disclosed HIV status to neighbor	1731	376.0	3086.0	0.012	− 251	− 1714.1	1212.1	0.736
Disclosed HIV status to employers	− 393.5	− 1586.1	799.1	0.516	− 505	− 1410.3	400.3	0.273
Disclosed HIV status to religious leaders	241.6	− 1675.6	2158.7	0.804	29	− 1120.3	1178.3	0.96
Get useful advice about importat things in life	− 112.7	− 1430.0	1204.6	0.866	16.4	− 400.5	433.4	0.938
Help with household duties	− 315.2	− 1460.0	829.6	0.588	− 226.4	− 556.1	103.4	0.178
Get financial help during emergency	779.3	− 291.9	1850.6	0.153	245.0	− 304.7	794.7	0.381
Get transportation help when needed	− 1143.3	− 1914.3	− 372.4	0.004	− 6.6	− 377.8	364.7	0.972
Get general help when sick	212.3	− 560.5	985.1	0.589	74.1	− 478.3	626.5	0.792

Table 4. Regression analysis between nevirapine and efavirenz plasma concentrations and HIV stigma variables.

the relationship between the three sociopsychological factors and NVP/EFV plasma concentrations and not a causal conclusion. Such outcomes can be confirmed in a longitudinal study.

Conclusions

This study, conducted in one of the oldest and largest cosmopolitan treatment centers in Kenya, shows that HIV stigma, lack of disclosure and inadequate social support are still noticeable among HIV-infected patients in Kenya. The NVP plasma concentrations were highly heterogeneous, with a significant proportion of patients having supratherapeutic and subtherapeutic plasma concentrations compared to those on EFV regimens. Social-psychological factors negatively impact adherence and are associated with increased NVP plasma concentration compared with EFV.

Data availability

All data will be stored at figshare at the moment submitted as electronic data.

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Author contributions
M.O.N., M.O. conceived the study. M.O.N. collected samples and conducted laboratory analysis. M.O., W.D.B. and F.A.O. supervised laboratory analysis. M.O.N. analyzed the data and prepared the draft manuscript. M.O., W.D.B. and F.A.O. provided guidance and mentorship during the implementation of the study. All authors reviewed and approved the final manuscript.

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The authors declare no competing interests.

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RESEARCH ARTICLE

Effects of cytochrome P450 2B6 and constitutive androstane receptor genetic variation on Efavirenz plasma concentrations among HIV patients in Kenya

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Abstract

The effects of genetic variation of cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) on efavirenz (EFV) plasma concentration was evaluated among 312 HIV patients in Nairobi Kenya. The EFV plasma concentration at steady-state were determined using ultra-high-performance liquid chromatography with a tandem quadruple mass spectrometer (LC-MS/MS). Thirteen CYP2B6 (329G>T, 341T>C, 444 G>T/C, 15582C>T, 516G>T, 548T>G, 637T>C, 785A>G, 18492C>T, 835G>C, 1459C>T and 21563C>T) and one CAR (540C>T) single nucleotide polymorphisms (SNPs) were genotyped using real-time polymerase chain reaction. HIV drug resistance mutations were detected using an in-house genotypic assay. The EFV concentration of patients ranged from 4 ng/mL to 332697 ng/mL (median 2739.5 ng/mL, IQR 1878–4891.5 ng/mL). Overall, 22% patients had EFV concentrations beyond therapeutic range of 1000–4000 ng/mL (4.5%% < 1000 ng/mL and 31.7% > 4000 ng/mL). Five SNPs (15582C>T, 516G>T, 785A>G, 983T>C and 21563C>T) were associated with higher EFV plasma concentration while 18492C>T with lower EFV plasma concentration ($p<0.05$). Strong linkage disequilibrium (LD) was observed for 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T. Sixteen haplotypes were observed and `CTGCTTCC`, `CTGCTTCT`, `TTGCTTCT` and `CGACCCCT` were associated with high EFV plasma concentration. In multivariate analysis, factors significantly associated with EFV plasma concentration included; the presence of skin rash (β = 1379, 95% confidence interval (CI) = 3216.9–3416.3; $p < 0.039$), T allele of CYP2B6 516G>T (β = 1868.9, 95% CI 3216.9–3416.3; $p < 0.018$), the C allele of CYP2B6 983T>C (β = 2638.3, 95% CI = 1348–3929; $p < 0.0001$), T allele of CYP2B6 21563C>T (β = 1737, 95% CI = 972.2–2681.9; $p < 0.0001$) and the presence of 5 to 7 numbers of SNPs per patient (β = 570, 95% CI = 362–778; $p < 0.0001$) and HIV viral load $\leq$1000 cells/mL (β = -4199.3, 95% CI = -7914.9 –-483.6; $p = 0.027$). About 36.2% of the patients had EFV plasma concentrations beyond therapeutic window, posing high risk of treatment failure or toxicity. The SNPs of CYP2B6 516G>T, CYP2B6 983T>C, 21563C>T, presence of higher numbers

cytochrome P450 2B6 and constitutive androstane receptor genetic variation on Efavirenz plasma concentrations among HIV patients in Kenya. figshare. Dataset. https://doi.org/10.6084/m9.figshare.15026289.v1.

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of SNPs per patient and haplotypes CTGCTTCC, CTGCTTCT, TTGCTTCT and CGACCCCT could efficiently serves as genetic markers for EFV plasma concentration and could guide personalization of EFV based ART treatment in Kenya.

Introduction

Efavirenz (EFV) is primarily metabolized to 8-hydroxyefavirenz by cytochrome P450 2B6 (CYP2B6) [1] and to a lesser extent to 7-hydroxy-EFV by CYP2A6 [2,3]. The direct N-glucuronidation of EFV metabolites for excretion by UDP-glucuronosyltransferase (UGT) isoforms (including UGT1A1 and 2B7) represent minor metabolic pathway [4,5]. The transcription factors pregnane X receptor (PXR, *NR1I2*) and constitutive androstane receptor (CAR, *NR1I3*) act on genes involved in xenobiotic metabolism and excretion [6–8]. EFV has the ability to autoinduce its own metabolism through the activation of PXR and CAR [7,9].

High genetic polymorphism has been observed in the CYP2B6 gene with several non-synonymous, synonymous and promoter SNPs identified [10]. Currently, about 38 CYP2B6 alleles (*1A [wild-type] to *38) associated with either increased, decreased or abolished enzymatic activity have been defined [11,12]. A number of CYP2B6 SNPs influencing EFV plasma levels such as 516G>T, 785A>G, 983T>C, and 1459C>T have been studied in details [13–15]. Nonetheless, investigating an individual SNP often do not offer satisfactory data predicting inter and intra personal variations in EFV plasma levels. To provide a more accurate data on the influence of SNPs on EFV plasma levels, studies are recommending evaluating a battery of SNPs that reduce the metabolic function of CYP2B6 [16]. This study evaluated the effect of thirteen CYP2B6 (329G>T, 341T>C, 444 G>T/C, 15582C>T, 516G>T, 548T>G, 637T>C, 785A>G, 18492C>T, 835G>C, 1459C>T and 21563C>T) and one CAR (540C>T) single nucleotide polymorphisms (SNPs) on efavirenz plasma concentrations. Additionally, the association of CYP2B6 and CAR polymorphisms and haplotypes with efavirenz plasma concentrations were also investigated.

Materials and methods

Study design and setting

This cross-sectional study was conducted between August, 2018 to January 2020. Consenting and enrollment was done for 312 HIV patients receiving HIV care and treatment at the Family AIDS Care and Educational Services (FACES) based at Kenya Medical Research Institute (KEMRI), Nairobi Kenya. Data presenting in this study was part of a study that aimed at evaluating the pharmacogenetic and pharmacoecologic etiology of sub-optimal responses to non-nucleoside reverse transcriptase inhibitor (NNRTI) for the purpose of individualization of HIV treatment in Kenya. Other than receiving ART treatment in FACES-KEMRI, patients were recruited in this study if they were: (i) aged above 18 year (ii) consenting to the study, (iii) be on ARV treatment for 12 months, and (iv) had been receiving first line ARV (Zidovudine (AZT) or Abacavir (ABC), 3TC, and EFV. The detail of this cohort has been described in detail in our previous publication [17].

Ethical statement

This study was done according to the principles of the Declaration of Helsinki and was approved by the KEMRI Scientific Review Unit (SERU) (SSC No. 2539). Before recruitment in this study, all patients filled in a written informed consent for study participation.

Data collection

A detailed, structured, face-to-face interview gathered information on patient's socio-demographic, ARV use and medical history. Blood samples (10 mL) at 12–16 h post ARV uptake were collected into three blood tube as follows: EDTA anticoagulant tube for immunological testing and CYP2B6 and CAR genotyping. Serum separating tube for clinical chemistry while Lithium heparin tube for HIV viral load and EFV plasma level quantification. The samples were stored at −80˚C after collection awaiting analysis.

Quantification of EFV plasma concentrations

Solutions. Efavirenz (purity: 99.0%) and internal standard C6-efavirenz (purity: 99.3%) were purchased from Alsachim (Strasbourg, France). The 200 μg/ml EFV stock solution was diluted with 50% methanol in water to concentration ranges of 523.56 to 62000.00 ng/ml. The internal standard was diluted in 50% methanol to give a working solution of 100 ng/mL Then 20μL working standard and 20μL IS was further diluted in 200μL drug free human plasma to prepare 6 plasma calibrators at 10-fold dilution.

Selectivity. The selectivity of endogenous plasma constituents was evaluated using six different sets of plasma samples by analyzing blanks and spiked samples at Low quality control (LQC) levels. The EFV in the plasma spiked at the LQC level and clinical samples was detected at its retention time with single peak an indication that the method was selective to EFV.

Method recovery and linearity. The data for absolute recovery of EFV for six replicates at Low quality control (LQC), middle quality control (MQC) and high-quality control (HQC) level were higher than 80% recovery, further showing the suitability of the method to analyze these two drugs.

Method accuracy and precision. Intra-day and inter-day accuracy and precision was evaluated at three different concentrations (LQC, MQC and HQC) for EFV. For inter-batch, three runs and for intra-batch, a single run was assayed. Each run consisted of six replicates. Both the intra- and inter- day accuracy and precision values were within the acceptance ranges. For EFV, intra-day accuracy ranged from 92.1% to 102% with an inter- day accuracy of 96.7% to 101.4%. The EFV intra-day precision ranged from 5.1% to 7.7% with an inter-day variation of 6.9% to 9.2%.

Viral inactivation. The quantification was achieved first by the inactivation of HIV virus as follows. The 50μl of plasma of each sample and 5μl internal standard in a 1.5ml Eppendorf tube was heated at 65˚C for 10 minutes and subsequently cooled at room temperature for 10 minutes. A 100μl cold methanol (-20˚C) was then added and kept at -20˚C for 10 minutes. The samples were then centrifuged at 20,000g at 20˚C for 8 minutes and the supernatant collected in a clean 1.5ml Eppendorf tube. The 850μl ammonium acetate buffer (pH = 3.00) was added to the supernatant and briefly centrifuged. The sample was considered safe to be handled in a non P3 laboratory.

Solid phase extraction using C18 Cartridge and quantification of EFV. The EFV plasma concentrations were measured using a tandem quadrupole mass spectrometer designed for ultra-high performance: Xevo TQ-S (Waters Corporation, U.S.A) as described by Reddy *et al.* [18]. The Bond Elute C18 cartridges were prepared and placed onto the Visiprep Vacuum Manifold with Standard Lid (Merck, Germany). The Bond Elute C18 150 × 4.6 mm, 5-μm column was conditioned by first passing through 1 ml methanol followed by 1 ml ultrapure water. Each column was then charged with 150μl samples containing 850μl ammonium acetate buffer (pH = 3.00) followed by twice cleaning using 1 mL ultrapure water. The first cleaning was collected into clean separate tube while the second water cleaning collected in the waste tubes. The columns were vacuum dried (5–10 kpa in Hg). The efavirenz elution at a flow

rate of 1 ml/min was then done twice using methanol 500μl with vacuum drying between the two elution. Elutes were then completely evaporated using Thermo Scientific™ Reacti-Vap™ Evaporators (Thermo Fisher Scientific Inc, USA) at 37˚C for 30 min. This was then reconstituted using 100μl of equal parts 1:1 acetonitrile and water, vortexed briefly and transferred into 50 ml capped vials and placed into Xevo TQ-S (Waters Corporation, U.S.A) for quantification. About 1μl of the samples was injected automatically into the LC/MS/MS instrument and quantified within 5 minutes. The EFV plasma concentration was categorized as <1000 ng/ml considered below therapeutic range, 1000 to 4000ng/ml considered therapeutic range and >4000 ng/ml considered supratherapeutic level [19,20].

CYP2B6 and CAR genotyping

DNA preparation. Genomic DNA was extracted from EDTA collected blood using automated NucliSENS® easyMAG® system (BioMérieux—Boston, USA) according to the manufacturer's instructions. The quality of DNA was measured using a ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Real time PCR genotyping. Genotyping was carried out on an ABI 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA). SNPs were analyzed using the validated Taqman Genotyping Assays for *CYP2B6* 516G>T (rs3745274), CYP2B6 983T>C (rs28399499), CYP2B6 15582C>T (rs2279345), CYP2B6 18492 C>T (rs2279345), CYP2B6 21563 C>T (rs8192719) and CAR 540C>T (rs2307424) applied Biosystem pre-validated assays were utilized. The assay IDs were C___7817765_60, C_60732328_20, C__26823975_10, C__26823975_10, C__22275631_10 and C__25746794_20 respectively. These assays were done according to the manufacturer's instructions. Briefly, in a final volume for each reaction of 20ul, was 2X TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 20X Drug Metabolism Genotyping Assay and 10ng genomic DNA. The PCR consisted of an initial step at 95˚C for 10 minutes and 50 cycles at 92˚C for 15 seconds and 90˚C for 60 seconds.

The primers for CYP2B6 329 G>T (rs186335453), CYP2B6 341T>C (rs139801276), CYP2B6 444 G>T (rs1053569), CYP2B6 548 T>C (ss539003292), CYP2B6 637 T>C (ss539003292), CYP2B6 785A>G (rs2279343), CYP2B6 835 G>C(rs139029625) and CYP2B6 1459 C>A (ss539003296) are listed in Table 1. These SNPs were also genotyped using an ABI 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as described by Radloff et al., [21]. Briefly, in a final volume for each reaction of 20ul, was 2X TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), primers forward (10um) and reverse (10 uM), wildtype and mutant probes (10 uM each), H_2O and 10ng genomic DNA. The PCR consisted of an initial step at 50˚C for 2 minutes, 95˚C for 10 minutes and 45 cycles at 95˚C for 15 seconds and 60˚C for 60 seconds. The plates were read using the allelic discrimination settings. The SNP assay was set up using SDS, version 1.3.0 as an absolute quantification assay. Post-assay analysis was done using SDS software. The results for these SNPs were defined as either homozygous wild type, heterozygous and homozygous mutant.

Blood chemistry. The CD4 cell counts were measured using a FACSCount TM flow cytometer (BD Biosciences, San Jose, USA) while HIV-1 RNA was measured using Generic HIV Viral Load® (Biocentric, Bandol, France). These assays were done according to manufactures instructions.

HIV drug-resistant genotyping. The presence of HIV drug-resistant mutation was tested using an in-house genotypic method previously described by Lehman *et al*., [22]. Resistance mutations were identified using the Stanford University and International AIDS Society-USA website Interpretation Algorithm.

Table 1. List of CYP2B6 SNPs primers used for genotyping in this study (Radloff et al., 2013) [21].

SNP	Region	Variant (rs number)	Direction	Sequence (5'– 3') c
329 G>T	Exon 2	rs186335453	F	CGACCCATTCTTCCGGGTATATGGTGTGATCTTTG
			R	CAAAGATCACACCATATACCCGGAAGAATGGGTCG
341 T>C	Exon 3	rs139801276	F	CCGGGGATATGGTGTGACCTTTGCCAATGGAAACC
			R	GGTTTCCATTGGCAAAGGTCACACCATATCCCCGG
444 G>T	Exon 3	rs1053569	F	GGAGCGGATTCAGGATGAGGCTCAGTGTCTG
			R	CAGACACTGAGCCTCATCCTGAATCCGCTCC
548 T>C	Exon 4	ss539003292	F	CATCATCTGCTCCATCGGCTTTGGAAAACGATTCC
			R	GGAATCGTTTTCCAAAGCCGATGGAGCAGATGATG
637 T>C	Exon 4	ss539003294	F	CTTTTTCACTCATCAGCTCTGTACTCGGCCAGCTGT
			R	ACAGCTGGCCGAGTACAGAGCTGATGAGTGAAAAAG
785 A>G	Exon 5	rs2279343	F	AGGCAAGTTTACAAAAACCTG
			R	CCCTCCCTAGTCTTTCTTCTTCC
835 G>C	Exon 6	rs139029625	F	GAAAAAGAGAAATCCAACCCACACAGTGAATTCAGCC
			R	GGCTGAATTCACTGTGTGGGTTGGATTTCTCTTTTTC
1459 C>A	Exon 9	ss539003296	F	CAACATACCAGATCAGCTTCCTGCCCCGC
			R	GCGGGGCAGGAAGCTGATCTGGTATGTTG

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Statistical analysis. Statistical analyses were done using Stata version 13 (StataCorp. LP, College Station, USA). Steady-state efavirenz plasma concentrations were tested for normality by the Shapiro- Wilk test. Evaluation of Hardy-Weinberg equilibrium (estimation of p-values was calculated using the Markov chain method) for the 13 CYP2B6 SNPs and 1 CAR genetic variation, Linkage Disequilibrium, allele, genotype and haplotype frequency and differences in the SNP/allele frequencies between groups/ populations using the SNPStats software -free web tool for SNP analysis [23]. Wright's F statistics was applied to evaluate the expected level of heterozygosity. Variation in log10-transformed EFV plasma levels across clinical and genetic factors were on EFV plasma concentrations was determined using Kruskal-Wallis test and Dunn's test by ranks. Quantile regression analysis was used to evaluate pharmacogenetical factors associated with EFV plasma levels. The significance level was set at $P \leq 0.05$.

Results

Baseline characteristics of study participants

A total of 312 patients were evaluated, with a median age of 40 years (interquartile range (IQR) = 34–47 years), 179 (57.4%) were female, 206(66%) were bantus, 60(19.2%) had a previous partner who had died due to HIV infection. Twenty (6.4%) of the patients had skin rash, 18 (5.8%) were smokers while 54(17.3%) were consuming alcohol. The majority of the patients 187(59.9%) were taking lamivudine/efavirenz/ tenofovir based ART regimen with a 207 (66.3%) non-adherence rate in the past 30 days. The median CD4 was 404.5 cell/mL (IQR = 273.5–543.5 cells/mL), with a median body mass index (BMI) of 24.6 kg/m2 (IQR = 21.5–29.2 kg/m2), median ALT of 25UL (IQR = 19–39.5 UL), median AST of 28 UL (IQR = 20–38 UL) (Table 2).

Efavirenz plasma concentration

The steady-state EFV plasma concentrations varied widely among patients, ranging from 4 ng/mL to 332697 ng/mL (median 2739.5 ng/mL, IQR 1878–4891.5 ng/mL). The patients on efavirenz plasma concentration were distributed as follows, the majority 199(63.8%) had plasma

Table 2. Summary of patient demographics and clinical characteristics of patients.

Variables	All patients	Efavirenz plasma concentration			*p* value
		<1000ng/ml	1000–4000ng/ml	>4000ng/ml	
		Sub-therapeutic range	Therapeutic range	Above therapeutic range	
		14 (4.5%)	199 (63.8%)	99 (31.7%)	
Age (years), Median (IQR)	40(34–47)	45.5(40–52)	40(35–47)	39(32–46)	0.083
Gender Female, n (%)	179(57.4)	7(50)	120(60.3)	52(52.5)	0.376
Ethnicity, Bantus, n (%)	206(66.1)	11(5.3)	130(63.1)	65(31.6)	0.753
Living with partner, n (%)	199(63.8)	5(2.5)	124(62.3)	70(35.2)	0.032
Age of sexual debut (Years), Median (IQR)	18(17–20)	17(16–20)	18(17–20)	18(16–19)	0.531
Duration with HIV (Years), Median (IQR)	10(8–13)	10(8–15)	10(8–13)	10(8–14)	0.216
Skin rash, Yes n (%)	20(6.4)	3(15)	13(65)	4(20)	0.045
Duration ART use (Months), Median (IQR)	24(12–28)	22(6–27)	24(12–27)	24(9–29)	0.983
Current ARV Type, n (%)					0.329
lamivudine, efavirenz, Abacavir	1 (0.3)	0	1(100)	0	
lamivudine, efavirenz, tenofovir	187 (59.9)	12(6.4)	116(62)	59(31.6)	
lamivudine, efavirenz, zidovudine	124 (39.7)	2(1.6)	82(66.1)	40(32.3)	
Missed ART scheduled visit, n (%)	25 (8.1)	3(12)	12(48)	10(40)	0.079
Non-adherence in the past 30 days, n (%)	207(66.3)	8(3.9)	139(67.2)	60(28.9)	0.197
HIV-RNA copies/mL, n (%)					*0.0001*
Failure (>1000copies/mL)	12 (3.9)	4(33.3)	7(58.3)	1(8.3)	
Responders (<1000copies/mL)	300 (96.1)	10 (3.3)	192(64)	98(32.7)	
Presence of HIV drug resistant mutation	10(3.2)	3(30)	6(60)	1(10)	*0.005*
CD4 Cells/mL, Median (IQR)	404.5(273.5–543.5)	285.5(126–510)	408(279–538)	403(278–563)	0.77
AST (U/L), Median (IQR)	28(20–38)	23(18–34)	28(20–38)	28(19.2–38)	0.688
ALT (U/L), Median (IQR)	25(19–39.5)	22(13–36)	25(19–41)	26(19–41)	0.342
BMI (kg/m2), Median (IQR)	24.6(21.5–29.2)	23.8(22–26.5)	24.7(21.3–29)	24.6(21.7–30.1)	0.346

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concentrations between 1000 to 4000 ng/mL considered levels for viral mutant selection windows followed by 99(31.7%) had supra-therapeutic EFV plasma levels (>4000 ng/mL) while 14(4.5%) had plasma concentrations of <1000 ng/ml considered levels for poor viral suppression window and ($p < 0.05$) (Table 2).

Patients with EFV plasma level within therapeutic 1000 and 4000 ng/mL were those living with their partners (62%) compared to patients with EFV plasma level of <1000 ng/mL or >4000 ng/mL (2.5% and 35.2% respectively; $p = 0.032$). For patients with skin rash 20% had EFV plasma level of >4000 ng/mL compared to 15% of the patients with EFV plasma level of <1000ng/mL; ($p = 0.045$). Among patients with virologic failure (>1000copies/mL) although the majority (58.3%) had EFV plasma level between 1000 ng/mL-4000ng/mL, a significant number (33.3%) had sub-optimal EFV plasma level of <1000ng/mL compared to 8.3% with supra-therapeutic EFV plasma level >4000ng/mL; ($p = 0.0001$). Similarly, among the 10 patients who had NNRTI drug resistant mutations, although the majority (60%) had EFV plasma level between 1000 ng/mL-4000ng/mL, a significant number (30%) had sub-optimal EFV plasma level of <1000ng/mL compared to 10% with supra-therapeutic EFV plasma level >4000ng/mL; ($p = 0.005$). No differences were observed with regards to gender, ART regimen type, non-adherence, median Age, duration with HIV, duration on ART, CD4 count, AST and ALT levels and BMI in patients with supratherapeutic EFV levels > 4000 ng/mL when compared to patients with sub-optimal EFV level. A trend was observed in the different strata with regards to missing ARV scheduled appointment and median age (Table 2).

Allele and genotype frequencies of CYP2B6 gene and CAR SNPs

The heterozygous or homozygous mutant or both were not detected for five CYP2B6 SNPs including: CYP2B6 329G>T, 341T>C, 444 G>T/, 637T>C, 835G>C, 548T>G and were not analyzed further. Seven CYP2B6 SNPs (516G>T, 785A>G, 983C>TA, 18492C>T, 21563C>T, 1459C>T and 15582 C>T) and one CAR SNP (540C>T) conformed to Hardy–Weinberg equilibrium and were further analyzed. The allele and genotype frequencies of these SNPs and association with EFV concentrations are summarized in Table 3. Kruskal-Wallis test analysis showed that the homozygous mutant for 155882C>T, 516G>T, 785A>G, 983T>C, 21563C>T are associated with significantly high median (IQR) EFV plasma concentration ($p<0.05$). Only SNP 18492C>T had both heterozygous and homozygous mutant significantly associated with lower median (IQR) EFV plasma concentration ($p<0.05$). The median (IQR) plasma EFV levels was not significantly associated with 1459C>T and CAR 540C>T (Table 3 and S1 Fig).

CYP2B6 and CAR haplotype frequency and association with EFV concentrations

We quantified the extent of LD among the CYP2B6 and CAR SNP pairs among study patients. Strong LD, defined by high values for both D' (≥0.8) and r2 (≥0.5) parameters, was only observed between SNP pairs CYP2B6 516–785, 516–21563 and 785–2156. The 516–18492 pair, 785-18492pair and 18492–21563 pair had high D' (>0.8) and moderate r2 (0.12–0.351) value. The CAR 540–18492 pair, had high D' (≥0.8) and moderate r2 (0.155) values. All other SNP pairs had highly variable D' (0–0.8) and low r2 (<0.1) values among the patients (S2 Fig). These findings indicate strong linkage disequilibrium among 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T, resulting in 16 haplotypes among which CTGCTTCC was the most common occurring 101(32.4%) with CGGCTTCC and TGATTTCC reported in 1(0.3%) patient each. The haplotypes (`CTGCTTCC`, `CTGCTTCT`, `TTGCTTCT` and `CGACCCCT`) were associated with higher EFV plasma concentration (Table 4).

Quartile regression model

In the final multivariate analysis, factors associated with a higher EFV plasma concentration included: presence of skin rash (β = 1379, 95% confidence interval (CI) = 3216.9–3416.3;

Table 3. Allele and genotype frequencies of CYP2B6 gene and CAR SNPs and their relationship with EFV plasma concentrations.

SNPs	Allele	Allele		Genotype			Median EFV Concentration (IQR) -μg/mL			P value
		Frequency–n (%)		Frequency–n (%)						
	A1/A2	A1	A2	A1/A1	A1/A2	A2/A2	A1/A1	A1/A2	A2/A2	
CYP2B6 15582C>T	C/T	560(0.9)	64(0.1)	255(0.82)	50(0.16)	7(0.02)	2747 (1918–5204)	2402(1633–3519)	43788(2539–9313)	0.07
CYP2B6 516G>T	G/T	395(0.63)	229(0.37)	128(0.41)	139(0.45)	45(0.14)	2037.5(1501–3170)	2754(1985–4487)	8282(504–13564)	0.0001
CYP2B6 785A>G	A/G	394(0.63)	230(0.37)	127(0.41)	140(0.45)	45(0.14)	2043(1548–3182)	2743(1968–4403)	8282(504–13564)	0.0001
CYP2B6 18492C>T	C/T	511(0.82)	113(0.18)	207(0.66)	97(0.31)	8(0.003)	3300(2014–6745)	2059(1718–3095)	2391(1434–3034)	0.0001
CYP2B6 983T>C	T/C	578(0.93)	46(0.07)	268(0.86)	42(0.13)	2(0.01)	2580(1836–4420)	3810(2520–10554)	8523(7572–9473)	0.002
CYP2B6 21563C>T	C/T	394(0.63)	230(0.37)	126(0.4)	142(0.46)	44(0.14)	2038(1548–3182)	2760(1985–4487)	7970(4978–13188)	0.0001
CYP2B6 1459C>T	C/T	621(0.99)	3(0.005)	310(0.99)	1(0.003)	1(0.003)	2739(1870–4872)	332701(332701–332701)	2019(2019–2019)	0.19
CAR 540C>T	C/T	574(0.92)	50(0.08)	264(0.85)	46(0.15)	2(0.01)	2687(1878–5025)	3017(1833–4872)	2640(2019–3262)	0.966

n—number; %—percentage; IQR—Interquartile range; p value—Significant level.

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Table 4. Relationship between haplotypes and EFV plasma concentrations.

Haplotypes	n (%)	Efavirenz plasma concentration			
		<1000ng/ml	1000–4000 ng/ml	>4000ng/ml	Median (IQR) ng/ml
		14 (4.5%)	26 (6.9%)	255 (67.6%)	
CTGCTTCC	101 (32.4)	2(1.9)	43(42.6)	56(55.5)	3001 (1925–5773)
CGATTCCC	52 (16.7)	2(3.9)	45(86.5)	5(9.6)	2529.5(1903–4063)
CGACTCCC	35 (11.2)	1 (2.9)	28(80)	6(17.1)	1991(1252–3095)
CTGTTTCC	35 (11.2)	2(5.7)	28(80)	5(9.6)	2368(1740–4713)
TGACTCCC	25 (8)	2(8)	18(72)	5(20)	3572(2526–6116)
TTGCTTCC	22 (7.1)	1(4.5)	15(68.2)	6(27.3)	3432.5(2127–11541)
CTGCTTCT	18 (5.8)	1(5.6)	8(44.4)	9(50)	2427.5(1679–3700)
CTGTTTCT	6 (1.9)	1(16.7)	3(50)	2(33.3)	2619(1868–5909)
CGACTCCT	5 (1.5)	1(20)	4(80)	0	3424(2592–3661)
TGATTCCC	3 (1)	0	3(100)	0	3067(1784–5139)
TGACTCCT	2 (0.6)	0	1(50)	1(50)	2805(2517–3093)
TGATTCCT	2 (0.6)	0	2(100)	0	3514.5(2765–4264)
TTGCTTCT	2 (0.6)	0	0	2(100)	4464(1422–7506)
CGACCCCT	2 (0.6)	0	0	2 (100)	2697(2237–3157)
CGGCTTCC	1 (0.3)	0	1(100)	0	3695 (3695–3695)
TGATTTCC	1 (0.3)	0	1(100)	0	3727(3727–3727)

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$p < 0.039$), T allele of CYP2B6 516G>T (β = 1868.9, 95% CI 3216.9–3416.3; $p < 0.018$), the C allele of CYP2B6 983T>C (β = 2638.3, 95% CI = 1348–3929; $p < 0.0001$), T allele of CYP2B6 21563C>T (β = 1737, 95% CI = 972.2–2681.9; $p < 0.0001$) and the presence of 5 to 7 numbers of SNPs per patient (β = 570, 95% CI = 362–778; $p < 0.0001$). Having HIV viral load ≤1000 cells/mL was associated with lower EFV plasma levels (β = -4199.3, 95% CI = -7914.9 –-483.6; $p = 0.027$). A trend was also observed for non-adherence in the past 30 days (β = -419.1, 95% CI = -916–77.9; $p = 0.098$) in association with lower EFV plasma levels (Table 5).

Discussion

This is among the first studies with sufficient samples size reporting the association between efavirenz plasma concentrations and expanded CYP2B6 genetic variants and one CAR in one of the largest cosmopolitan ARV treatment centers in Nairobi Kenya. Although majority of patients' EFV plasma concentrations (63.8%) were within the therapeutic window, 4.5% of the patients had suboptimal EFV concentrations. This outcome is significant given EFV is the only NNRTI still retained as part of first line ART treatment in Kenya [24]. Further, these results are significant given that the use of EFV is associated with treatment discontinuation due to neurotoxicity [25] implying an existing challenge in the management of HIV patients receiving this regimen in Kenya.

The acceptable minimum EFV plasma concentration required to attain virologic suppression is indicated as >1000 ng/mL [26]. Sub-optimal EFV plasma levels reduces viral suppression and is associated with the development ART resistant mutations [20,27,28]. In agreement with our study, about 33.3% of the patients with virologic failure also had suboptimal EFV plasma concentration. The presence of HIV resistant mutation was associated with lower EFV plasma levels. Thirty percent (30%) of the 10 patients with drug resistant mutation had sub-optimal plasma level with some 10% having EFV plasma level considered supra-therapeutic levels. The dynamic nature in the frequency of specific HIV drug resistant mutations conferring resistance to NNRTI including (K103N, Y181C and G190A) as a results of long-term

Table 5. Quartile regression analyses of factors associated with EFV plasma concentrations.

Variable	Univariate analysis				Multivariate analysis			
	Unadjusted β	(95% CI)		P-value	Adjusted β	(95% CI)		P-value
Age	-14	-38.1	10.8	0.273	-7.4	-33.7	19	0.582
Gender	-35	-545.9	475.9	0.893	-325.8	-925	273.3	0.285
Alcohol use	330	-296.1	956.1	0.3	293.5	-632.2	1219.2	0.533
Smoking	-715	-2413.9	983.9	0.408	-55.7	-1589.6	1478.2	0.943
Skin rash	788	114.2	1461.8	0.022	1379	72.5	2685.5	0.039
ART regimen	318	-148.5	784.5	0.181	316.6	-326.2	959.4	0.333
None-adherence	-400	-826.7	26.7	0.066	-419.1	-916	77.9	0.098
Months since ART initiation	-2.3	-16.1	11.5	0.743	-8.6	-35.2	18	0.526
HIV drug resistant mutation	1388	304.8	2471.2	0.012	1314.5	-937.4	3566.3	0.252
HIV-RNA <1000 cps/mL	-1390	-2676.2	-103.8	0.034	-4199.3	-7914.9	-483.6	0.027
CD4 count	0.04	-1.5	1.6	0.953	0.4	-0.2	1.1	0.18
AST	1.9	-4.6	8.4	0.558	0.1	-7.4	7.5	0.984
ALT	6.96	-2.4	16.3	0.144	7.1	-10.4	24.6	0.427
BMI	-26.9	-90.7	36.9	0.408	-11.4	-74.4	51.6	0.722
15582C>T	54	-422.9	530.9	0.824	293.7	-727.6	1315.1	0.572
516G>T	1583	1137.1	2028.9	0.0001	1868.9	321.6	3416.3	0.018
785A>G	1570	1061	2079	0.0001	711	-9053.1	10475.1	0.886
18492C>T	-896	-1398.5	-393.5	0.001	-444.4	-1360.7	471.9	0.341
983T>C	2068	467.9	3668.1	0.02	2638.3	1348	3929	0.0001
21563C>T	1577	1083.5	2070.5	0.0001	1737	792.2	2681.8	0.0001
1459C>T/A	364	-267177	266449	0.998	135	-318857	319126.6	0.999
540C>T	275	-559.5	1109.5	0.542	230.5	-817.3	1278.4	0.665
Number of SNPs per patient	517	364	670	0.0001	570	362	778	0.0001
Haplotypes	**107**	-26.1	240.1	0.115	16.4	-64.6	97.4	0.69

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ART treatment play a big role in the treatment outcome [29]. Inevitably, drug resistant mutations whether transmitted, acquired or archived are crucial in determining the treatment outcomes especially for the NNRTIs which have been shown to have a relatively low genetic barrier to resistance [30].

Though we reported no association between EFV with hepatotoxicity (ALT and AST), there was evidence towards increased ALT and AST levels among patients with supra-therapeutic EFV plasma concentrations. Studies have associated patients with supra-therapeutic plasma NNRTI, with a high risk of developing drug toxicity, a common cause of ART treatment non- adherence and discontinuation medications [31].

The prevalence of variant alleles for CYP2B6 516T, 983C, CYP2B6 18492T, CYP2B6 21563T, CYP2B6 1459T and CYP2B6 785G in this population was similar to that reported for other African populations and Kenyan ethnic groups [32–34]. For the CAR 540T C>T, the frequency of the T variant allele was 14.9% which was similar to those reported by Wyen, *et al* [35] which showed 15% variant alleles in black population. The allele frequencies of 516G>T and 785A>G have been consistently higher in the Asian populations, such as Chinese populations [36], Japanese and Korean [37], but lower than that in African populations [14,38]. Further, the allele frequencies of CYP2B6 983C was 14.1% which is consist to other studies showing predominant occurrence of this allele in African subjects [38,39]. Studies have demonstrated the role of polymorphisms in the CYP2B6 gene on enzymatic activity with concomitant effect on EFV concentrations [36,40]. In this study were reported an increased EFV

plasma level with the presence of the T and C allele at the position c.516 and of 983 CYP2B6 respectively in agreement with previous studies [33,36,40]. For CYP2B6 983T>C genotype, both the homozygosity and heterozygosity for mutant were associated with higher EFV plasma concentration than those patients with wild-type 983CC. The effect was noteworthy for its magnitude [41]. The presence of T allele at the position c.18492 of CYP2B6 18492 was associated lower plasma efavirenz concentrations, which is consistent with previous studies [34,40,42]. Studies have shown the presence of this CYP2B6 18492T>C SNP together with coadministration of a strong CYP inducer may increase the likelihood of subtherapeutic plasma efavirenz concentrations [34].

This is the first report in Kenya to assess the role of constitutive androstane receptor (CAR) 540T C>T in the concentrations for EFV. The frequency of the T variant allele for CAR 540 C>T was 12.2% in agreement, Wyen *et al*., [35] reported the prevalence of T variant in 32.7% among the Caucasians and 15% among the blacks. In Ghana Sarfo et al., [43] reported slightly lower the frequency of variant allele for CAR 540C>T at 7% among patients. In our study, though not significant in linear regression analysis, the homozygous and heterozygous for mutation for CAR 540T C>T had higher EFV plasma concentrations. This was consistent with the two reports which observed a trend towards association between plasma efavirenz concentration and CAR 540C>T [35,43].

Haplotype analysis evaluates the interactions of multiple SNPs, leading to a decrease or increase in the metabolic function of CYP2B6 or CAR. Haplotype might accurately predict ARV drug pharmacokinetics than a single SNP [16]. In this study, linkage disequilibrium among 15582C>T, 516G>T, 785A>G, 18492C>T, 983T>C, 21563C>T, 1459C>T and CAR 540C>T was observed, resulting in 16 haplotypes among which `CTGCTTCC` and `CGGCTTCC` or `TGATTTCC` had the highest and the lowest frequency, respectively. Compared to `CTGCTTCC`, 4 haplotypes (`CTGCTTCC`, `CTGCTTCT`, `TTGCTTCT` and `CGACCCCT`) were associated with higher EFV plasma concentration). Meng et al., (2015) [36] showed the predictive accuracy of the average EFV plasma concentration was higher among the haplotypes than with each single SNPs.

This study has three major limitations. First, the cross-sectional nature of this study had the potential of introducing confounding factors as well as only permitting describing the relationship between EFV plasma concentrations, patient genetics and a few pharmacoecologic factors and not a causal conclusion. Such outcomes can be confirmed in a longitudinal study. Second, although we excluded patients on tuberculosis, hepatitis B or C virus co-infection was not tested during the study. The prevalence of hepatitis B or C virus coinfection is generally high among HIV patients [44,45]. Studies have reported the influence of HCV co-infection on nevirapine plasma levels [28], with the effect of this HCV coinfection being minimal among patient with a normal liver function [46]. Hepatitis co-infection and potential influence on EFV plasma levels particularly among patients with hepatotoxicity cannot be ruled out in this study. Third, even though the study was conducted in a cosmopolitan treatment center, the outcome from this geographically define subset of patients may not be generalized to other patients majorly because ethnicity and environmental factors influences variations in drug levels. One of the strengths of this study was that, blood samples were collected among patients who had been on ARV treatment for 12 months. Further, sample collections were done between 12 and 16 h after EFV administration. These two factors might have mitigated the effects of non-compliance and inter-individual variability [47].

Given these limitations, the following conclusions can be drawn from these data. Broader interindividual variability in efavirenz plasma concentrations was reported with a relatively large percentage (36.2%) of the patients having EFV plasma concentrations beyond therapeutic window, posing high risk of treatment failure or toxicity. Other than *CYP2B6* c.516G>T

and CYP2B6 983T>C polymorphism, four *CYP2B6* gene (785A>G, 18492C>T, 21563C>T and 15582C>T) and one CAR (540T>C) are potential predictors of efavirenz plasma levels Haplotype analysis suggested strong association of CTGCTTCC, CTGCTTCT, TTGCTTCT and CGACCCCT and EFV plasma concentration.

Supporting information

S1 Fig. The differences in log_{10}-transformed EFV plasma concentrations by genotypes of 7 CYP2B6 and 1 CAR SNPs. 15582C>T, 516G>T, 785A>G, 983T>C, 21563C>T and 18492C>T significantly influence EFV plasma concentration ($p<0.05$) but not 1459C>T and CAR 540C>T.
(PDF)

S2 Fig. Linkage disequilibrium analysis of 7 SNPs of CYP2B6 and 1 CAR. Dark red squares: strong evidence of LD, dark yellow/orange squares: uninformative, light yellow squares: strong evidence of recombination. SNP1-15582C>T; SNP2 - 516G>T; SNP3 - 785A>G; SNP4-18492C>T; SNP5- 983T>C; SNP6-21563C>T; SNP7- 1459C>T and SNP8—CAR 540C>T.
(PDF)

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