EFFECT OF ABCB1 C3435T POLYMORPHISM ON CLINICAL OUTCOMES IN HIV PATIENTS ON LOPINAVIR-BASED ANTIRETROVIRAL THERAPY AT KENYATTA NATIONAL HOSPITAL

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U52/75740/2014

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY
UNIVERSITY OF NAIROBI

DECEMBER, 2017

DECLARATION

I, Richard Kagia Njung'e, do hereby declare that this thesis is my original work carried out in part-fulfillment of the requirements for the award of the degree of Master of Science in Molecular Pharmacology of the University of Nairobi. This work has not been presented for the award of any other degree or to any other university.

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DEDICATION

I dedicated this thesis to my dear lovely wife Dorothy Makena and my daughter Talia Wakera and my son Immanuel Njung'e

ACKNOWLEDGEMENTS

I hereby give gratitude to the University of Nairobi because of awarding me a scholarship to pursue this course. I also thank my supervisors Dr. Margaret N. Oluka, Dr. Faith A. Okalebo and Dr. Anne Njoroge for having sacrificed their precious time and resources to guide me till I successfully completed this thesis.

My special thanks also go to Mr. Daniel Juma of the School of Pharmacy, Department of Pharmacology and Pharmacognosy and Mr. Meshack Wadegu of the Center of Public Health and Research, Walter Reed project for their sacrifice in guiding me through DNA extraction and genotyping. Also special thanks to Martin Maina of Africa Biosystems, for technical support throughout the genotyping assays. May God bless them for their immense support.

I am also deeply grateful to my lecturers for their guidance. I also thank my parents, my wife, my daughter, my friends and colleagues for their constant encouragement, moral support and prayers. May God bless them immensely.

Finally, I thank God for his grace, mercy and favour which have enabled me to reach this far.

RESEARCH OUTPUTS

Published Articles

1. Kagia Richard, Okalebo Faith, Oluka Margaret, Njoroge Anne and Bulimo Wallace (2017). Effect of ABCB1 C3435T Polymorphism on Clinical Outcomes in Kenyan HIV Patients on Lopinavir-Based Regimens. *Journal of Pharmacy and Pharmacology* 7(7): 478-488. doi: 10.17265/2328-2150/2017.07.013.

2. Kagia Richard, Oluka Margaret, Okalebo Faith and Njoroge Anne (2017). Socio-demographic and treatment-related variables associated with CD4 cell counts in Kenyan HIV patients on second-line regimens. *Afr. J. Pharmacol. Ther.* 6(3): 142-148. http://www.uonbi.ac.ke/journals/kesobap/

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LIST OF ABBREVIATIONS AND ACRONYMS

ABC	ATP Binding Cassette
ABCB	ATP Binding Cassette sub-family B
ABCB1	ATP Binding Cassette sub-family B member 1/ Permeability glycoprotein
ABCC	ATP Binding Cassette sub-family C
ADR	Adverse Drug Reaction
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Transaminase
ART	Antiretroviral Therapy
ARV	Antiretroviral Drugs
ATP	Adenosine Triphosphate
ATV/r	Atazanavir/ritonavir
AZT	Zidovudine
BBB	Blood Brain Barrier
BMI	Body Mass Index
bp	base pair
CCC	Comprehensive Care Centre
CCR5	HIV-co receptor C-C chemokine receptor type 5
CD4	Cluster of differentiation antigen 4
CYP450	Cytochrome P450
D4T	Stavudine
DNA	Deoxyribonucleic Acid
DRV/r	Darunavir/ritonavir
EFV	Efavirenz
EMEA	European Medicine Agency
FDA	Food and Drug Administration Agency
FDC	Fixed Dose Combination
HAART	Highly Active Anti-retroviral Therapy
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IC_{50}	Inhibitory Concentration at 50% of the maximal effect

KNH	Kenyatta National Hospital
LPV/r	Lopinavir/ritonavir
MDR1	Multidrug Resistance Protein 1
MOH	Ministry of Health, Kenya
NASCOP	National AIDS and STI Control Program
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NVP	Nevirapine
SLCO	Solute Carrier Organic Anion Transporter family
SNP	Single Nucleotide Polymorphism
TB	Tuberculosis
TDF	Tenofovir
UNAIDS	United Nations Acquired Immune Deficiency Syndrome report
UON	University of Nairobi
WHO	World Health Organization
3TC	Lamivudine

DEFINITION OF OPERATIONAL TERMS

Allele: one of two or more alternative forms of a gene found at the same place on a chromosome and arise by mutation

Baseline characteristics: important attributes of the participants when enrolled at the start of the study.

Clinical outcomes: they include immunologic response (represented by CD4 cell counts), liver function (represented by ALT levels), kidney function (represented by creatinine levels) and blood profile (represented by Haemoglobin levels).

Confounder: is a third variable that can make it appear (sometimes incorrectly) that an observed exposure is associated with an outcome.

Co-variate: is a variable that is possibly predictive of the outcome under study.

Gene: Is the basic physical and functional unit of heredity passed from parent to offspring.

Genotype: Is the DNA sequence of an individual or an organism that determines a specific characteristic of that individual or organism.

Genotyping: Is the process of determining the genetic constitution of an organism.

Haplotype: Is a group of genes which is inherited together by an offspring from a single parent.

Mutation: Is a permanent change of the nucleotide sequence of the genome of an organism.

Pharmacogenetics: The convergence of genetics and pharmacology which deals with responses to drugs that are genetically determined.

Pharmacogenomics: The convergence of pharmacology and genomics which deals with genetically determined responses to drugs.

Phenotype: The observable biochemical or physical characteristics of an organism as determined by both environmental influences and genetic makeup.

Polymorphism: Natural variation in a gene, DNA sequence or chromosomes that have no adverse effect on the individual and occur with a frequency of 1% or more in the general population.

Sanctuary sites: Areas in the body that are poorly penetrated by pharmacological agents, for example latently infected (resting) CD4 cells, macrophages, central nervous system and testes.

Single nucleotide polymorphism: A variation in a DNA sequence at a single position among individuals.

ABSTRACT

Background

Genetic variation is important consideration in drug disposition and overall clinical response in patients on antiretroviral therapy. ABCB1 affects disposition of many drugs and thus affects the pharmacokinetics of drugs and ultimately treatment response. Polymorphisms of ABCB1 especially ABCB1 C3435T polymorphism may affect pharmacokinetics of lopinavir by interfering with efflux of lopinavir from sanctuary sites and thus affect CD4 treatment response and other clinical outcomes of patients infected by human immunodeficiency virus (HIV).

Objectives

The main objective was investigating effects of ABCB1 C3435T polymorphisms on clinical outcomes in HIV patients on lopinavir-based antiretroviral therapy (ART) regimens at the Comprehensive Care Centre (CCC) of Kenyatta National Hospital (KNH).

Method

The study design was a historical cohort carried out among Kenyan HIV patients on lopinavir-based regimens attending KNH CCC clinic and entailed collection of patient data. Patient clinical and demographic information were abstracted from the medical records using a data abstraction form. Blood samples were drawn from the participants. PureLink® genomic DNA extraction mini kit was used for the extraction and purification of genomic DNA. TaqMan® drug genotyping assay and protocol was used in the DNA amplification and genotyping by use of real time polymerase chain reaction. Data analysis was conducted by use of STATA software version 10.

Results

There were 84 study participants comprising 59.5% female and 40.5% male. All were on a lopinavir-based regimen. Prevalence of the ABCB1 3435CC wild-type genotype was 64 (76.2%) while that of the heterozygous CT genotype was 19 (22.6%), and the TT variant genotype was 1 (1.2%). ABCB1 C3435T polymorphism was significantly associated with creatinine levels ($p=0.001$) 6 months after therapy on lopinavir-based regimens. Study participants with the CT genotype had lower creatinine levels after 6 months on lopinavir-based regimens than those with the CC genotype. In addition, study participants with the CT genotype had mean change of CD4 count of 71 after six months on lopinavir-based regimens compared to those with the CC genotype who had a mean change of CD4 count of 28. ABCB1 C3435T genotypes had no significant association with haemoglobin levels, ALT levels and body mass index.

Conclusion

This study reveals that the patients with heterozygous ABCB1 3435 CT genotype exhibit better immunological profile and better renal function. Therefore, genotyping for ABCB1 C3435T polymorphism would help predict patients who would respond effectively to lopinavir-based regimens. However, more genotypic studies on other ABCB1 polymorphisms need to be done.

Chapter 1

INTRODUCTION

1.1 Background

Human Immunodeficiency virus (HIV) is no longer a severe and fatal disease due to the success of the antiretroviral therapy (ART) which has led to reduced incidence of opportunistic infections, admissions in hospitals and mortality (NASCOP, 2014a). Between 2011 and 2014, adults living with HIV in Kenya were initiated on ART if they were in the World Health Organisation (WHO) stage III or IV or their CD4 cell count was not more than 350 or had HIV and Tuberculosis (TB) co-infection (NASCOP, 2011). From 2014, patients were started ART if they had a CD4 cell count of less than 500, were in WHO stage IV or III or had HIV and TB co-infection or were pregnant or breastfeeding or in a sero-discordant relationship (NASCOP, 2014a). Based on CD4 cell count of less than 350, adults in need of ART were estimated to have reached 760,000 (NASCOP, 2012), however, this number increased to 888,000 (766,000 to 1,009,000) using the 2013 WHO treatment guidelines (NASCOP, 2014b). Coverage of ART among all HIV-infected Kenyan adults and adolescents was 60.5% (NASCOP, 2014b). However, the Kenyan Guidelines on management of HIV in 2016 recommend that all individuals with confirmed HIV infection are eligible for ART provided that they are ready and prepared to take and be adherent to treatment. Age, CD4 cell count levels, WHO clinical stage, co-infection, pregnancy or breastfeeding status or any other criteria should not be considered in initiating ART (NASCOP, 2016).

Patients may fail first-line ART regimen and this necessitates change of regimen to second line ART regimens. Failure may either be immunological, clinical or virological. Causes of treatment failure may include pre-existing drug resistance, non-adherence to treatment, impaired drug absorption and regimens with low potency (NASCOP, 2011). Altered drug pharmacokinetics may also cause failure due to insufficient drug concentrations in the body. There was a 40% lower risk of virologic failure among whites compared to blacks which was not explained by patient demographic characteristics in a study in the United States; thus indicating that genetic factors could play a role in virologic failure (Ribaudo et al., 2013). Another study done in the United States found that protease inhibitors therapeutic drug monitoring appeared

beneficial in black and Hispanic patients and not whites showing that genetic variability is critical in protease inhibitors disposition (Demeter et al., 2009).

ABCB1 is a membrane transporter that pumps out drugs from cells and thus interferes with the pharmacokinetics and pharmacodynamics of many drugs and it may affect oral bioavailability and prevent penetration into sanctuaries protected by ABCB1. Lopinavir, which is an important drug in second line ART regimens, is a substrate of ABCB1. Therefore, pharmacogenetics of ABCB1 may affect lopinavir plasma levels and thus affect treatment outcomes.

ABCB1 3435 C>T Single Nucleotide Polymorphism (SNP) leads to decreased mRNA and protein levels and reduced function and expression of ABCB1. The homozygous mutant genotype of ABCB1 3435TT shows low expression of ABCB1 indicating that 3435 C>T SNP leads to reduced activity of ABCB1. Therefore, ABCB1 3435 C>T SNP may affect the pharmacokinetics of lopinavir and thus affect the immunological response. A study done in Africans concluded that 3435T alleles resulted to a higher effective renal plasma flow and glomerular filtration rate and lower renal resistance (Bochud et al., 2008). This indicates that ABCB1 C3435T polymorphism may affect renal function.

1.2 Research problem

The prevalence of ABCB1 polymorphisms in Kenya is unknown. Protease inhibitors and lamivudine are substrates of ABCB1 (Rathbun and Liedtke, 2011; Zhu et al., 2013). In addition, protease inhibitors are inducers of ABCB1 (Hughes et al., 2011) and thus polymorphism of ABCB1 is likely to affect the levels of these drugs which are used in second line antiretroviral therapy. This may interfere with treatment outcomes.

ABCB1 C3435T polymorphism has an allele frequency of 10-27% in African populations (Lam and Cavallari, 2013) and thus would affect the pharmacokinetics of lopinavir in Kenyan populations. In addition, there is no agreement among various studies done worldwide on the effect of ABCB1 polymorphisms on immunological response and virological response in HIV patients (Bakshi et al., 2008; Coelho et al., 2013; Winzer et al., 2005; Zhu et al., 2013).

1.3 Study justification

A study in the Henan cohort in China reported that genotyping for ABCB1 genotypes could assist in predicting HIV treatment response; there was an association between 3435 C>T genotypes and CD4+ T cell count (Zhu et al., 2013). Another study in South India reported that

differences in the frequency of ABCB1 3435 C>T genotypes would have an impact on antiretroviral (ARV) response and progression of HIV-1 disease. However, another study in Germany reported no significant differences in immunological and virological response among ABCB1 C3435T genotypes. ABCB1 C3435T variant was likely to cause reduced risk of hepatotoxicity in patients on nevirapine (Aceti et al., 2015); however, another study reported increases in liver enzymes (Pavlos and Phillips, 2011). A study done in Africans concluded that 3435T alleles were associated with a higher effective renal plasma flow and glomerular filtration rate and lower renal resistance compared to the wildtype genotype (CC) (Bochud et al., 2008).

These inconsistent results in different studies indicate that the effect of ABCB1 C3435T genotypes is not well characterized. Among the studies done with regard to effect of ABCB1 polymorphisms, only a few have been done in African populations; none so far has been done to investigate effect of ABCB1 on African HIV patients on second-line ART. It is critical to identify the effects of ABCB1 polymorphisms in HIV patients especially among those taking second-line ART since ABCB1 polymorphisms could affect treatment outcomes.

1.4 Research question

Does the prevalence of ABCB1 C3435T genotypes in Kenyan HIV patients conform to Hardy-Weinberg proportions?

Is the ABCB1 C3435T SNP predictive of clinical outcomes in Kenyan HIV patients taking second line lopinavir-based ART?

What are the factors affecting CD4 cell counts in Kenyan patients on lopinavir-based regimens?

1.5 Objectives

1.5.1 Main objective

The main objective was to investigate the effects of ABCB1 C3435T genotypes on clinical outcomes in HIV patients on lopinavir-based second line ART regimens at the Comprehensive Care Centre of Kenyatta National Hospital.

1.5.2 Specific objectives

They were to:

i. Determine the prevalence of ABCB1 C3435T genotypes in Kenyan HIV patients on second line lopinavir-based ART regimen at the CCC of Kenyatta National Hospital.

ii. Determine the effects of ABCB1 C3435T genotypes on clinical outcomes (CD4 cell counts, ALT levels, creatinine and hemoglobin).

iii. Describe factors affecting CD4 cell counts from time of ART initiation in Kenyan HIV patients on second line lopinavir-based ART regimen at the CCC of Kenyatta National Hospital.

1.6 Significance of the study

The prevalence of ABCB1 C3435T genotypes in Kenya is presented in this study and thus provides critical information for other studies done in Kenya analyzing the effect of ABCB1 3435 C>T genotypes.

This study has reported the effect of ABCB1 C3435T genotypes on clinical outcomes in HIV patients on lopinavir-based regimens. This will enable identification of patients who would respond effectively to lopinavir through genotyping of ABCB1 C3435T polymorphism.

Chapter 2

LITERATURE REVIEW

2.1 Background of HIV

United Nations Acquired Immune Deficiency Syndrome (UNAIDS) report estimated that people living with HIV were more than 35 million people in 2013. In 2012, those that had died of Acquired Immune Deficiency Syndrome (AIDS)-related causes worldwide were 1.6 million. In middle and low-income countries, around 9.7 million HIV-positive patients had access to ART in 2012 (UNAIDS, 2013). In 2015, there were 36.7 million people who were HIV-positive and 17 million had access to ART (UNAIDS, 2015).

There were 24.7 million HIV-positive individuals in sub-Saharan Africa in 2013 and there were 1.2 million AIDS-related deaths in 2012 (UNAIDS, 2013). In Kenya, 1.6 million individuals were HIV-positive in 2013 while in 2015, there were 1.5 million. The HIV prevalence in Kenya among people aged 15-49 was 6% in 2013 and 5.9% in 2015. Prevalence was higher in women compared to men; in women the prevalence was 7.6% while in men it was 5.6% (NASCOP, 2014b).

The current Kenyan Guidelines on Management of HIV recommend that all individuals with confirmed HIV infection are eligible for ART provided that they are ready and willing to adhere to and take ART. CD4 cell count levels, age, WHO clinical stage, co-infection, pregnancy or breastfeeding status or any other criteria are not considered in initiating ART (NASCOP, 2016). This is contrary to the guidelines recommended in 2011 and 2014 which had some specific criteria (NASCOP 2011; 2014a). Initiation of ART early ensures that patients have better outcomes and this necessitates provision of ARVs to all individuals with HIV (NASCOP, 2016).

2.2 Longitudinal clinical data collected during ART

Patients on ART are routinely followed up. CD4 cell count determination is done during initiation and every six months especially where viral load testing is not available. Viral load determination is done after 6 and 12 months of initiating therapy and thereafter annually (NASCOP, 2014). Clinical evaluation, adherence checks and TB screening is done during every visit. Evaluation for haemoglobin levels is done during initiation of therapy and thereafter depending on clinical symptoms of the patient and regularly for patients on zidovudine. Liver

function tests, for example, ALT determination, are done on initiation and thereafter depending on clinical symptoms of the patient and regularly for patients on nevirapine (NASCOP, 2014). Fasting lipid profile and glucose testing is done for patients taking protease inhibitors annually. Creatinine levels are evaluated during initiation and thereafter depending on clinical symptoms of the patient (NASCOP, 2014).

2.3 Failure to Antiretroviral Therapy

According to the Kenya ART Guidelines 2016, patients to be changed to second line regimen were those who experienced treatment failure. Treatment failure can either be clinical failure whereby there is a new onset of significant opportunistic infections, recurrence of opportunistic infections even after six months of treatment with HAART except TB or downgrading of WHO classification during follow up (NASCOP, 2014). It can also be immunological failure whereby from peak value of treatment the CD4 cell count falls by 30% or more, falls below pre-ART level or remains persistently below 100cell/mm^3. Alternatively, it can be virological failure whereby viral load is greater than 1000 copies/ml (NASCOP, 2014). In Haiti, patients failing first-line ART because of immunologic or clinical failure and were not switched were more likely to die than those switched after failure (Charles et al., 2012).

2.4 Second line ART regimens in Kenya

National AIDS and STI Control Program (NASCOP) formulate guidelines for application of ART in Kenya. These guidelines are normally adapted from WHO guidelines and they guide changing from first-line ART regimen to second-line. Recommended second-line ART regimens for adults in 2011 and 2014 were as shown in Table 2.1.

Table 2.1: Second line ART regimens for adults as recommended in 2011 and 2014 (NASCOP, 2011; 2014a).

First line Regimen	Second-line Regimen according to 2011 ART guidelines	Second-line Regimen according to 2014 ART guidelines
TDF + 3TC + EFV or NVP	AZT + 3TC + LPV/r or ATV/r	AZT + 3TC + ATV/r or LPV/r
AZT + 3TC + EFV or NVP	TDF + 3TC + LPV/r or ATV/r	TDF + 3TC + ATV/r or LPV/r
D4T + 3TC + EFV or NVP	TDF + 3TC + LPV/r or ATV/r	TDF + 3TC + ATV/r or LPV/r
TDF + 3TC + ATV/r or LPV/r (according to 2014 guidelines)		AZT + 3TC + DRV/r

Abbreviations: TDF, Tenofovir; LPV/r, Lopinavir/ritonavir; D4T, Stavudine; AZT, Zidovudine; EFV, Efavirenz; NVP, Nevirapine; DRV/r, Darunavir/ritonavir; 3TC, Lamivudine; ATV/r, Atazanavir/ritonavir.

Adult patients who were less likely to develop treatment failure in South Africa were those on second-line regimen containing Tenofovir (Wandeler et al., 2012). A study done in Western Kenya also found out that use of stavudine, age and baseline CD4 cell count were important predictors of change to second-line therapy (Inzaule et al., 2014). In cases of second-line ART regimen failure, the options for third-line are limited but include integrase inhibitors like raltegravir, new-generation NNRTIs like etravirine, protease inhibitors like darunavir and recycling of drugs that confer benefit like lamivudine and tenofovir (NASCOP, 2011). Initiation of third line regimen is determined by resistance patterns of ARVs (NASCOP, 2014a).

Lopinavir boosted with ritonavir is a critical component of second-line ART regimens. Almost all adult Kenyan HIV patients are given lopinavir/ritonavir together with two NRTIs as second-line ART regimen; the remaining few are given atazanavir/ritonavir instead of lopinavir/ritonavir.

2.5 Chemistry of lopinavir

The chemical structures of lopinavir and ritonavir are presented below.

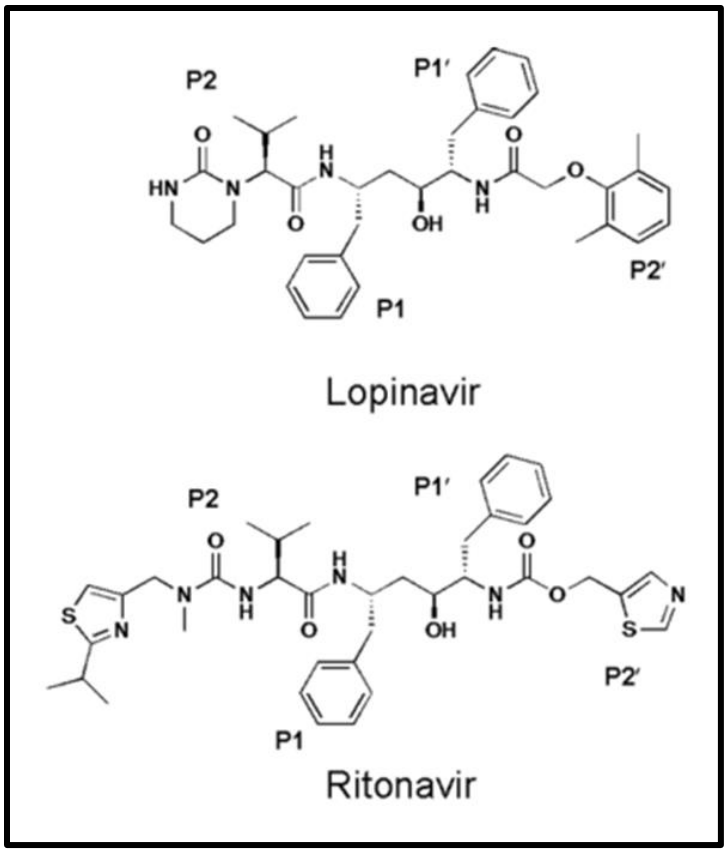

Figure 2.1: Chemical structures of lopinavir and ritonavir (Yedidi et al., 2014)**.**

P1 and P1' para-fluoro phenyl groups showed improved pharmacological properties of lopinavir and enhanced binding when structrure based virtual screening was done (Yedidi et al., 2014).

2.6 Pharmacodynamics of lopinavir

Lopinavir is a protease inhibitor used as an antiretroviral drug for both HIV-2 and HIV-1. It binds specifically to the protease enzyme (Hurst and Faulds, 2000). It inhibits the protease enzyme and thus prevents cleavage of the gag–pol and gag polyproteins and thus inhibits assembly and maturation of HIV polyproteins (Hughes et al., 2011). Common adverse effects of lopinavir/ritonavir include hypercholesterolemia, insulin resistance, lipodystrophy, hypertriglyceridemia and hyperglycemia (Pau and George, 2014).

2.7 Pharmacokinetics of lopinavir

Lopinavir has limited oral bioavailability which is improved markedly when lopinavir is administered with ritonavir. Bioavailability can also be improved with ingestion of foods

containing moderate to high fat. It is highly bound to proteins found in plasma especially alpha-1-acid glycoprotein and albumin. Distribution of lopinavir is determined by organic anion transporting polypeptides (OATP) (Schipani et al., 2012) and ABCB1 which promotes accumulation in sanctuary locations and decreases sequestration in tissues and target organs (Van Waterschoot et al., 2010). Experiments done in rats reported that orally administered lopinavir/ritonavir is widely distributed in the liver, adrenal glands and thyroid (EMEA, 2005).

Lopinavir undergoes significant oxidative metabolism mediated by CYP3A4 (Van Waterschoot et al., 2010). It is normally co-formulated with ritonavir which is a potent CYP3A4 inhibitor. Ritonavir strongly inhibits lopinavir metabolism especially by inhibiting CYP 3A4 and thus increases lopinavir concentration in the bloodstream. Lopinavir and ritonavir are inducers and substrates of ABCB1, CYP 2D6 and CYP 2C9 (Hughes et al., 2011).

2.8 Pharmacogenetics of lopinavir

There is great inter-individual differences in lopinavir pharmacokinetics among HIV-positive individuals (Kohlrausch et al., 2010). These differences in the pharmacokinetics could be due to the pharmacogenetics of protease inhibitors. Polymorphisms of membrane transporters like ABCB1, ABCC2 and SLCO1B1 and metabolizing enzymes like CYP3A affect pharmacokinetics of lopinavir.

ABCB1 polymorphism rs1045642 (3435C>T) is significantly associated with virological failure in patients on protease containing ART regimen (Coelho et al., 2013). In people with ABCBB1 3435CC genotype, access of lopinavir to major cellular sites expressing ABCB1 is impaired and this could affect its use (Aceti et al., 2015). Genetic polymorphism of SLCO1B1 521T>C (rs4149056) SNP leads to reduced activity and transport of lopinavir (Schipani et al., 2012). It also led to an increase in lopinavir plasma levels (Kohlrausch et al., 2010; Rakhmanina et al., 2011).

Genetic polymorphism CYP3A4*22 is associated with increased blood levels of lopinavir (Olagunju et al., 2014). The pregnane X receptor SNP 63396C→T affects CYP3A4 activity and pregnane X receptor expression. Higher lopinavir clearance is associated with homozygous PXR 63396 TT genotype, likely mediated through expression of ABCB1, SLCO1B1 and CYP3A4 which are important in lopinavir clearance (Pavlos and Phillips, 2011). A study among Caucasians identified two functional single nucleotide polymorphisms in SLCO1B1, a SNP in

CYP3A and one functional SNP in ABCC2 which affected lopinavir pharmacokinetics. The study concluded that genetic variations described 5% of lopinavir variability (Lubomirov et al., 2010).

2.9 Structure of ABCB1

ABCB1 belongs to the ABC transporter superfamily. ABC proteins contain a nucleotide-binding domain which possesses highly conserved motifs, for example, the Q and H loops, Walker A and B sequences and ABC signature motif. In addition, ABC transporters possess hydrophobic α – helices and trans- membrane domains (Vasiliou, 2009). The core unit of ABC transporter contains two transmembrane domains which are involved in translocation across the lipid membrane and substrate recognition and two nucleotide binding domains which attach to and break down ATP (Vasiliou, 2009). ABC transporters use energy generated by ATP hydrolysis to carry out transmembrane movement of substrates (Vasiliou et al., 2009).

The human genome has 49 ABC genes as shown below. ABCB1 genes are a subfamily of the ABC gene family.

Table 2.2 Human ABC gene subfamilies (Vasiliou and Nebert, 2009).

Subfamily name	Other names	Number of genes	Number of pseudogenes
ABCA	ABC1	12	5
ABCB	MDR*	11	4
ABCC	MRP	13	2
ABCD	ALD	4	4
ABCE	OABP	1	2
ABCF	GGN20	3	2
ABCG	White	5	2

**ABCB1 is also called Multi-Drug Resistant Protein and has 13 genes*

ABCB1 is also known as MDR1 and mediates the ATP-dependent removal of drugs from cells. ABCB1 is found in low quantities in several tissues, at intermediate quantities in the rectum, colon, lower jejunum, liver and lung; but at high quantities in the kidney, BBB and adrenal gland (Giacomini et al., 2012). ABCB1 has many drug binding sites which interact with many substrates; it can also interact stereo selectively with some of its substrates (Safa, 2004). The nuclear receptor constitutive androstane receptor regulates at the BBB the functional expression of ABCB1(Slosky et al., 2013). The structure and model of transport of ABCB1 is shown in Figure 2.2.

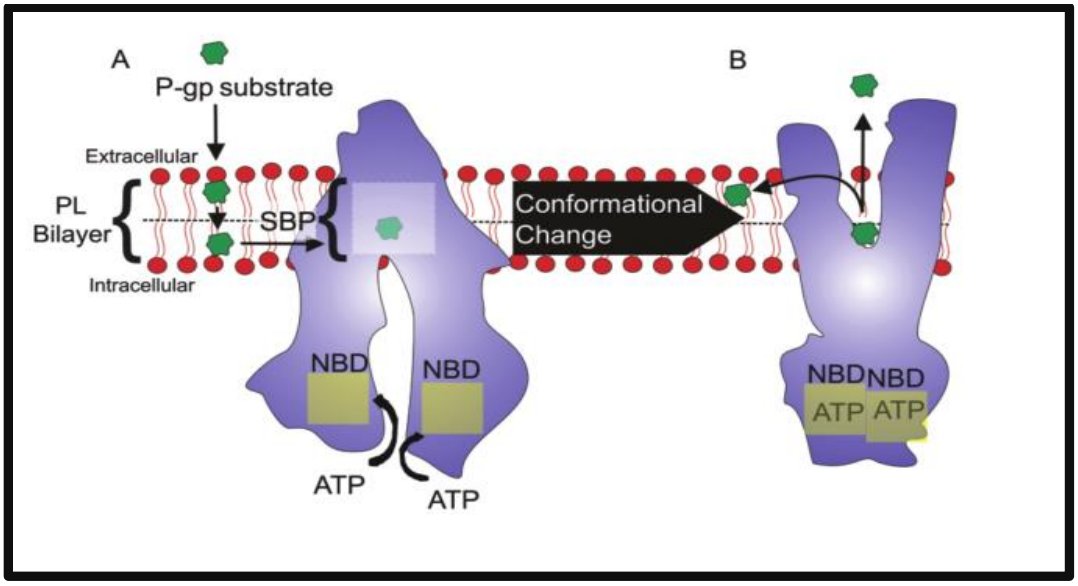

Figure 2.2: Model of ABCB1 substrate transport (O'Brien et al., 2012)

2.10 Effects of ABCB1 on drugs pharmacokinetics

ABCB1 binds many structurally unrelated compounds, however, most of its substrates are generally hydrophobic amphipathic compounds (O'Brien et al., 2012). ABCB1 transport protein confer drug resistance and affects pharmacokinetics and pharmacodynamics of protease inhibitors by decreasing bioavailability, reducing sequestration in tissues and target organs and increasing accumulation at sanctuary locations. ABCB1 interferes with intracellular concentrations of lopinavir and its oral bioavailability in the blood-testis barrier, brain and intestine (Brouwer et al., 2011). ABCB1 is important in reducing transport of atazanavir in the testes and brain (Robillard et al., 2014).

ABCB1 may also affect the disposition of other drugs like lamivudine (Zhu et al., 2013) and efavirenz (Ngaimisi et al., 2013). It also affects the entry of antiepileptic drugs through BBB (Ma et al., 2013). Other drugs that are affected by ABCB1 are shown in the Table 2.3. Some drugs normally inhibit ABCB1 and thus interfere with the pharmacokinetics of ABCB1 substrates. Examples of the inhibitors are shown in the Table 2.3.

Table 2.3 Substrates and inhibitors of ABCB1 (O'Brien et al., 2012).

Selected examples of ABCB1 substrates and inhibitors	
Drug class	**Examples of substrates**
Antiretroviral drugs	Lopinavir, ritonavir, saquinavir, indinavir, efavirenz, lamivudine
Antidiarrheal agents	Loperamide
H_2 receptor antagonists	Ranitidine, Cimetidine,
Anticancer agents	Doxorubicin, vincristine, vinblastine, paclitaxel, methotrexate, imatinib
Antidepressants	Nortriptyline, citalopram, fluvoxamine, fluoxetine, venlafaxine, Amitriptyline
Cardio active drugs	Verapamil, diltiazem, digoxin, Amiodarone,
B blockers	Talinolol
Antiemetics	Ondansetron, Domperidone,
Antihistamines	Desloratidine, fexofenadine, Cetirizine
Antipsychotics	Risperidone
ABCB1 Inhibitors	**Examples of inhibitors**
First generation	Cyclosporine, quinidine, nifedipine, verapamil, Amiodarone,
Second generation	Biricodar, valspodar, elacridar, Dexverapamil,
Third generation	Zosuquidar, tariquidar, laniquidar,

Protease inhibitors and NNRTIs are ligands for nuclear receptors like pregnane X receptor and constitutive androstane receptor which cause activation of transcriptional factor which results in induction of ABCB1. This may lead to decreased systemic exposure and therapeutic failure (Rathbun and Liedtke, 2011). A study done by Dumond indicated that prolonged exposure of tipranavir/ritonavir causes induction of intestinal ABCB1 (Dumond et al., 2010). Paracetamol activates the nuclear receptor constitutive androstane receptor at the BBB and increases ABCB1 functional expression (Slosky et al., 2013).

2.11 Genetic polymorphisms of ABCB1

The highly polymorphic *ABCB1 or MDR1* gene which has three insertion/deletion and more than 50 SNPs reported encodes for ABCB1. The *ABCB1* gene is localized to chromosome 7p21.1, contains 28 (or 29) exons, spans a region of ~200 kb and is highly polymorphic (Benish et al., 2010). The SNPs that occur commonly include 1236C>T (rs1128503), the 3435 C>T (rs1045642) and the 2677 G >A/T (rs2032583). The c.2677G>A/T (rs2032583) polymorphism is a non-synonymous mutation giving rise to a change in amino acid sequence p.A893T (2677 G >A) SNP or p.A893S (2677 G >T) SNP. Haplotypes containing of C3435T, 2677G>A/T and

C1236T have been reported and they are in strong linkage disequilibrium (Lam and Cavallari, 2013).

Studies conducted on effects of ABCB1 1236T>C polymorphism on drug response found inconsistent results; some reported that the 1236 CC genotype was associated with increased drug response while others 1236 TT genotype was associated with increased drug response (Hodges et al., 2011). Other studies found inconsistent results with regard to effect of 2677T>G/A variant (Hodges et al., 2011). ABCB1 3435 C>T SNP leads to decreased mRNA and protein levels and reduced function and expression of ABCB1 (Bakshi et al., 2008). The homozygous mutant genotype of ABCB1 3435TT shows low expression of ABCB1 indicating that 3435 C>T SNP leads to reduced activity of ABCB1 (Aceti et al., 2015).

2.12 The ethnic distribution of ABCB1 polymorphisms

The distribution of the various SNPs in different ethnic groups is shown below.

Table 2.4 Ethnic distribution of ABCB1 variants (Lam and Cavallari, 2013)

Genes	**Allele variants, Amino acid change**		**Frequency (%)**		
		Activity	Caucasians	Asians	Africans
ABCB1	3435C>T	Reduced	48-59	37-66	10-27
	1236C>T	Inconsistent	34-42	60-72	15-21
	2677G>T, A893S	Inconsistent	38-47	32-62	<15
	2677G>A, A893T	Inconsistent	1-10	3-22	-
	1236C>T/2677G>T/A/3435C>T haplotype		23-42	28-56	4.5-8.7

The frequency of 2677 GG genotype is 10–32% in Mexicans, Caucasians, Asians, Italians and American Indians but more than 81% in African populations (Hodges et al., 2011). There are different frequencies of variant alleles of ABCB1 in different African populations (Ikediobi et al., 2011), for example, the prevalence of ABCB1 c.4036A>G genotypes is significantly higher in Tanzanians compared to Ethiopians (Ngaimisi et al., 2013).

2.13 Clinical pharmacogenomics of ABCB1

A study in the Henan cohort in China reported that genotyping for ABCB1 genotypes may assist in predicting HIV treatment response (Zhu et al., 2013). 3435 C>T genotypes was significantly associated with CD4+ T cell count. Patients with CT or CC genotype tended to have significantly lower CD4 cell count than those with the TT genotype. However, viral load was not

significantly associated with the genotype (Zhu et al., 2013). In another study in South India, differences in the ABCB1 3435 C>T genotypes distribution was reported to impact on ARV response and progression of HIV-1 disease; higher plasma efavirenz concentrations was associated with the CC genotype (Bakshi et al., 2008). In north eastern Brazil, ABCB1 3435C>T (rs1045642) genotypes was significantly associated with virological failure in patients on protease inhibitor containing ART regimen was reported (Coelho et al., 2013). ABCB1 C3435T polymorphism associated with higher atazanavir plasma levels (Pavlos and Phillips, 2011). Lower viral loads and greater CD4 cell counts have been reported in several studies among HIV-positive individuals with 3435TT genotype of ABCB1 than they with CT or CC genotype probably due to lack of efflux of the antiretroviral drugs from sanctuary sites by ABCB1 (Aceti et al., 2015). However, another study in Germany reported that immunological and virological response were not affected by ABCB1 2677TT, 3435TT genotypes and 2677/3435 haplotype (Winzer et al., 2005).

ABCB1 3435 C>T variant was likely to cause reduced risk of hepatotoxicity in patients on nevirapine (Aceti et al., 2015); however, another study reported increases in liver enzymes among patients on nevirapine-based regimens (Pavlos and Phillips, 2011). A study done in Africans concluded that 3435T alleles resulted to a higher effective renal plasma flow and glomerular filtration rate and and lower renal resistance (Bochud et al., 2008).

A study among Ethiopian and Tanzanian HIV-positive individuals noted that ABCB1 A4036G polymorphism is a predictor of intracellular efavirenz concentration and leads to higher plasma efavirenz concentrations (Ngaimisi et al., 2013). The ABCB1 1236CT and 1236TT genotypes led to high efavirenz concentrations while ABCB1 4036AG and 4036GG genotypes led to low plasma efavirenz concentrations. A haplotype ABCB1T-G-T-A led to high plasma efavirenz concentrations (Swart et al., 2012). In Burundi, ABCB1 polymorphisms especially C3435T and C1236T genotypes were not significantly associated with nevirapine plasma concentrations (Calcagno et al., 2011). Association of ABCC2 and/or ABCB1 polymorphisms with concentrations of drug in blood is difficult to interpret and correlate effects of ABCB1 3435 genotypes with regard to drug activity in the body. However, immunosupressants and ARVs intralymphocyte concentrations demonstrated a direct influence by ABCB1 polymorphisms on intracellular or target tissue drug concentrations (Haufroid, 2011).

ABCB1 2677G > A/T was significantly associated with obesity in a Japanese population. Individuals with the TT genotype had a greater body mass index than those with the GG genotype (Ichihara et al., 2008).

2.14 Other genetic factors associated with immunological response

Other host genetic factors can also affect immunological response. A study in Argentina concluded that absence of CCR5-HHA and HLA-A*01/*23 and presence of CCR5-CF2, HLA-B*53, HLA-A*24/*33 resulted to lower baseline CD4 cell count. In addition, absence of HLA-B*07/*39 and HLA-A*01 and presence of HLA-B*53, HLA-A*24 led to lower 6-month CD4 T-cell count. Presence of HLA-C*08, CCR5-CF2, HLA-B*14, HLA-A*33 and lack of HLA-C*07, HLA-B*07 led to lower 12-month CD4 T-cell count (Coloccini et al., 2014).

2.15 Non-genetic factors associated with immunological response

Adherence to ART is critical in determining the immunological response. Patients who adhere to ART tend to have higher CD4 cell count than those who do not (Abrogoua et al., 2012). Psychosocial factors like depression and singlehood can affect adherence and hence affect CD4 cell count and eventually treatment response (Langford et al., 2007). In a study done in Ethiopia, lack of co-trimoxazole prophylaxis, low body weight, anemia and WHO stage III or IV were associated with death in HIV patients. In the same study, sex was not a predictor of mortality (Alemu and Sebastián, 2010). In a study in South Africa, factors that significantly affected immunological response were CD4 cell count at start of antiretroviral therapy and older age while body mass index, baseline haemoglobin, sex, concurrent TB co-infection and ART regimen did not affect immunological response significantly (Julg et al., 2012).

Nutritional supplements taken with ART improve immune response in HIV –positive patients (Evans et al., 2013). Alcohol abuse leads to lower CD4 cell counts (Iralu et al., 2010). Use of ART, changing doctors, hospital treatment, use of illicit drugs, smoking and patient's age affects the CD4 cell count (Montarroyos et al., 2014). Low CD4 cell counts were correlated to high incidence of anaemia, lymphopenia and thrombocytopenia (Parinitha and Kulkarni, 2012). A study in Ethiopia concluded that higher educational status, old age and low baseline CD4 cell count led to reduced CD4 cell count leading to immunological treatment failure (Teshome and Assefa, 2014). Another study in Ethiopia indicated that WHO Stage IV/III or higher baseline CD4 cell count are risks for immunological failure (Yirdaw and Hattingh, 2015).

Chapter 3

METHODS

3.1 Study design

This was a retrospective cohort study and entailed collection of participants' data from the time the ART was initiated up to when blood samples were obtained for genotyping.

3.2 Study site

It was done at the CCC of Kenyatta National Hospital, the biggest hospital in East and Central Africa, with a diverse inter-ethnic population of patients. It has an accident and emergency department, 24 theaters (16 specialized), 22 out-patient clinics and 50 wards. It has a bed capacity of 1800. The CCC is an outpatient clinic within the hospital that offers specialized care and treatment for HIV infected patients in addition to being a research centre. Patients are reviewed every 3-6 months; during which blood samples are drawn for estimating viral load and CD4 cell counts. The clinic has approximately 10,000 active patients.

3.3 Study population

It was made up of HIV patients on any lopinavir-based second-line ART regimen seen at the KNH CCC between January 2016 and April 2016.

3.4 Inclusion and Exclusion criteria

3.4.1 Inclusion criteria

Patients included were HIV infected patients on any lopinavir-based second-line ART, at least 6 months of second-line ART, of either sex, gave informed consent and were above 18 years of age.

3.4.2 Exclusion criteria

Participants who were not included were those who declined to give consent, were on second-line ART for not more than 6 months and aged below 18 years.

3.5 Sample size calculation

The expected main outcome of interest is change of CD4 levels. Consequently the Twisk (2003) formula for estimation of sample size of a continuous outcome variable in a cohort study was used.

$$N = \frac{\left(Z_{(1-\alpha/2)} + Z_{(1-\beta)}\right)^2 \sigma^2 (r+1)[1+(T-1)\rho]}{v^2 r T}$$

Where, N is the sample size,

$Z\ (1-\beta)$ is the $(1-\beta)$ percentile point of the standard normal distribution,

$Z\ (1-\alpha/2)$ is the $(1-\alpha/2)$ percentile point of the standard normal distribution,

v is the difference in mean value of the outcome variable between the groups.

ρ is the association coefficient of the repeated measurements

T is the number of follow-up measurements

r is the ratio of the number of subjects in the compared groups, and

σ is the standard deviation of the outcome variable,

Therefore, the sample size needed to make a 0.3 difference in a continuous outcome variable with a power of 80% and statistically significant on a 5% level with different within-subject association coefficients (ρ) of 0.5 and four repeated measurements was 59. To accommodate for expected missing files or incomplete data entries of about 20%, the calculated sample size was inflated by 20%. Therefore, a minimum sample size of 71 participants was targeted.

3.6 Sampling and participant recruitment

Patients meeting the inclusion criteria were conveniently sampled as they refilled their prescriptions at the CCC pharmacy. Two trained pharmaceutical technologists offered study information to prospective participants and obtained written informed consent from willing patients. This was done in a side room to maintain privacy and confidentiality.

3.7 Data collection

Data collection was divided into three sections: data abstraction from patient files, blood sample collection, processing and genotyping.

3.7.1 Retrospective review of files

Participant medical records were retrieved. Data was abstracted from the files using a data collection tool. Demographic data collected included marital status, body mass index, sex, age, occupation, education, ethnicity, alcohol use and smoking. Clinical data collected included second-line ART regimen and duration, first-line ART regimen and duration, WHO staging, CD4 cell count at different time points, viral load, adherence, haemoglobin, ALT (Alanine transaminase) and creatinine levels. This data was collected at ART initiation, ART switch, 6 months after ART switch and at recruitment when collecting blood samples.

3.7.2 Blood sample processing

Blood samples were obtained from participants satisfying the inclusion criteria. Blood collection was done in the bleeding room of the CCC laboratory by a phlebotomist. From each patient 5ml of blood was obtained from the antecubital vein and was immediately transferred to an EDTA containing tube. The blood was centrifuged; 3ml plasma was separated and stored at -20^0C and approximately 2ml of whole blood was used for genotyping.

3.7.3 Genotyping

3.7.3.1 DNA Extraction

This was done at the African Institute of Biomedical Science and Technology (AiBST) laboratory, University of Nairobi situated at the Department of Pharmacognosy and Pharmacology, School of Pharmacy.

Equipment

The equipment used included a heat block, sterile micro centrifuge tubes, pipettes, spin columns and collection tubes (supplied with the kit), a vortexing machine (Thermal Electron Corporation, Denley VibroMix), a centrifuge (Biofuge Pico, Heraeus Instruments) and a water bath.

Materials and reagents

The PureLink® Genomic DNA Kit (K182002) was used for DNA extraction in accordance to manufacturer's instructions and it was composed of Proteinase K (20 mg/mL in storage buffer), RNAse A (20 mg/mL in 10 mM EDTA, pH 8.0, 50 mM Tris-HCL) and buffers. The buffers included PureLink® Genomic Wash buffer 1 and 2 which were dissolved in ethanol; PureLink® Genomic Lysis/ Binding buffer and PureLink® Genomic Elution buffer (0.1 mM EDTA, pH 9.0, 10 mM Tris-HCL).

DNA extraction was done from thawed whole blood using PureLink® Genomic DNA Kit described above as per the manufacturer's protocol (Life Technologies Carlsbad CA). About 200µL of the thawed whole blood was pipetted into sterile micro centrifuge tubes. Twenty microlitre of proteinase K was added to the 200µl of whole blood. This lysed the proteins. This was followed by hydrolysis of RNA by the addition of 20µl of RNAse A. The mixture was incubated and vortexed at 25^0C for 120 seconds to ensure that the contents were well mixed. Approximately 200µL of PureLink® Genomic Lysis buffer was mixed with the contents and for ten minutes, the lysate was incubated at 55^oC. To the lysate, 200µL of absolute ethanol was added and vortexed briefly to obtain a homogenous mixture.

Six hundred and fifty microlitre of the lysate was put in a sterile PureLink® Spin Columns in collection tubes provided with the kit. The contents were centrifuged at 25^0C for 1 minute at 10,000 x g. The collection tube containing the filtrate was discarded and the spin column was placed into a sterile PureLink® Collection tube.

Five hundred microlitre of wash buffer 1 was put into the spin columns which were centrifuged at 25^0C for 60 seconds at 10,000 x g. Resultant filtrate was discarded while the spin columns were put into sterile collection tubes. Five hundred microliter of wash buffer 2 was put into the spin columns and the columns centrifuged at 25^0C for 180 seconds at maximum speed and the collection tubes discarded.

Fifty microlitre of PureLink® Genomic Elution Buffer (0.1 mM EDTA, pH 9.0, 10 mM Tris-HCL) was added to the spin columns which were already put into 1.5-mL micro centrifuge tubes. The contents were subsequently incubated at 25^0C for approximately 60 seconds and the columns centrifuged at 25^0C for 60 seconds at maximum speed (13,000 x g) to complete the elution of the DNA. The eluted purified genomic DNA was contained in the 1.5-mL micro centrifuge tube. The spin column was discarded. The DNA was stored in a freezer at -20^{o}C for further processing.

3.7.3.2 DNA amplification

Genotyping was done at the Walter Reed Project situated at Kenya Medical Research Institute (KEMRI), Centre for Public Health and Research and was achieved on 7500 Fast Real Time PCR machine (Applied Biosystems, Foster City, California).

The reaction mix was composed of 20X TaqMan Drug Metabolism Genotyping Assay, nuclease-free water and TaqMan Genotyping Master Mix and. The 2X TaqMan Genotyping Master Mix was swirled gently to mix the contents. The Drug Metabolism Genotyping Assay was vortexed and centrifuged so that it could mix properly. A total volume of 105µl of 20X TaqMan Drug Metabolism Genotyping Assay, 1.05ml of 2X TaqMan Genotyping Mix and 777µl of nuclease-free water were pipetted into sterile tubes and capped then vortexed briefly to mix the components. The air bubbles were eliminated from the solution by centrifuging and spinning down the contents. The reaction mix for each well was prepared as shown in Table 3.1. This was sealed with transparent adhesive tape and gently tilted and vortexed to ensure uniform mixing of the reaction solution.

Table 3.1 Preparation of the reaction mix

Components	**96- well plate (25 µL reaction)**
2X TaqMan® Genotyping Master Mix	12.50 Ml
20X TaqMan® Drug Metabolism Genotyping Assay	1.25 µL
Nuclease free water	9.25 µL
Genomic DNA	2 µL
Total volume per well	25 µL

The reaction plate was then introduced into the reaction chamber of the 7500 fast real time PCR machine and software for the sequence detection was activated. Allelic discrimination pre-read test in a reaction volume of 25µl was carried out at 60^{o}C for 1 minute.

DNA amplification was achieved under the following conditions: an initial hold cycle for 10 minutes at 95^{o}C, denaturing for 50 cycles for 15 seconds at 92^{o}C and then annealing and extending at 60^{o}C for 180 seconds.

After real time PCR amplification was over, allelic discrimination post-read using sequence detection software was performed at 60^{o}C for 1 minute to characterize the distribution of ABCB1 C3435T alleles in the study population.

3.8 Variables and definitions

The main outcome variable in this study was clinical outcomes which included CD4 cell count (to represent immunological response), ALT levels (to represent liver function), creatinine levels (to represent renal function) and haemoglobin (to represent haematological profile). The main predictor variable of interest was the influence of ABCB1 C3435T genotypes. Other co-variates that may have acted as confounders included phenotype and other participant characteristics like sex, ethnicity, age, education level, ART regimens, body mass index and duration of therapy.

3.9 Data management

Data was entered in an Excel spreadsheet and the data transferred to a STATA database version 10. The data was double checked by the investigator during data entry. This was also done for

data generated during genotyping. Any document linking the collected data to the patient files including the raw data was kept under lock and key and only accessible by the researcher or on request by regulatory teams like the Ethics and Research Committee (ERC) and the quality control team for audit purposes. The final report was subjected to inspection and quality audit as per the investigators set standards and available protocols.

3.10 Data analysis

All variables were subjected to descriptive data analysis. Normal distribution of continuous variables was assessed by use of shapiro-wilk test. Standard deviation and mean and were used to give a summary of variables that were normally distributed and those that were not normally distributed were expressed as the median and inter- quartile range.

Exploratory data analysis was then conducted; this was done to identify key association and associations between variables. In order to identify factors associated with baseline CD4 count, CD4 count at ART switch and current CD4 count, multi-linear regression was done. Generalized linear modelling with adjustment for clustering within the patients was done to identify the factors associated with rate of CD4 cell count change. Manual forward stepwise model building was then done in all regression analyses. Linear regression was also done to assess the effect of ABCB1 C3435T genotypes on various clinical characteristics like CD4 cell count, ALT levels, creatinine levels, haemoglobin and body mass index (BMI). STATA version 10 software was used to do data analysis. The significance level was set at 0.05. Hardy-Weinberg Equilibrium was done to test if ABCB1 3435 CC, CT and TT genotypes conformed to the Hardy-Weinberg proportions.

3.11 Ethical considerations

Permission to do the study was given by Kenyatta National Hospital/University of Nairobi Research and Ethics committee (Ref: KNH-ERC/A/499). The letter that granted ethical approval is attached in Appendix C. The nature of the study was fully disclosed to the participants. Patients signed informed consent and data collected was handled with confidentiality.

3.12 Dissemination of research findings

A soft copy of the thesis write-up will be deposited in the electronic repository of University of Nairobi. In addition, two articles have been published in peer-reviewed journals.

Chapter 4

RESULTS

4.1 Baseline clinical and socio-demographic characteristics

All the study participants were on a lopinavir-based regimen. The study involved 84 study participants, 52 (61.9%) were on TDF + 3TC + Lopinavir/ritonavir (LPV/r) while 25 (29.8%) were on AZT + 3TC + LPV/r and 7 (8.3%) were on Abacavir (ABC) + 3TC + LPV/r. They were on second-line antiretroviral therapy since they had experienced either immunological failure or virological failure on first line therapy.

There were 50 (59.5) female study participants and 34 (40.5) male. There were 53 (63.1%) participants from the Bantu ethno-linguistic group, 30 (35.7%) from the Nilotes and finally Cushites with 1(1.2%) participant. Median baseline body weight was 61 [interquartile range (IQR) 54.2 – 71.2]. There were 5 (6.0%) study participants with a baseline body mass index (BMI) of less than 18.5 while 34 (40.5%) had normal BMI. Median age was 36 years [IQR 32 – 44]. Sixteen (19%) study participants were single while the married study participants were 45 (53.6%). Only 16 (24.2%) study participants had tertiary education and above.

The median duration of therapy during first line ART treatment was 2 years [IQR 0 – 5] while that during second line was 4 years [IQR 2 – 6]. The median creatinine levels at HAART initiation was 79. 5 [IQR 64.5 – 93] while the median ALT levels was 23 [IQR 16 – 44]. The first line regimens that the study participants had previously used included 20 (23.8%) on Zidovudine (AZT) + Lamivudine (3TC) + Efavirenz (EFV), 19 (22.6%) on Tenofovir (TDF) + 3TC + Nevirapine (NVP), 15(17.9%) on Stavudine (d4T) + 3TC + NVP, 11 (13.1%) on AZT + 3TC + NVP. The study participants who had been on nevirapine-based regimens previously were 45 while those who had been on efavirenz-based regimens were 39. A summary of the baseline characteristics is presented in Table 4.1.

Table 4.1 Baseline socio-demographic and clinical characteristics

Variables	n (%) or Median [IQR]
Sex	
Male	34 (40.5)
Female	50 (59.5)
Age At diagnosis in years	36 [32 – 44]
Weight at diagnosis (kg)	61 [54.2 – 71.2]
Height at diagnosis (cm)	165 [158 – 171]
BMI at HAART initiation	
< 18.5	5 (6.0)
18.5 - 24.9	34 (40.5)
> 24.9	18 (21.4)
Missing values	27 (32.1)
Ethnolinguistic Groups	
Bantu	53 (63.1)
Nilotes	30 (35.7)
Cushites	1 (1.2)
Education level	
Primary level and below	13 (19.7)
Secondary level	37 (56.1)
Above tertiary level	16 (24.2)
Regimens at initiation of HAART	
TDF + 3TC + NVP	19 (22.6)
TDF + 3TC + EFV	9 (10.7)
d4T + 3TC + EFV	8 (9.5)
d4T + 3TC + NVP	15 (17.9)
AZT + 3TC + EFV	20 (23.8)
AZT + 3TC + NVP	11 (13.1)
d4T + DDI + EFV	2 (2.4)
First-line Duration of therapy (yrs)	2 [0 – 5]
Second-line Duration of therapy (yrs)	4 [2 – 6]
ALT at HAART initiation	23 [16 – 44]
Normal (≤ 40U/L)	22 (26.2)
Elevated (> 40U/L)	11 (13.1)
Missing values	51 (60.7)
Creatinine Levels	79. 5 [64.5 – 93]
Normal (≤ 120µmol/l)	32 (38.1)
Elevated (>120µmol/l)	4 (4.8)
Missing values	48 (57.1)
CD4 Cell Counts at diagnosis (cells/mm^3)	88 [19 – 284]
≤ 200	49 (58.3)
> 200	19 (22.6)
Missing values	16 (19.1)

4.2 ABCB1 C3435T alleles and genotypes

4.2.1 Prevalence of ABCB1 C3435T alleles and genotypes

The frequency of the C allele of the ABCB1 C3435T alleles was 147 (87.5%) while that of T allele was 21 (12.5%) as shown in Table 4.2. The prevalence of the ABCB1 3435CC homozygous wild-type genotype was 64 (76.2%) while that of the heterozygous CT genotype was 19 (22.6%), and the TT homozygous variant genotype was 1 (1.2%). Comparison between observed and expected genotype frequencies was in conformity with Hardy-Weinberg equilibrium indicating that they conformed to the Hardy-Weinberg proportions (p=0.755).

Table 4.2: C3435T allele and genotype frequencies in the study population

ABCB1 C3435T	n	%	p-value*
Allele			
C	147	87.5	
T	21	12.5	
Total	168	100	
Genotype			
CC	64	76.2	
CT	19	22.6	0.755
TT	1	1.2	
Total	84	100	

*Test for Hardy-Weinberg equilibrium of the genotype

4.2.2 ABCB1 C3435T genotypes and allele frequencies in the ethno-linguistic groups

The distribution of ABCB1 C3435T genotype was compared across the Bantus, Nilotes and Cushites which are the major Kenyan ethno-linguistic groups. Bantu participants with the ABCB1 3435CC genotype were 39 (75%) while those with the CT genotype were 12 (23.1%) and only 1 Bantu had the rare TT genotype. The Nilotes with the CC genotype were 24 (77.4%) and 7 (22.6%) had the CT genotype. One Cushite (100%) had the CC genotype. The prevalence of ABCB1 C3435T genotypes among Bantus, Nilotes and Cushites conformed to the Hardy-Weinberg equilibrium The difference in the distribution of the genotypes among Bantus, Nilotes and Cushites was not statistically significant as presented in Table 4.3.

The frequency of ABCB1 3435C allele was 90 (86.5%) among Bantus while that of the T allele was 14 (13.5%). Distribution of the C allele was 55 (88.7%) among Nilotes while that of the T allele was 7 (11.3%). Distribution of the C allele was 2 (100%) among Cushites. The difference

in the distribution of the T and C alleles among Bantus, Nilotes and Cushites was not statistically significant.

Table 4.3 Prevalence of ABCB1 C3435T genotypes and allele frequencies in the ethno-linguistic groups in the study population

	Ethnicity, n (%)				
	Bantus	**Nilotes**	**Cushites**	**Total**	**p-value**
C3435T Alleles					
C	90 (86.5)	55 (88.7)	2 (100.0)	147	
T	14 (13.5)	7 (11.3)	0 (0.0)	21	
N	104	62	2	168	
C3435T Genotype					
CC	39 (75)	24 (77.4)	1 (100)	64	0.919
CT	12 (23.1)	7 (22.6)	0 (0.0)	19	
TT	1 (1.9)	0 (0.0)	0 (0.0)	1	
N	52	31	1	84	

4.2.3 Prevalence of ABCB1 C3435T genotypes and alleles in males and females

There were more females than males with the wild-type CC genotype. The males who expressed the CC genotype were 25 (73.5%) while those with the CT genotype were 9 (26.5%). The females with the CC genotype were 39 (78%) and those with the CT genotype were 10 (20%). For the homozygous mutant allele, only one female expressed it. The distribution of the ABCB1 C3435T genotypes in the two genders conforms to the Hardy-Weinberg equilibrium. Distribution of ABCB1 C3435T genotypes by sex was not statistically significant (p=0.575).

Table 4.4 Prevalence of ABCB1 C3435T genotypes by sex in the study population

C3435T Genotype	**Sex, n (%) Male**	**Female**	**Total**	**p-value**
CC	25 (73.5)	39(78)	64	
CT	9 (26.5)	10 (20)	19	0.575
TT	0	1 (2)	1	
N	34	50	84	

4.3 Effect of ABCB1 C3435T genotypes on clinical outcomes

4.3.1 Effect of the polymorphism on CD4 cell count

The median baseline CD4 cell count of the participants with ABCB1 CC genotype was 86 [19 – 276] while that of those with ABCB1 CT genotype was 150.5 [43 – 361] as presented in Table 4.5. Only one participant had ABCB1 TT genotype and the participant had the lowest baseline median CD4 cell count of 18. Therefore, the patients with the CT genotype had the highest baseline CD4 cell counts. On univariate regression analysis, participants with the CT genotype had higher log baseline CD4 cell counts by 0.218 units [crude β=0.218(-0.658, 1.096)] compared to those with the CC genotype. However, there was no statistical significance (p=0.620).

Table 4.5 Effect of ABCB1 C3435T genotypes on CD4 cell count

ABCB1 C3435T Genotypes	Baseline CD4 cell counts, cells/mm^3 median [IQR]	CD4 cell counts at ART switch, cells/mm^3 median [IQR]	CD4 cell counts 6 months after ART switch, cells/mm^3 median [IQR]	CD4 cell counts at recruitment, cells/mm^3 median [IQR]
CC genotype	86[19 – 276]	275[85.5-435]	303[125-464]	414[244-617]
CT genotype	150.5[43-361]	173[89-264]	244[204-806]	454.5[304.5-749.5]
TT genotype	18[18-18]	624[624-624]	346[346-346]	346[346-346]
p-value	0.620	0.789	0.230	0.636

At ART switch, the CD4 cell count of the study participant with the TT genotype was 624. Study participants with the CC genotype had higher CD4 cell counts with a median of 275[85.5-435] compared to those with the CT genotype who had a median of 173[89-264] as illustrated in Table 4.5. On univariate regression analysis, participants with the CT genotype had lower square root of CD4 cell counts by 0.629 units [crude β=-0.629(-5.341, 4.083)]. However, this did not have statistical significance (p=0.789).

After 6 months of antiretroviral therapy with lopinavir-based regimens, the median CD4 cell count of the study participants with the CC genotype was 303[125-464] and this was higher than those with the CT genotype who had a median of 244[204-806] as illustrated in Table 4.5. Study participants with the CT genotype had mean change of CD4 count of 71 after 6 months on lopinavir-based regimens compared to those with the CC genotype who had a mean change of

CD4 count of 28. On univariate regression analysis, participants with the CT genotype had higher square root of CD4 cell counts by 2.572 units [crude β=2.572(-1.681, 6.826)]. However, this did not have statistical significance (p=0.230).

The study participants with the CT genotype have higher CD4 cell counts at recruitment with a median of 454.5[304.5-749.5] compared to those with the CC genotype who have a median of 414[244-617]. The participant with the TT genotype had a lower CD4 cell counts at recruitmentthan that at ART switch as shown in Table 4.5. On univariate regression analysis, participants with the CT genotype had higher square root of CD4 cell counts by 0.802 units [crude β=0.802(-2.566, 4.169)]. However, this did not have statistical significance (p=0.636).

The effect of education was controlled by generating two different plots of the rate of CD4 cell counts change vs C3435T genotype; one for participants with tertiary education and the other for those without tertiary education. The study participants with the heterozygous CT genotype and no tertiary education had consistently higher log CD4 cell counts than those with the CC genotype and no tertiary education. However, the rate of CD4 cell count change appeared to be similar as illustrated in Figure 4.4. The study participants with the heterozygous CT genotype and tertiary education and above had consistently higher log CD4 cell counts than those with the CC genotype and tertiary education and above. However, the rate of CD4 cell count change was similar between the two groups as illustrated in Figure 4.1. The slopes were parallel and thus indicate that the rate of change was constant regardless of the genotype.

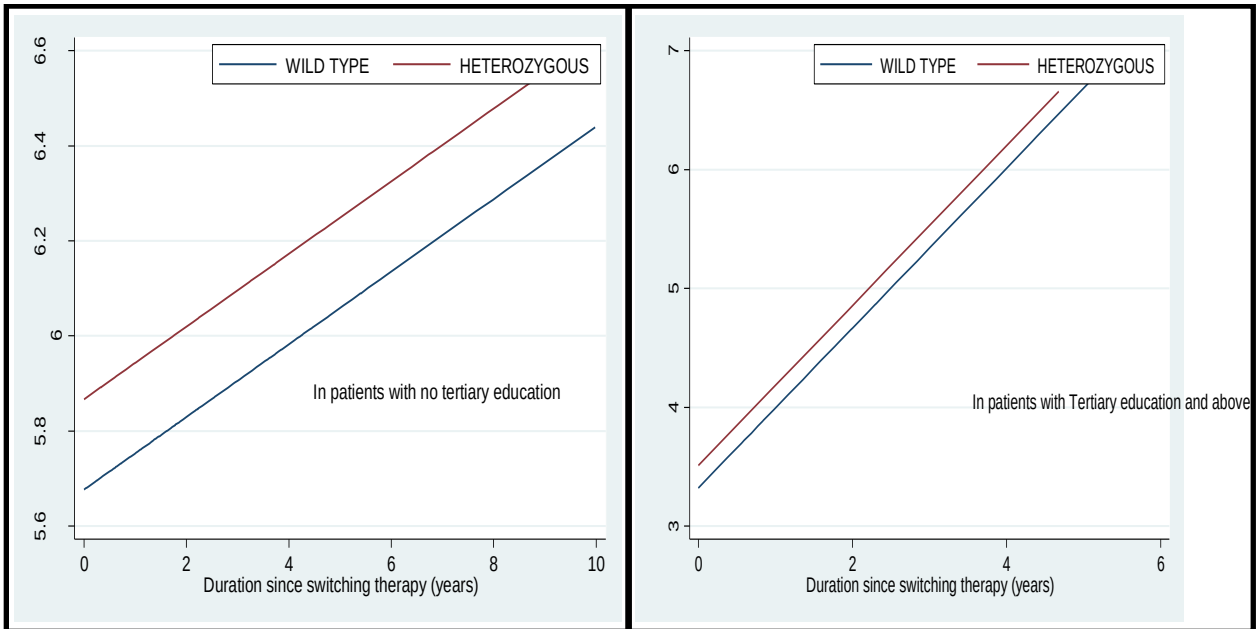

Figure 4.1 Effect of ABCB1 C3435T genotypes on rate of change of CD4 cell count

4.3.2 Effect on ALT levels

The study participants with the CC genotype had a higher median baseline ALT level of 23[17-35.5] compared to those with the CT genotype who had a median of 22.7[16-37]. The study participant with the TT genotype had a baseline ALT level of 14. On univariate regression analysis, the effect of ABCB1 C3435T genotypes did not have statistical significance (p=0.515)

At ART switch, the median ALT level of the study participants with the CC genotype was 24[22-43] which was higher than those with the CT genotype who had a median ALT level of 20.5[13.5-27]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on log ALT levels was not statistically significant (p=0.081). When adjusted for baseline ALT levels, the effect of ABCB1 C3435T genotypes on log ALT levels was not statistically significant (p=0.122).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had lower median ALT levels of 16.7[13-21] compared to those who had the CT genotype with a median ALT level of 25.5[17-39]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on log ALT levels was not statistically significant (p=0.257). Compared to levels at ART switch, the study participants with the CC genotype had a decrease in median ALT levels while those with CT genotype had an increase in median ALT levels as shown in the table below.

Table 4.6 Effect of ABCB1 C3435T genotypes on ALT levels

ABCB1 C3435T Genotypes	**Baseline ALT levels, Units/L median [IQR]**	**ALT levels at ART switch, Units/L median [IQR]**	**ALT levels 6 months after ART switch, Units/L median [IQR]**
CC genotype	23[17-35.5]	24[22-43]	16.7[13-21]
CT genotype	22.7[16-37]	20.5[13.5-27]	25.5[17-39]
TT genotype	14[14-14]	-	-
p-value	0.515	0.122*	0.257

*when adjusted for confounding by baseline ALT levels

4.3.3 Effect on Creatinine levels

The study participants with the CC genotype had a lower median baseline creatinine level of 81[72-101] compared to those with the CT genotype who had a median of 88[63-97.3]. The study participant with the TT genotype had a baseline creatinine level of 82. On univariate regression analysis, the effect of ABCB1 C3435T genotypes did not have statistical significance (p=0.200).

At ART switch, the study participants with the CC genotype still had a lower median creatinine level of 83.3[72.5-117] compared to those with the CT genotype who had a median creatinine level of 86[81.75-103.5] as presented in Table 4.7. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on creatinine levels was not statistically significant (p=0.479).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had higher median creatinine levels of 87.5[72-101] compared to those who had the CT genotype with a median creatinine level of 67.5[56.5-73.15]. Compared to levels at ART switch, the study participants with the CC genotype had an increase in median creatinine levels while those with CT genotype had a marked decrease in median creatinine levels. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on creatinine levels was statistically significant (p=0.001). When adjusted for baseline creatinine levels, the effect of ABCB1 C3435T genotypes was still statistically significant (p=0.009); the participants with the CT genotype had a decrease of creatinine levels by 25.093 units [crude β=-25.093(-42.985, -7.202)] compared to those with CC genotype.

Table 4.7 Effect of ABCB1 C3435T genotypes on Creatinine levels

ABCB1 C3435T Genotypes	**Baseline creatinine levels, µmol/l median [IQR]**	**Creatinine levels at ART switch, µmol/l median [IQR]**	**Creatinine levels 6 months after ART switch, µmol/l median [IQR]**
CC genotype	81[72-101]	83.3[72.5-117]	87.5[72-101
CT genotype	88[63-97.3]	86[81.75-103.5]	67.5[56.5-73.15]
TT genotype	82[82-82]	-	-
p-value	0.200	0.479	0.009*

*when adjusted for confounding by baseline creatinine levels and BMI

4.3.4 Effect on Hemoglobin levels

The study participants with the CC genotype had a slightly lower median baseline haemoglobin of 12.4[11-14.3] compared to those with the CT genotype who had a median of 12.7[11.7-14.3] as presented in Table 4.8. The study participant with the TT genotype had baseline haemoglobin of 13.5. On univariate regression analysis, the effect of ABCB1 C3435T genotypes did not have statistical significance (p=0.154).

At ART switch, the study participants with the CC genotype still had a slightly lower median haemoglobin of 12.2[10-14.2] compared to those with the CT genotype who had a median haemoglobin of 12.55[11.35-14.2]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on was not statistically significant (p=0.827).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had higher median haemoglobin of 12.85[11-14.5] compared to those who had the CT genotype with a median creatinine level of 12.5[10.8-15.3]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on was not statistically significant (p=0.878).

Table 4.8 Effect of ABCB1 C3435T genotypes on haemoglobin levels

ABCB1 C3435T Genotypes	**Baseline haemoglobin, g/dl median [IQR]**	**Haemoglobin at ART switch, g/dl median [IQR]**	**Haemoglobin 6 months after ART switch, g/dl median [IQR]**
CC genotype	12.4[11-14.3]	12.2[10-14.2]	12.85[11-14.5]
CT genotype	12.7[11.7-14.3	12.55[11.35-14.2]	12.5[10.8-15.3]
TT genotype	13.5[13.5-13.5]	-	
p-value	0.154	0.827	0.878

4.3.5 Effect on Body Mass Index

The median baseline body mass index (BMI)of the study participants with the CC genotype was 23.1[19.7-26.6] which was higher than those with the CT genotype who had a median of 20.7[18.6-23.3]. The study participant with the TT genotype had baseline BMI of 21.9. On univariate regression analysis, the effect of ABCB1 C3435T genotypes was almost statistically

significant (p=0.055). However, on multivariate analysis, when adjusted for age and gender, the effect of ABCB1 C3435T genotypes did not have statistical significance (p=0.081).

At ART switch, the median BMI of the study participants with the CC genotype was 24.4[21.1-27.3] and this was still higher than those with the CT genotype who had a median BMI of 22.5[21.1-25.8]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes was not statistically significant (p=0.068). On adjusting for marital status and baseline creatinine levels, the effect of ABCB1 C3435T genotypes was still not statistically significant (p=0.925).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had higher median BMI of 25.3[21.6-27.5] compared to those who had the CT genotype with a median BMI of 23.6[20.6-27.8]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes did not have statistical significance (p=0.278) as shown below.

Table 4.9 Effect of ABCB1 C3435T genotypes on Body Mass Index

ABCB1 C3435T Genotypes	**Baseline BMI, median [IQR]**	**BMI at ART switch, median [IQR]**	**BMI 6 months after ART switch, median [IQR]**
CC genotype	23.1[19.7-26.6]	24.4[21.1-27.3]	25.3[21.6-27.5]
CT genotype	20.7[18.6-23.3]	22.5[21.1-25.8]	23.6[20.6-27.8]
TT genotype	21.9[21.9-21.9]	-	-
p-value	0.081[a]	0.925[b]	0.278

[a] when adjusted for confounding by age and gender
[b] when adjusted for confounding by baseline creatinine levels and marital status

4.4 Longitudinal changes of CD4 cell counts

4.4.1 Baseline CD4 cell counts

The baseline, CD4 cell counts at switch and at recruitment are presented in Table 4.10. There were 49 (58.3%) study participants with a CD4 cell count of not more than 200 and 19 (22.6%) with more than 200 at the HAART initiation. Baseline CD4 cell counts for sixteen participants were not reported. Male participants had lower baseline CD4 cell counts than female participants; the male participants had a median CD4 cell count of 57.5 [IQR 15 – 254] while female participants had a median of 131 [IQR 33-445.5]. Those aged less than 40 years had a higher median CD4 cell count than those with more than 40 years. The patients with ALT levels lower than 40 UI/L had a higher median CD4 cell count than those with higher ALT levels. The study participants with creatinine levels less than 120µmol/l had higher median baseline CD4 cell counts than those with higher creatinine levels.

Table 4.10 CD4 cell counts at Baseline, ART switch and at recruitment attained in the study population

Patient Characteristics	Baseline CD4 cell counts, cells/mm^3 median [IQR]	CD4 cell counts at ART switch, cells/mm^3 median [IQR]	CD4 levels at recruitment, cells/mm^3 median [IQR]
Sex			
Male	57.5 [15 – 254]	173.5 [56 – 294.5]	340[221-441]
Female	131 [33-445.5]	374 [179.5-491]	554[334-722]
Age			
≤ 40	115[30-288]	303[173-473]	437[485-608]
> 40	88[11-254]	189.5[85-414]	358[244-619.5]
BMI at initiation			
Underweight(< 18.5)	4[2-132]		340[296-374]
Elevated (18.5 - 24.9)	69[19-288]		470[309-617]
Overweight or Obese (> 24.9)	94[30-180]		419.5[164-514]
First-line regimens			
TDF+3TC+NVP	-	329[167.5-444]	366[297-441]
AZT+3TC+NVP	-	464[313-606]	514[346-781]
AZT+3TC+EFV	-	134[70-222]	689[689-689]
D4T+3TC+NVP	-	473.5[197-706]	150[32-245]
D4T+3TC+EFV	-	270[218-365]	597.5[400-722]
TDF+3TC+EFV	-	9[6-42]	440.5[374.5-655.5]
D4T+DDI+EFV	-	254[254-254]	420[253.5-574]
Current Regimens			
ABC + 3TC + LPV/r	-	-	243[150-554]
AZT + 3TC + LPV/r	-	-	497.5[114.5-683.5]
TDF + 3TC + LPV/r	-	-	414[305-617]

4.4.2 CD4 cell counts at ART switch

The CD4 cell counts at ART switch were higher than baseline CD4 cell counts. The median CD4 cell count at ART switch was 244[87.5-414.5] while at baseline the median was 88[19-284]. Male participants still had lower CD4 cell counts than the females while the elderly patients had lower CD4 cell counts than younger participants. The participants with high ALT levels had lower CD4 cell counts at ART switch than those with normal liver functions while those with impaired kidney function had lower CD4 cell counts than those with normal kidney function. Study participants who had been on TDF+3TC+EFV had the lowest median CD4 cell counts at ART switch while those on D4T+3TC+NVP and AZT+3TC+NVP had the highest median CD4 cell counts as shown in Table 4.10.

4.4.3 CD4 cell counts at recruitment

The median CD4 cell count at recruitment was 423.5[256-622] while at ART switch, the median CD4 cell counts was 244[87.5-414.5] and that of baseline which was 88[19-284]. Study participants on AZT + 3TC + LPV/r had the highest CD4 cell counts at recruitment while the participants on ABC + 3TC + LPV/r had the lowest CD4 cell counts at recruitment. The patients who had been on zidovudine in first line therapy also had high CD4 cell counts at recruitment. Males, those with impaired liver function and impaired kidney function still had lower CD4 cell counts at recruitment compared to their respective counterparts.

4.5 Factors associated with CD4 cell counts

4.5.1 Factors associated with baseline CD4 cell counts

The factors are as shown in Table 4.11. The factor that had a significant negative association on univariate analysis with log baseline CD4 cell count included baseline creatinine [crude β= -0.006 (-0.008, -0.004)] while the variable that had a significant positive association with log baseline CD4 cell count was weight [crude β=0.038 (0.001, 0.075)]. Low baseline creatinine levels were significantly associated with higher log baseline CD4 cell counts. Higher baseline weight was significantly associated with higher log baseline CD4 cell counts

Baseline creatinine was the most important predictor for CD4 response at baseline and it was still significant when adjusted for confounding by weight, BMI, age, ALT, sex and WHO staging. There was a negative association between creatinine levels and the baseline log CD4 counts. Increased creatinine levels by one unit led to decreased baseline log CD4 levels by 0.004 units (-0.006, -0.003).

Table 4.11 Factors associated with log baseline CD4 cell count

Variables	Univariate analysis Beta (95% CI)	p-value	Multivariate analysis Beta† (95% CI)	p-value
Age	-0.032 (-0.071, 0.008)	0.113		
Sex	0.429 (-0.654, 1.510)	0.429		
Baseline Creatinine	-0.006 (-0.008, -0.004)	**0.000**	-0.004 (-0.006, -0.003)	**0.000**
Baseline ALT	-0.020 (-0.040, 0.003)	0.099		
Baseline BMI	0.028 (-0.007, 0.063)	0.118		
Weight	0.038 (0.001, 0.075)	**0.043**		
WHO stage	-0.277 (-0.673, 0.119)	0.165		
Marital status	0.171 (-0.363, 0.706)	0.522		
Education level	-0.330 (-0.950, 0.290)	0.290		
Ethnicity	0.245 (-0.722, 1.211)	0.613		
Baseline Hb	0.075 (-0.136, 0.286)	0.477		

4.5.2 Factors associated with CD4 cell counts at ART switch

Factors with a significant negative association with square root CD4 cell count at ART switch included baseline weight [crude β= -0.196 (-0.378, -0.015)], Education level [crude β= -3.333 (-5.762, -0.904)], AZT+3TC+EFV [crude β= -4.386 (-8.379, -0.393)], TDF+3TC+EFV [crude β= -11.416 (-14.987, -7.845)] and efavirenz-based regimens [crude β= -6.603 (-10.263, -2.944)]. The study participants with low baseline weight and lower education level had a significant association with higher square root of CD4 count at ART switch. Study participants on efavirenz-based regimens had lower square root of CD4 count at ART switch compared to those on nevirapine-based regimens. The factors that had a significant positive association with square root CD4 count at ART switch were baseline CD4 cell count [crude β=0.038 (0.001, 0.075)], sex [crude β=5.015 (1.140, 8.887)] and AZT+3TC+NVP [crude β=6.441 (2.385, 10.497)]. There was a significant association between high baseline CD4 counts and high square root of CD4 count at ART switch. Female study participants also had higher square root of CD4 counts than male study participants. The other factors are as presented in Table 4.12.

On adjusting for confounding, baseline weight [adjusted β=-0.145 (-0.290, -0.000)] and TDF+3TC+EFV [adjusted β=-8.887 (-13.623, -4.150)] had a significant negative association with square root of CD4 count at ART switch. Study participants who were previously on TDF+3TC+EFV had lower square root of CD4 count compared to other regimens by 8.887 units. An increase of weight by one kilogram resulted to a decrease of square root of CD4 count by 0.145 units. On the other hand, baseline CD4 count was significantly associated with CD4 count at ART switch; increasing baseline CD4 cell count by one unit led to an increase in square root of CD4 count at ART switch by 0.012 units. Males had significantly lower square root of CD4 count at ART switch compared to females by 4.201 units.

Table 4.12 Factors associated with CD4 cell count at ART switch

Square root of CD4 cell count at switch	Univariate analysis Beta (95% CI)	p-value	Multivariate analysis Beta† (95% CI)	p-value
Sex	5.015 (1.140, 8.887)	**0.012**	4.201 (0.528, 7.875)	**0.027**
Baseline CD4 count	0.017 (0.009, 0.025)	**0.000**	0.012 (0.003, 0.021)	**0.013**
Baseline Creatinine levels	-0.045 (-0.134, 0.044)	0.301	-	-
Baseline ALT	0.013 (-0.229, 0.255)	0.912	-	-
Baseline BMI	-0.027 (-0.092, 0.145)	0.653	-	-
Baseline Weight	-0.196 (-0.378, -0.015)	**0.035**	-0.145 (-0.290, -0.000)	**0.050**
Baseline WHO stage	-0.869 (-3.249, 1.510)	0.463	-	-
WHO stage at switch	-1.660 (-4.241, 0.921)	0.198	-	-
Age at switch	-0.058 (-0.187, 0.071)	0.372	-	-
Marital status	-0.436 (-3.810, 2.942)	0.796	-	-
Education level	-3.333 (-5.762, -0.904)	**0.008***	-	-
Ethnicity	-2.333 (-6.312, 1.649)	0.245	-	-
Baseline Hb	0.258 (-1.203, 1.719)	0.720	-	-
ABCB1 C3435T genotype	-0.629 (-5.341, 4.083)	0.789	-	-
Duration of first-line therapy	0.497 (-0.349, 1.343)	0.243	-	-
First-line regimen	-1.080 (-2.191, 0.028)	**0.056**	-	-
TDF+3TC+NVP	1.893 (-2.301, 6.087)	0.368	-	-
AZT+3TC+NVP	6.441 (2.385, 10.497)	**0.003**	-	-
AZT+3TC+EFV	-4.386 (-8.379, -0.393)	**0.032**	-	-
D4T+3TC+NVP	6.326 (-0.070, 12.722)	0.052	-	-
D4T+3TC+EFV	2.437 (-1.330, 6.204)	0.199	-	-
TDF+3TC+EFV	-11.416 (-14.987, -7.845)	**0.000**	-8.887 (-13.623, -4.150)	**0.001**
D4T+DDI+EFV	0.835 (-1.283, 2.953)	0.432	-	-
Efavirenz-based regimens	-6.603 (-10.263, -2.944)	**0.001***		-

*The factors with an asterisk seemed to have an effect though they were not included in the final model

4.5.3 Factors associated with rate of change of the CD4 counts

CD4 cell counts logarithm was obtained and regressed against covariates using generalized linear modelling. The factors associated with the rate of CD4 count change after ART switch are presented in Table 4.13. The factors that had a significant positive association with rate of change included sex [crude β= 0.822 (0.373, 1.271)], CD4 count at ART switch [crude β= 0.003 (0.001, 0.004)], and D4T+3TC+EFV [crude β= 0.382 (0.018, 0.745)]. The factors that had a significant negative association with the rate of change of CD4 count included education level especially education above tertiary level [crude β= -1.314 (-2.176, -0.452)] and TDF+3TC+EFV [crude β= -2.167 (-2.937, -1.398)].

Table 4.13 Factors associated with rate of log CD4 cell counts change

Variables	Univariate analysis Beta (95% CI)	p-value	Multivariate analysis Beta† (95% CI)	p-value
Age	-0.005 (-0.032, 0.022)	0.701	-	-
Sex	0.822 (0.373, 1.271)	**0.000**	0.610 (0.325, 0.894	**0.000**
Baseline CD4 cell count	0.002 (0.001, 0.003)	**0.002**	-	-
CD4 count at ART switch	0.003 (0.001, 0.004)	**0.000**	-	-
Baseline Creatinine	-0.001 (-0.002, -0.000)	**0.002**	-	-
Baseline ALT	-0.002 (-0.014, 0.009)	0.700	-	-
Baseline BMI	0.002 (-0.013, 0.017)	0.831	-	-
Baseline Weight	-0.022 (-0.050, 0.006)	**0.129**	-	-
Baseline WHO stage	-0.082 (-0.313, 0.148)	0.484	-	-
WHO stage at ART switch	-0.152 (-0.410, 0.105)	0.245	-	-
Marital status	-0.133 (-0.318, 0.515)	0.157	-	-
Education level above tertiary	-1.314 (-2.176, -0.452)	**0.003**	-1.451 (-2.216, -0.685)	**0.000**
Ethnicity	-0.136 (-0.605, 0.332)	0.568	-	-
Hb at enrollment	-0.048 (-0.198, 0.102)	0.527	-	-
First-line regimen	-0.214 (-0.386, -0.042)	**0.015**	-	-
TDF+3TC+NVP	0.279 (-0.123, 0.680)	0.173	-	-
AZT+3TC+NVP	0.481 (-0.039, 1.001)	**0.070**	-	-
AZT+3TC+EFV	0.114 (-0.295, 0.523)	0.586	-	-
D4T+3TC+NVP	-0.060 (-0.562, 0.441)	0.813	-	-
D4T+3TC+EFV	0.382 (0.018, 0.745)	**0.039**	-	-
TDF+3TC+EFV	-2.167 (-2.937, -1.398)	**0.000**	-1.133 (-1.740, -0.527)	**0.000**
D4T+DDI+EFV	0.553 (0.323, 0.784)	**0.000**	-	-
Second-line regimen	-0.032 (-0.382, 0.319)	0.860	-	-
Interaction between duration and education above tertiary level			0.446 (0.193, 0.699)	**0.001**

The interpretation of the findings of generalized linear regression analysis were improved by generating predictive plots for each of the variables that were significant.

4.3.3.1 Effect of Education on rate of change of CD4 cell count

From generalized linear modelling, education affected the rate of change because the interaction between duration and education above tertiary level was statistically significant as shown above.

Study participants who had tertiary education had lower CD4 cell counts at ART switch but a higher rate of log CD4 count change while study participants who did not have tertiary education had a higher CD4 cell counts at ART switch but a lower rate of log CD4 cell count change. This is illustrated in Figure 4.2.

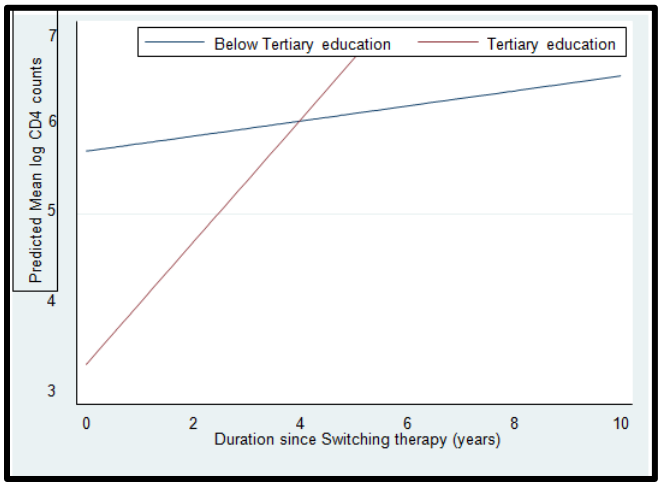

Figure 4.2 Effect of education on rate of change of CD4 cell count

4.3.3.2 Effect of Sex on rate of CD4 cell count change

Females had consistently higher log CD4 cell counts compared to males. However, males had a slightly higher rate of log CD4 cell count change than females as illustrated in Figure 4.3.

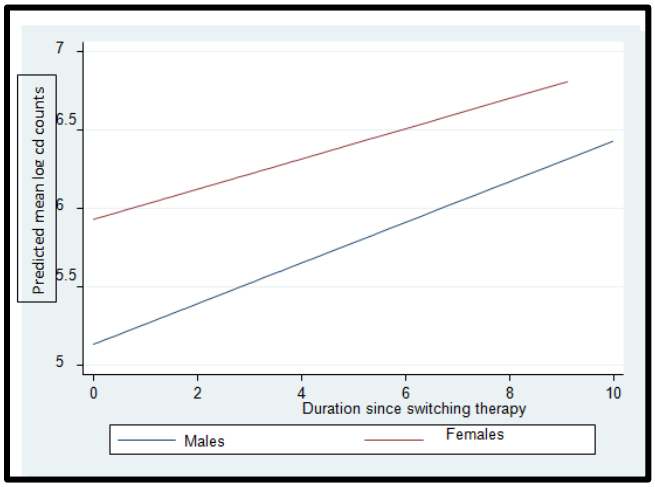

Figure 4.3 Effect of Sex on rate of change of CD4 cell count

4.3.3.3 Effect of first-line regimen on rate of CD4 cell count change

In order to control for the effect of education, two different plots of the rate of CD4 count change vs first line regimen were generated; the first for participants without tertiary education and the second for those with tertiary education. Patients who had been on TDF+3TC+EFV had consistently lower CD4 cell counts after change of therapy to second-line antiretroviral therapy compared to those who had been on other regimens. However, they seemed to have a higher rate

of change of CD4 than those previously on other regimens as illustrated in Figure 4.4. The study participants with tertiary education still had a higher rate of CD4 count change than those without tertiary education.

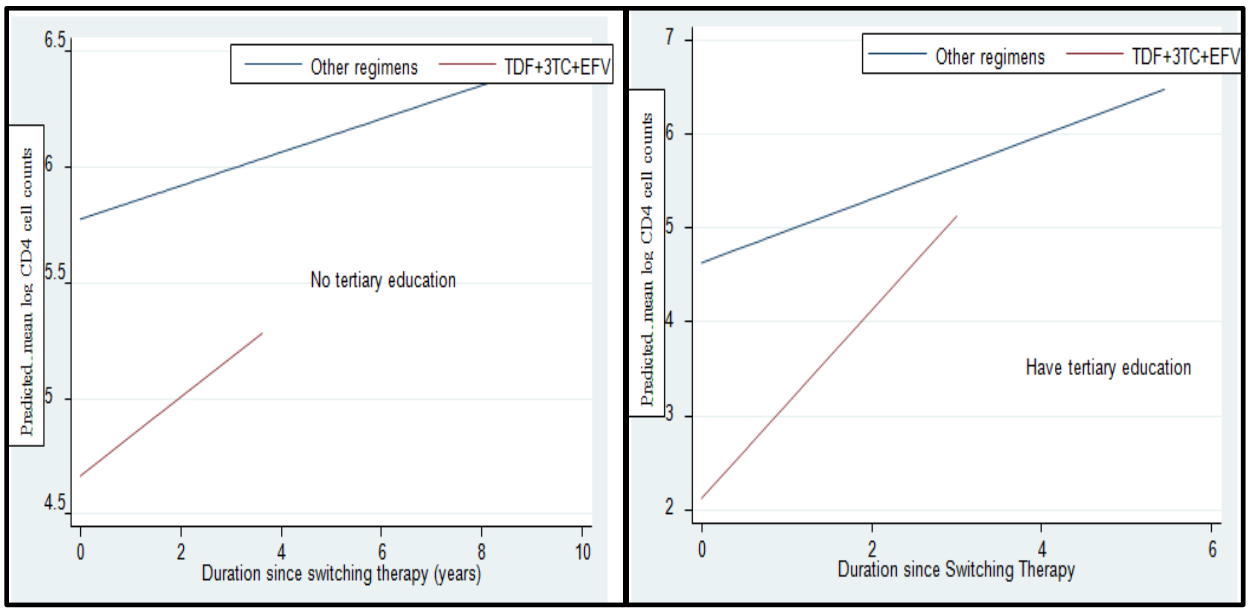

Figure 4.4 Difference in the rate of change in log CD4 counts of patients on TDF+3TC+EFV and other regimens

4.5.4 Factors associated with current CD4 counts

CD4 cell counts at recruitmentsquare root was computed and regressed against the covariates as presented in Table 4.14. The variables that had a significant negative association with square root of CD4 cell counts at recruitmentincluded baseline weight [crude β= -0.109 (95% CI: -0.226, 0.007)], education level [crude β= -2.241 (-3.788, -0.694)], baseline creatinine levels [crude β= -0.109 (-0.226, 0.007)] and type of second-line regimen [crude β= -2.322 (-4.646, 0.007)]. Higher baseline weight, higher baseline creatinine levels, those with high education level and those on ABC+3TC+LPV/r were significantly associated with lower square root of current CD4 counts.

Variables that had a significant positive association with CD4 cell counts at recruitment square root were CD4 count at ART switch [crude β=0.0119 (0.005, 0.019)], sex [crude β=3.351 (0.703, 6.004)] and baseline WHO stage [crude β=1.662 (-0.066, 3.391)]. Low CD4 counts at ART switch and low baseline WHO stage were significantly associated with low square roots of CD4 cell counts at recruitment. Study participants with high CD4 counts at ART switch and those with a high baseline WHO stage were associated with high CD4 cell counts at recruitment square roots. Females had a higher CD4 cell counts at recruitment compared to males. Other factors are as presented in Table 4.14.

On adjusting, CD4 cell count at ART switch [adjusted β=0.009 (0.000, 0.018)], education level [adjusted β=-3.561 (-6.660, -0.463)] and baseline WHO stage [adjusted β=2.244 (0.244, 4.243)] were important predictors of CD4 cell counts at recruitment. The study participants who had tertiary education and above had lower square roots of CD4 cell counts at recruitment compared to those without tertiary education. An increase in CD4 cell count at ART switch by one unit led to an increase in CD4 cell counts at recruitment square root by 0.009 units (0.000, 0.018). An increase in baseline WHO stage by one unit led to an increase in CD4 cell counts at recruitment square root by 2.244 units (0.244, 4.243).

Table 4.14 Factors associated with square root of CD4 cell counts at recruitment in the study participants

Variables	Bivariable analysis Beta (95% CI)	p-value	Multivariate analysis Beta† (95% CI)	p-value
Sex	3.351 (0.703, 6.004)	**0.014**	-	-
Baseline CD4 cell count	0.006 (-0.002, 0.014)	0.139	-	-
CD4 count at ART switch	0.0119 (0.005, 0.019)	**0.001**	0.009 (0.000, 0.018)	**0.048**
Baseline Creatinine	-0.010 (-0.016, -0.004)	**0.001**	-	-
Baseline ALT	-0.025 (-0.111, 0.062)	0.567	-	-
Baseline BMI	-0.043 (-0.137, 0.051)	0.362	-	-
Baseline Weight	-0.109 (-0.226, 0.007)	**0.066**	-	-
Baseline WHO stage	1.662 (-0.066, 3.391)	**0.059**	2.244 (0.244, 4.243)	**0.029**
WHO stage at ART switch	1.219 (-1.127, 3.565)	0.300	-	-
Current Age	0.058 (-0.058, 0.175)	0.321	-	-
Marital status	-1.651 (-3.490, 0.191)	0.078	-	-
Education level above tertiary	-3.733 (-6.810, -0.656)	**0.018**	-3.561 (-6.660, -0.463)	**0.026**
Ethnicity	-1.042 (-3.795, 1.717)	0.455	-	-
Baseline Hb	0.251 (-0.731, 1.232)	0.609	-	-
Duration of first-line therapy	0.155 (-0.282, 0.593)	0.481	-	-
First-line regimen	-0.871 (-1.891, 0.151)	0.094	-	-
Duration of second-line therapy	0.153 (-0.399, 0.704)	0.582	-	-
Second-line regimen	-2.322 (-4.646, 0.007)	**0.051**	-	-
TDF+3TC+LPV/r	2.594 (-0.589, 5.778)	0.109	-	-
AZT+3TC+LPV/r	-1.000 (-4.528, 2.527)	0.573	-	-
ABC+3TC+LPV/r	-4.750 (-10.315, 0.816)	0.093	-	-

Chapter 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

This study is the first in Kenya to detail the prevalence of ABCB1 C3435T genotypes and their allele frequencies. The allele distribution of the variant T allele was 12.5% while that of C allele was 87.5%; the prevalence of ABCB1 3435CC wild-type genotype was 76.2% while that of the heterozygous CT was 22.6% and the TT variant was 1.2%. These findings conform to Hardy-Weinberg proportions and thus indicates that the frequencies would not change from one generation to another if there are no other evolutionary influences.

The study also reported a significant association between ABCB1 C3435T genotypes and creatinine levels of participants who had been on lopinavir-based regimens for 6 months. Study participants with the CT genotype had lower creatinine levels than those with the CC genotype. In addition, the study participants with the CT genotype had consistently higher CD4 cell counts than those with the CC genotype from the time of ART switch.

5.1 ABCB1 C3435T Single Nucleotide Polymorphism variability

The prevalence in Burundi was 1.5% TT genotype, 20.1% CT and 78.4% CC while the prevalence in Ethiopia was 4.9% (13 patients) TT genotype, 34.1% (90) CT, 61% (161 participants) CC genotype and in Tanzania it was 1.1% (2 patients) TT genotype, 29.0% (53) participants CT genotype and 69.9% (128 participants) CC genotype (Lam and Cavallari, 2013; Ikediobi et al., 2011; Calcagno et al., 2011; Ngaimisi et al., 2013).

Table 5.1 Distribution of ABCB1 C3435T genotypes in various African populations

	Number of study participants (%)			
ABCB1 C3435T Genotypes	Kenya	Tanzania	Burundi	Ethiopia
CC genotype	64 (76.2)	128 (69.9)	160 (78.4)	160 (61)
CT genotype	19 (22.6)	53 (29)	41 (20.1)	90 (34.1)
TT genotype	1 (1.2)	2 (1.1)	3 (1.5)	13 (4.9)
Total study participants	84 (100)	183 (100)	204 (100)	263 (100)

Our findings were similar to the prevalence reported in Burundi and Tanzania but slightly different from the prevalence in Ethiopia.

One patient in our study had the variant TT genotype (1.2%) while a study done in Burundi found three patients (1.5%) with the variant TT genotype (Calcagno et al., 2011). Another study done among Tanzanians had only two patients (1.1%) with the variant TT allele while among Ethiopians, there were thirteen patients (4.9%) with the variant TT allele (Ngaimisi et al., 2013). A study done in South India reported seventy eight (44%) of the study population with the TT genotype (Bakshi et al., 2008). This was very different from our findings and thus indicates racial differences in the frequencies of the polymorphism of ABCB1 C3435T.

5.2 Effect of ABCB1 C3435T genotypes on clinical outcomes

A study done in China reported an association which was significant between ABCB1 C3435T polymorphism and CD4 count but our study did not report a significant association (Zhu et al., 2013). This is probably because a mutation on ABCB1 at position 3435 would reduce the activity of the MDR protein and thus prevent efflux of lopinavir from sanctuary sites. This increase in lopinavir in sanctuary sites would lead to higher CD4 cell counts.

A study done among Africans concluded that 3435T allele was correlated to higher effective renal plasma flow and glomerular filtration rate and lower renal resistance than the CC genotype (Bochud, 2008). This was similar to our findings in this study. This is probably because those with the CT genotype had reduced activity of the MDR1 protein. Since MDR1 is found at the distal and proximal tubule epithelium apical membrane, it would limit the flow of the drug to the tubule for those with the CC genotype and hence reduce excretion of lopinavir leading to reduced kidney function. The participants with the CT genotype would have reduced function of the MDR1 protein and thus lopinavir would easily flow to the tubule and hence excreted.

Some studies reported that ABCB1 3435 C>T variant was likely to cause reduced risk of hepatotoxicity in patients on nevirapine (Aceti, 2015) while others reported increases in liver enzymes among patients on nevirapine-based regimens (Pavlos, 2011). This was different from our study which had no significant association with ALT levels. This is probably because lopinavir does not have an effect on the liver while nevirapine does (Chou et al., 2010; Kityo et al., 2010).

5.3 Variables associated with CD4 cell response

5.3.1 Sex

A study done among Kenyan study participants found out that males had lower baseline CD4 counts and this was statistically significant (Angima, 2015). In our study, this finding was not statistically significant probably because we had a smaller sample size.

A study done in Nigeria reported that males had a higher likelihood of loss to follow up (Odafe et al., 2012). This suggests that males are more likely to have poor adherence and this may lead to decline in CD4 cell response. Another study done in Burkina Faso indicated that men were strongly associated with virologic failure probably due to poor adherence (Penot et al., 2014). Another study done in Myanmar also found out that being male was a risk factor for loss to follow up or death (Thida et al., 2014). This was in agreement with our study. These studies were an indication that men were more likely to have poor adherence and this could explain their low CD4 cell count at ART switch.

5.3.2 ART regimens

A study done in Uganda indicated that nevirapine was more likely to be associated with treatment failure than efavirenz (Sebunya et al., 2013). This was different from our study which indicated that efavirenz-based regimens were associated with immunological failure when switching from first-line to second-line. These findings suggest that current treatment guidelines need to be critically reviewed with regard to selection of NVP viz a viz EFV based regimens. The available scientific literature on the comparative effectiveness of these agents needs to be critically reviewed so as to guide treatment selection.

The second line regimens that the study participants were on did not have a significant association with the current CD4 counts. This was in agreement with a study that found no significant association between CD4 count and type of ART regimen done in Ghana (Barry et al., 2013).

5.3.3 Duration of therapy

A study done in Asia found out that longer duration of antiretroviral therapy was associated with failure of first-line antiretroviral regimen (Jiamsakul et al., 2014). Another study reported that high rates of virological failure on second-line therapy was associated with duration of previous drug regimens exposure (Ajose et al., 2012). Duration of therapy was significantly associated

with the rate of CD4 count change in our study. The longer the patient had been on antiretroviral therapy, the higher the likelihood of a faster increase in CD4 response.

5.3.4 Baseline CD4 cell count

Baseline CD4 count significantly affected CD4 count at ART switch. This was similar to a study done in Western Kenya which also found out that baseline CD4 count was a predictor of CD4 count at change of therapy to second-line therapy (Inzaule et al., 2014). Another study done in South Africa also reported that baseline CD4 cell counts were determinants for recovery (Julg et al., 2012). A study in Ethiopia concluded that immunological failure was associated with low baseline CD4 count hence reduced CD4 count at ART switch (Teshome and Assefa, 2014).

5.3.5 Other variables

Study participants with tertiary education and above had consistently lower CD4 cell counts compared to those without tertiary education probably because those without tertiary education were more likely to keenly follow the instructions from the health workers. A study in Ethiopia concluded that higher education was associated with reduced CD4 cell count leading to immunological treatment failure (Teshome and Assefa, 2014). However, study participants with tertiary education and above had a higher rate of change of CD4 cell count after ART switch than those without tertiary education. This is probably because they were more likely to understand the impact of treatment failure and hence they would have improved adherence. This would have led to a higher rate of CD4 cell count change.

CD4 cell response had no significant association with age. This was unlike a study done in South Africa which noted a significant association between severe CD4 cell count decline and old age (Julg et al., 2012). Several studies have also shown an association between rate of CD4 count change and age (Montarroyos et al., 2014). A study in Ethiopia concluded that old age was associated with reduced CD4 cell count leading to immunological treatment failure (Teshome and Assefa, 2014)

Conclusion

Genotyping for ABCB1 C3435T polymorphism would help predict patients who would respond effectively to lopinavir-based regimens. The patients with heterozygous ABCB1 3435 CT genotype exhibit better immunological profile and better renal function. However, more genotypic studies on other ABCB1 polymorphisms need to be done.

Recommendations for policy, practice and further research

More studies should be carried out to compare effectiveness of efavirenz vis-à-vis nevirapine vis-à-vis integrase inhibitors. In addition, plasma concentrations of lopinavir need to be determined in order to infer whether they are affected by ABCB1 C3435T single nucleotide polymorphism. In addition, research on the effects of other ABCB1 single nucleotide polymorphisms on CD4 cell count need to be carried out.

Study limitations

There were challenges in controlling for some possible confounding factors like diet, drugs, comorbidities and social habits. Recruitment of study participants was also a challenge. In addition, other polymorphisms of drug metabolizing enzymes were not considered in this study and these may affect HIV treatment response. Information was obtained from records and hence incomplete and/or inaccurate records may have affected the quality of data.

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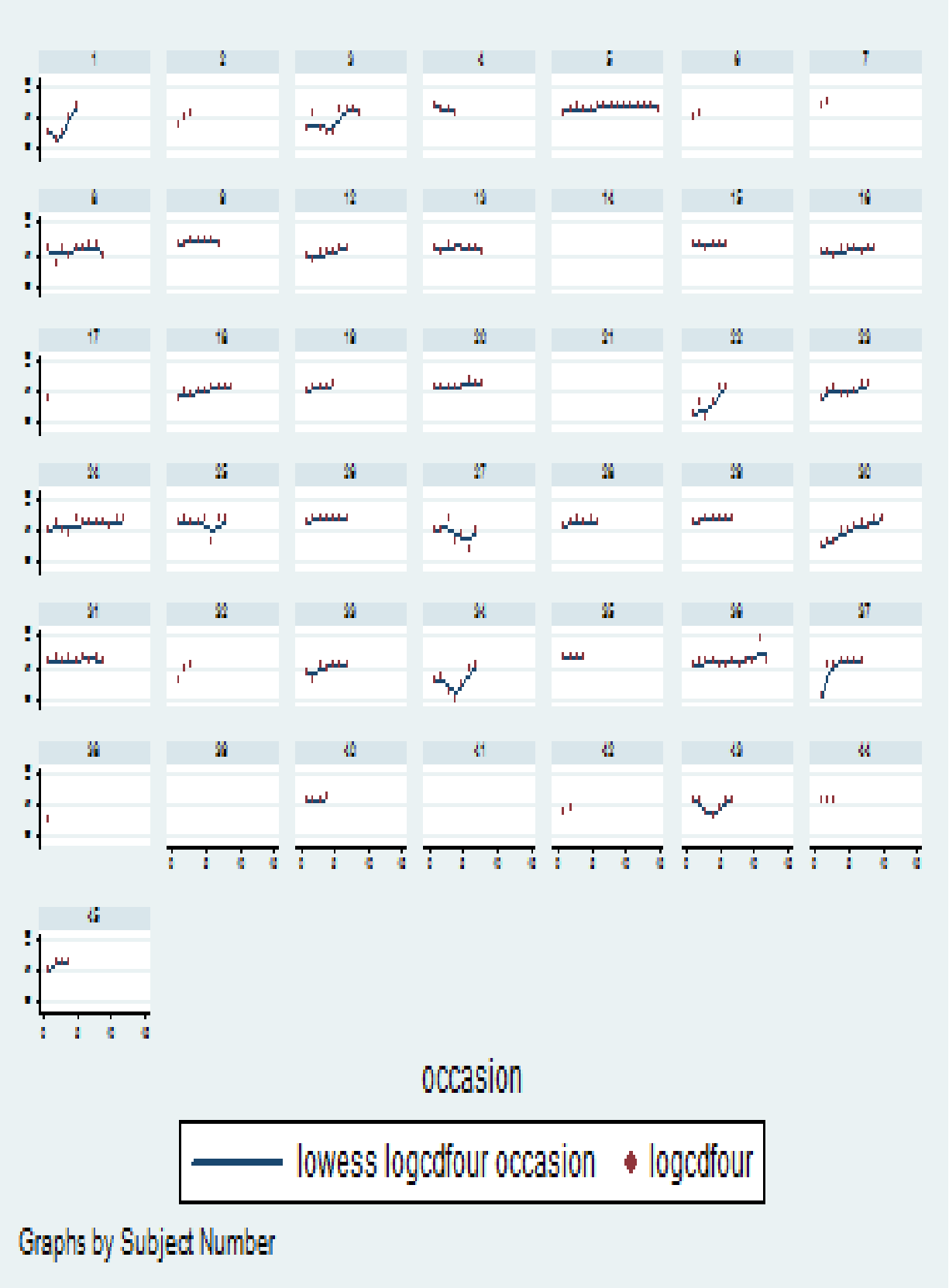
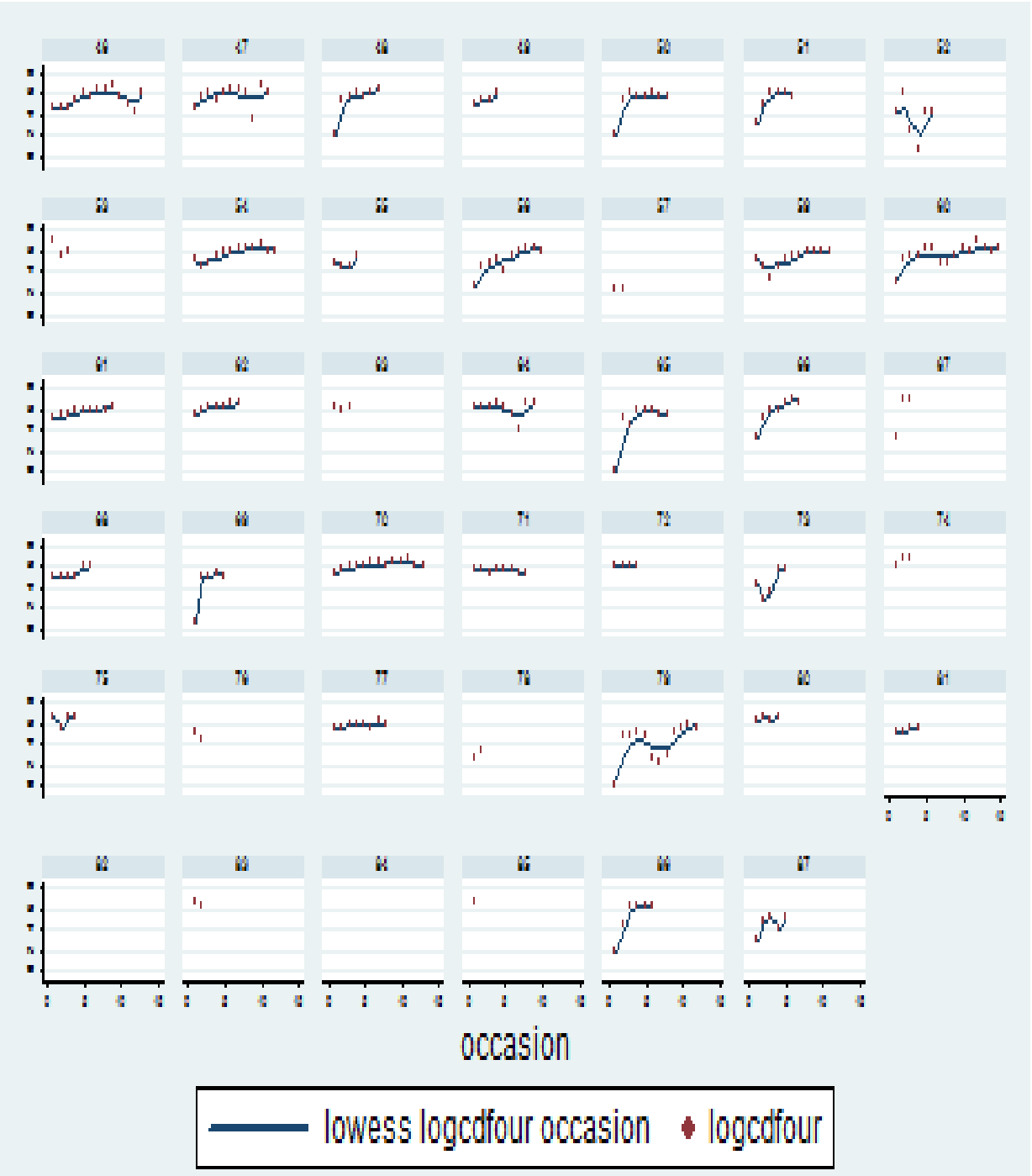
APPENDICES

APPENDIX A: EMPIRICAL PLOTS FOR EACH INDIVIDUAL

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Graphs by Subject Number

APPENDIX B : VOLUNTEER INFORMATION AND CONSENT FORM

Consenting process

This document is a consent form; it has information about the study and shall be discussed with you by the investigators. Please study it carefully and feel free to seek any clarification especially concerning terminologies or procedures that may not be clear to you. Once you understand and agree to take part, you are requested to sign your name on this form. You should understand the following general principles which apply to all participants in a medical research.

i. Your agreement to participate in this study is voluntary.
ii. You may withdraw from the study at any time without necessarily giving a reason for your withdrawal.
iii. Refusal to participate in the research will not affect the services that you are entitled to receive in this Clinic.

Introduction to the study

Lopinavir is a protease inhibitor and a key component in HIV management especially second-line antiretroviral therapy. It is used in combination with other anti-retroviral drugs. However, patients may experience treatment failure of second-line antiretroviral therapy. This is critical since there are very few options of third-line antiretroviral therapy.

Treatment failure may be due to disposition of the drugs used in second-line antiretroviral therapy including lopinavir. ABCB1 is a transporter that removes drugs from the cells and it may affect the disposition of drugs used in second-line antiretroviral therapy. I am assessing the effects of ABCB1 polymorphisms on clinical outcomes in patients taking second-line antiretroviral therapy. Samples may be taken abroad for secondary analysis. Permission is requested from you to enroll in this medical research study.

Purpose of the study

The primary objective is to determine the effects of ABCB1 polymorphisms on clinical outcomes in participants on second-line antiretroviral therapy.

Procedures to be followed

With your permission we will go through your medical records to obtain information on laboratory investigations which have been conducted since you were initiated on ART. We will also check whether you have suffered any bad reactions to drugs.

You will be asked a few questions about your ethnicity, if you are using any other drugs (prescription or over the counter) or herbal products, whether you drink or smoke, how regularly you take medication and whether you have experienced any bad reactions to drugs that you are taking. We will also collect a blood sample from you.

Selection criteria

You will be selected to take part in this study if you meet the following criteria:

a) You have been on second-line ART for at least 6 months
b) You are aged above 18 years
c) You must have agreed to take part in the study.

Risks or/and discomfort.

There will be no risks involved in this study to you.

Rights and safety

To safeguard your rights and safety as a participant taking part in this study, the Kenyatta National Hospital/University of Nairobi Research and Ethics Committee will review the study protocol and the informed consent process before commencing the research.

Benefits

The study may be of benefit to you and other HIV patients in that it will be used to enhance detection of patients who may have ABCB1 polymorphisms and may experience treatment failure. It may also inform policy makers on the need to review guideline on pharmacogenomics testing.

Assurance on confidentiality

Utmost care will be taken to keep your participation in this study confidential. All information obtained from your file and laboratory investigation shall be used for the purpose of this study only and kept confidentially. Your name will not be used during data handling or in any resulting publications, codes will be used instead. Your medical records will be kept under lock and key and information will be accessible to authorized persons only.

Contacts

For any further information about this study you may contact me, my academic department or the Kenyatta National Hospital/University of Nairobi Ethics and research Committee using the contacts provided below:

Richard Kagia Njung'e,
Department of pharmacology and pharmacognosy
School of Pharmacy,
University of Nairobi
P.O Box, 19676- Nairobi. Tel: 0704-407405

Dr. Margret Oluka,
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School of Pharmacy,
University of Nairobi
P.O Box, 19676- Nairobi. Tel: 0737-434204

The chairperson,
The Kenyatta National Hospital/University of Nairobi Research and Ethics Committee,
P.O Box, 19676- Nairobi. Tel: 020-2726300 Ext 44102

FOMU YAKUIDHINISHA UTARATIBU WA KUKUSANYA UJUMBE NA MAAFIKIANO

Nambari ya usajili_____mtindo:01 _____ Tarehe: Januari, 2016

MADA YA UTAFITI: MADHARA YA ABCB1 POLYMORPHISM JUU YA MATOKEO YA KLINIKI YA WAGONJWA WA KENYA WENYE VIRUSI VYA HIV KATIKA HOSPITALI YA TAIFA YA KENYATTA.

A. <u>Ujumbe wa kuidhinisha maelezo</u>

Mada ya utafiti: Madhara ya ABCB1 polymorphism juu ya matokeo ya kliniki ya wagonjwa wa kenya wenye virusi vya HIV katika hospitali ya taifa ya kenyatta

Chuo: Idara ya famakolojia na famakognsia, chuo cha famasia behewa la Nairobi, SLP 30197-00400, Nairobi.

Idhinisho la kimaadili

Hospitali kuu ya Kenyatta/ Chuo Kikuu cha Nairobi, kamati ya utafiti, SLP 20723-00100 Nairobi. Simu 2726300 / 2716450 ext 4410. Tunakuomba ruhusu kwako ili ujisajili katika utafiti huu. Unafaa kuelewa mambo yafuatayo ya kimsingi yanayofaa kuzingatiwa na washiriki wote.

i. Mwitikio wako wa kushiriki katika utafiti huu ni wa hiari.
ii. Unaweza kujitoa kutoka kwa utaiti huu wakati wowote pasipo kuhitajika kutoa sababu za kujitoa.
iii. Baada ya kufanya maelezo tafadhali, una uhuru wa kuuliza maswali yoyote yatakayokuwezesha kuelewa vizuri aina tofauti.
iv. Usajili huu unakadiriwa kuchukua muda wa dakika 20-30.

Utangulizi: ABCB1 ni transporter ambayo huondosha dawa kutoka seli na inaweza kuathiri mwelekeo wa dawa za kurefusha maisha zinazotumika kwa HIV.

Lengo la utafiti: Lengo kuu ni kujua madhara ya ABCB1 polymorphism juu ya matokeo ya kliniki ya wagonjwa wa kenya wenye virusi vya HIV katika hospitali ya taifa ya kenyatta

Utaratibu: Kwa ruhusa yako, tutaangalia faili yako kudhibitisha madawa unayopewa na pia kujua habari yako kama miaka, lugha ya mama na mengineyo. Pia tutachukua damu yako. Ujumbe wote utashughulikiwa kwa siri.

Hatari: Hakuna hatari yoyote kwa kuwa atakayeshirikiwa hataumizwa lakini kunaweza kuwa na adhari za kisaikolojia au adhari za kihisia zinazohusiana na ujumbe ambaye anayesaidiwa atatoa wakati wa usajili. Tutapunguza au kumaliza hisia hizi kabisa kwa kuuweka ujumbe gushi na anaweza kuorodhesha baadhi ya vipengee kumwezesha kubaini aliyetoa. Wasaidizi wake

wowote watapata ujumbe na maelezo kutoka kwa msajili na ujumbe wenyewe hautakuwa na ushusiano wa moja na ule uliotolewa na aliyesajiliwa.

Umuhimu: Hakutakuwa pesa zozote zitakazotolewa ama faida ya moja kwa moja. Hata hivyo ,matokeo yatasaidia kuboresha utunzaji wa wagonjwa wenye HIV hasa wanaotumia dawa za mstari hasa pili tiba ya kurefusha maisha

Hakikisho la usiri: Ujumbe wote utakaochukuliwa kwako utahifadhiwa kama siri. Hakuna wakati jina lako litatajwa au litumike wakati wa kuwasilisha ujumbe au kuandika nakala ya ujumbe gushi. Nambari za siri zitatumika. Nambari za hospitali/wodi za wagonjwa zitaondolewa kutoka kwenye ujumbe gushi na nambari za siri zitumike kuwatambulisha. Ujumbe huo utafungwa na ufunguo utawekwa na ujumbe utafunguliwa tu na aliyeutafiti.

Mawasiliano: Iwapo ungetaka kuwasiliana nami, ama hospitali kuu ya Kenyatta chuo kikuu cha Nairobi maadili na kamati ambayo imeniruhusu kufanya utafiti huu, kuwa huru na utumie nambari za mawasiliano zilizoorodheshwa kwenye fomu hii.

FOMU YA IDHINI YA MSHIRIKI

Mimi niliyetia sahihi kwa hiari nakubali kushiriki utafiti huu. Nimeelezwa na kuelewa kinachohusiana na utafiti huu, majukumu yangu katika utafiti huu, athari zinazoweza kutokana na kujitolea kwangu na kuwa maswali yote yanayohusiana na utafiti huu nimeyajibu kama inavyotakikana. Naelewa kuwa naweza kuchagua kuacha kushiriki katika utafiti huu wakati wowote bila kupigwa penalti kwa njia yoyote. Naelewa kuwa ujumbe uliokusanywa utatumika kwa lengo la utafiti pekee na usiri wa hali ya juu utadumishwa.

Nitapokea nakala ya fomu hii iliyotiwa sahihi.

Jina na sahihi ya mshiriki wa utafiti _____tarehe_____

MIADI YA MTAFITI

Nimeelewa kuwa ujumbe katika fomu hii kwa mshirika huyu na nimewatia wote moyo ili kuuliza maswali ambayo nilichukua wakati kuyajibu. Nimetosheleza kila mshirika kabisa na naelewa vipengee vyote vya utafiti kama ilivyoelezwa katika fomu ya kudhibitisha ruhusa hapo juu.

Jina na sahihi ya anayeomba ruhusa _____ Tarehe _____

MAWASILIANO

Mtafiti: Richard Kagia SLP 40393-00100, Nairobi. Simu +254 704407405 .
Sekritari ,Hospitali kuu ya Kenyatta chuo kikuu cha Nairobi kamati ya maadili na utafiti, SLP 20723-00100, Nairobi. Simu 2726300-2716450 ext 44102.

APPENDIX C: KNH/UoN ETHICAL APPROVAL