UNIVERSITY OF NAIROBI

ADVERSE DRUG REACTIONS AND UNDERLYING METABOLOMIC PROFILES IN HIV PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY

BY

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DECLARATION

I affirm this thesis to be my original work and verify that I have not submitted it somewhere else for examination, honour of degree or publication. There was correct acknowledgement and referencing of other authors' work or my very own work, in accordance with the University of Nairobi's requirements.

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DEDICATION

I commit this thesis to my own particular family, my mother, siblings, and companions without their affection, support, and comprehension this work could not have been a declaration. Overall, we now surely understand that accomplishment resembles a chunk of ice; its tip is fabulous, however underneath are massive pain-staking sacrifices. I cherish all of you.

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ACRONYMS AND ABBREVIATIONS

AA	Arachidonic Acid
ABC	Abacavir sulphate
ADRs	Adverse Drug Reactions
AIDS	Acquired Immunodeficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APV	Amprenavir
ARV	Antiretroviral
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
ATV	Atazanavir
ATV/r	Ritonavir-Boosted Atazanavir
AZT	Azidothymidine
CCR5	Chemokine Co-Receptor Type 5
CD4+	Cluster of Differentiation 4 Positive
Cobi	Cobicistat
CVS	Comma Separated Value
CXCR4	Chemokine Receptor Type 4
CYP	Cytochrome P450
d4T	Stavudine
DLV	Delavirdine
DNA	Deoxyribonucleic Acid
DRG	Dorsal Root Ganglion
DSP	Distal Sensory Polyneuropathy
DTG	Dolutegravir
EDTA	Ethylenediaminetetraacetic Acid
EFV	Efavirenz
EPA	Eicosapentaenoic Acid

EpiA-S	Epiandrosterone Sulfate
ERC	Ethics Research Committee
ETR	Etravirine
EVG	Elvitegravir
FDF	Follicular Dendritic Cells
FIA	Flow Injection Analysis
FPV	Fosamprenavir
FTC	Emtricitabine
GC/MS	Gas Chromatography / Mass Spectrometry
GLUT	Glucose Transporters
GP	Glycoproteins
GP×	Glutathione Peroxidase
GSH	Glutathione
H_2O_2	Hydrogen Peroxide
HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HCV	Hepatitis C Virus
HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
HOCI	Hypochlorous Acid
HPLC	High-Performance Liquid Chromatography
HPO	Hydroperoxides
HSN	HIV-Associated Sensory Neuropathy
IDP	Inflammatory Demyelinating Polyneuropathy
IDV	Indinavir
IENFD	Intraepidermal Nerve Fibre Density
IFN-α	Interferon-Alpha
INSTIs	Integrase Strand Transfer Inhibitors
KACP	Kenya AIDS Control Project

KNH	Kenyatta National Hospital
LDL	Low-Density Lipoproteins
LOD	Limit of Detection
LPC	Lysophosphocholine
LPV	Lopinavir
LPV/r	Ritonavir-Boosted Lopinavir
LTNP	Long-Term Non-Progressors
MDA	Malondialdehyde
MetLIMS	Metabolomic Laboratory Information Management System Module
MNM	Mono-Neuropathy Multiplex
MNS	Mono-Neuropathy Simplex
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
mtDNA	Mitochondrial DNA
MVC	Maraviroc
NFV	Nelfinavir
NMR	Nuclear Magnetic Resonance
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NO	Nitric Oxide
NtRTI	Nucleotide Reverse Transcriptase Inhibitors
NVP	Nevirapine
O_2	Oxygen
OH	Hydroxyl Radicals
OH-NVP	Hydroxynevirapine
ONOO	Peroxynitrite
OXPHOS	Oxidative Phosphorylation
PI	Protease Inhibitor
PITC	Phenylisothiocyanate
PK	Pharmacokinetic
PN	Polyneuropathy

Pol-γ	Polymerase Gamma
PPRP	Phosphoribosyl-1-Pyrophosphonate
PUFA	Polyunsaturated Fatty Acids
PY	Person Years
RAL	Raltegravir
RPV	Rilpivirine
RTV	Ritonavir
SAM	Significance Analysis of Microarray
SMDB	Serum Metabolome Database
SOP	Standard Operating Procedure
SQV	Saquinavir
STIs	Sexually Transmitted Infections
SWOP	Sex Workers Outreach Programme
TB	Tuberculosis
TC	Total Cholesterol
TDF	Tenofovir Disoproxil Fumarate
TG	Triglyceride
tNRTI	Thymidine-analogue nucleoside reverse transcriptase inhibitor
uCP	Uncoupling Proteins
UDP	Uridine Diphosphate
UGT	Glucuronosyltransferase
UNITID	University of Nairobi, Institute of Tropical, and Infectious Diseases
VIP	Variable Importance in Projection
WHO	World Health Organization

ABSTRACT

Background: Adverse drug reactions (ADRs) associated with prolonged use of antiretroviral therapy (ART) are a serious hindrance to achieving optimal treatment outcomes. Evidence demonstrates that metabolites play an important role in the advancement of ART-related ADRs. So far, there is a paucity of information on the prevalence of long-term ART-related ADRs and the underlying molecular processes in the Kenyan populations.

The development of ART-related ADRs varies substantially between individuals and the role of important factors such as ethnicity that contribute to the heterogeneity among Africans and Europeans populations has not been adequately investigated.

Objective: To describe long-term ART-related ADRs and corresponding variations in plasma metabolites in HIV-infected Kenyan and German patients on long-term ART.

Methods: The description of common ADRs and related determinants was performed through a retrospective analysis of electronic medical records from the Sex Workers Outreach Program (SWOP) (n = 1,450) in Kenya. The study identified factors associated with polyneuropathy through a nested case-control study that involved HIV-infected patients who developed polyneuropathy (cases, 94) and those without polyneuropathy (controls, 212) after long-term ART. The description of the corresponding variations in plasma metabolites was achieved through an exploratory cohort (n = 65). The exploratory cohort comprised of HIV-infected Kenyan (n = 36) and German (n = 29) patients on long-term ART. The metabolomic profiling entailed the collection of blood samples from HIV-infected Kenyan patients, while in the case of the German cohort the study utilized archived plasma samples. Concentrations of plasma metabolites were determined using Liquid Chromatography coupled with Mass Spectrometric techniques (HPLC-MS).

To reveal common ADRs and associated factors, the statistical analyses involved both descriptive and inferential statistics. Further, a bivariate analysis was conducted to compare polyneuropathy related factors between cases and controls. Metabolomic data analysis involved the application of the Analysis of Variance, Partial Least Squares-Discriminant Analysis, Significance Analysis of Microarray and metabolic pathway mapping using Metaboanalyst Programme.

Results: In SWOP clinics, long-term use of ART-regimens containing either stavudine (d4T) or azidothymidine (AZT) or tenofovir disoproxil fumarate (TDF) combined with lamivudine (3TC) and efavirenz (EFV) or nevirapine (NVP) for a median duration of 4.3 years (1.7-5.3) related to the development of ADRs among HIV-infected Kenyan patients. The common ADRs in SWOP facilities included lipodystrophy 211 (41.7%), polyneuropathy 149 (29.4%), anaemia 78 (15.4%), hepatotoxicity 47 (9.3%), skin rash 14 (2.8%) and renal toxicity 7 (1.4%). The development of ADRs accounted for 287 (76%) of all ART regimen changes, mostly due to stavudine-related lipodystrophy (n = 204, 76%) and polyneuropathy (n= 54, 20%).

The risk of patients developing an ADR during 24 months of ART was statistically significant (Log-rank test, p = 0.044). While the use of tenofovir disoproxil fumarate (TDF) was protective [hazards ratio 0.50; 95% CI: 0.3-0.8], older patients (≥ 40 years) had an increased risk of developing an ADR [hazards ratio 1.0; 95% CI: 1.0-1.1]. Patients with polyneuropathy were significantly older (0 = 0.017) and had a higher systolic blood pressure (p = 0.025). Apparently, TDF was significantly related to the development of polyneuropathy (p = 0.017), suggesting an earlier exposure to stavudine before changing to TDF.

The metabolomic analysis revealed raised levels of phosphatidylcholine diacyl C42:0 (PC aa C42:0) in HIV-infected Kenyan patients with polyneuropathy (0.8±0.3) and without polyneuropathy (0.7±0.2) compared to HIV-infected German patients prior to ART (0.5±0.2) and after initiation of ART (0.5±0.2). Correspondingly, levels of

LysoPC a C17:0 were elevated in HIV-infected Kenyan patients with polyneuropathy (2.3±0.9) and without polyneuropathy (2.1±0.9) compared to HIV-infected German patients prior to ART (1.6±0.5) and after initiation of ART (1.5±0.5) (variable importance in projection > 1, $p = 0.05$). There were elevated levels of short-chain acylcarnitine (propionylcarnitine) in HIV-infected Kenyan patients with stavudine-related polyneuropathy (0.3±0.1) compared to HIV-infected polyneuropathy free, ART-naive German patients (0.28±0.1), polyneuropathy free German patients on ART (0.2±0.1) and polyneuropathy free Kenyan patients on ART (0.2±0.1) ($p < 0.001$).

Discussion and conclusion: Lipodystrophy and polyneuropathy were the most commonly encountered ADRs in SWOP clinics, accounting for most of the ART drug changes. Although the prevalence of ADRs vary from one study to another, factors such as variations in ADR reporting procedures and concomitant medication may have influenced these findings. Older age significantly related to an increased hazard of developing ADRs and a significantly increased risk of developing polyneuropathy. This suggests that the burden of ADRs such as polyneuropathy is likely to be high in older HIV-infected populations compared to younger populations on long-term ART.

Typical long-term ADRs were more likely to develop in older HIV-infected Kenyan patients compared to HIV-infected German patients. Although patients in SWOP clinics tolerated well the use of TDF-based regimens, a significant number of patients on these regimens ended up with polyneuropathy. The development of polyneuropathy in patients, who never used stavudine, suggests an ongoing burden of polyneuropathy even after the ban on stavudine. The findings of this study further demonstrate that many plasma metabolites undergo significant alterations in HIV-infected patients before and after long-term exposure to ART. The observed up-regulation of glycerophospholipids namely PC aa C42:0 and LysoPC a C17:0 and short-chain acylcarnitine namely propionylcarnitine (C3) suggests that glycerophospholipids and fatty acid oxidation metabolism could be the most

disturbed pathways in HIV-infected patients on long-term ART. The affected pathways seemed to correlates well with clinical manifestations such as polyneuropathy, lipoatrophy, pancreatitis, lactic acidosis and nephrotoxicity. The current results may serve as a starting point for the search of new biomolecules that could be potential biomarkers for early detection of adverse drug reactions. There is a need for further studies to confirm causal pathway between HIV-infection, metabolomic changes and clinical outcomes.

TABLE OF CONTENT

Contents	Page
DECLARATION	I
ACKNOWLEDGEMENTS	III
RESEARCH OUTPUTS	IV
SUBMITTED ARTICLES	IV
ORAL AND POSTER COMMUNICATION	IV
ACRONYMS AND ABBREVIATIONS	V
ABSTRACT	IX
TABLE OF CONTENT	XIII
LIST OF FIGURES	XVII
LIST OF TABLES	XVIII
CHAPTER ONE:	**1**
INTRODUCTION AND LITERATURE REVIEW	**1**
1.1. Synopsis of Human Immunodeficiency Virus	1
1.1.1. Prevalence of Human Immunodeficiency Virus	1
1.1.2. The Structure of Human Immunodeficiency Virus	3
1.1.3. The Life Cycle of Human Immunodeficiency Virus	4
1.1.4. Pathophysiology of HIV Disease	6
1.2. Chemotherapy of HIV-Infection	9
1.2.1. Reverse Transcriptase Inhibitors	9
1.2.2. Viral Entry Inhibitors	11
1.2.3. Integrase Strand Transfer Inhibitors	12
1.2.4. Protease Inhibitors	13
1.2.5. Pharmacokinetic Enhancers	15
1.3. Metabolism of Antiretroviral Drugs	15
1.3.1. Metabolism of Protease Inhibitors	16
1.3.2. Metabolism of Nucleoside Analogues	17
1.3.3. Metabolism of Integrase Inhibitors	18
1.3.4. Metabolism of Entry Inhibitors	18
1.4. Antiretroviral Drug Toxicity	19
1.4.1. Long-term Adverse Drug Reactions of ART	20
1.4.1.1. Fat Redistribution	20
1.4.1.2. Changes in Lipid Metabolism	22
1.4.1.3. Peripheral Neuropathy	23
1.4.1.4. Disorders of Glycolytic Metabolism	26
1.4.1.5. Hyperlactatemia and Lactic Acidosis	27
1.4.1.6. Mitochondrial Toxicity	28
1.4.1.7. Oxidative Stress	29
1.4.2. Prevalence of ART-Related Adverse Drug Reactions	30
1.4.2.1. ART-Related Adverse Drug Reactions in Developed Countries	31
1.4.2.2. ART-Related Adverse Drug Reactions in Sub-Saharan Africa	32
1.4.2.3. ART-Related Adverse Drug Reactions in Kenya	33
1.5. The Role of Metabolites in the Development of Adverse Drug Reactions	34
1.5.1. Drug Related Metabolites	35
1.5.1.1. Phosphorylated Metabolites of NRTIs/ NtRTIs' Analogues	35

1.5.1.2. Genetic Predisposition to NRTIs/ NtRTIs-Related Toxicity 37
1.5.1.3. Hydroxylated Metabolites of NNRTIs' Analogues 38
1.5.1.4. Genetic Predisposition to NNRTIs' Toxicity 40
1.5.2. Endogenous Metabolites 41
1.5.3. Metabolomic Platform for Measuring Drug Toxicity 43
1.5.3.1. Targeted Metabolomics 43
1.5.3.2. Untargeted Metabolomics 44
1.6. Study Rationale 45
1.7. Research Question 48
1.8. Main Objective 49
1.8.1. Specific Objectives 49

CHAPTER TWO: 50
THE PREVALENCE AND DETERMINANTS OF ADVERSE DRUG REACTIONS IN HIV-INFECTED PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY 50
2.1. INTRODUCTION 50
2.2. METHODS 50
2.2.1. Study Design 50
2.2.2. Study Sites 50
2.2.3. Study Population 51
2.2.4. Inclusion and Exclusion Criteria 52
2.2.5. Sample Size 52
2.2.6. Data Collection 53
2.2.7. Study Variables 55
2.2.8. Definition of Terms 55
2.2.9. Types of Adverse Drug Reactions Evaluated in the Study 56
2.2.10. Statistical Analysis 58
2.2.11. Ethical Considerations 59
2.3. RESULTS 59
2.4. DISCUSSION 67
2.5. CONCLUSIONS 71

CHAPTER THREE: 72
FACTORS INFLUENCING DEVELOPMENT OF POLYNEUROPATHY IN HIV-INFECTED PATIENTS IN THE SEX WORKERS OUT-REACH PROGRAMME IN KENYA 72
3.1. INTRODUCTION 72
3.2. METHODS 72
3.2.1. Study Design 72
3.2.2. Study Site 73
3.2.3. Study Population 73
3.2.4. Inclusion and Exclusion Criteria 73
3.2.5. Sample Size 74
3.2.6. Selection of Cases with Polyneuropathy 74
3.2.7. Selection of Controls 75
3.2.8. Data Collection 75
3.2.8.1. Anthropometric Measurements 76
3.2.9. Data Analysis 76

3.2.10. ETHICS CONSIDERATIONS 76
3.3. RESULTS 77
3.4. DISCUSSIONS 82
3.5. CONCLUSION 85

CHAPTER FOUR: 87
VARIATIONS IN METABOLOMIC PROFILES IN HIV-INFECTED PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY 87
4.1. INTRODUCTION 87
4.2. METHODS 87
4.2.1. STUDY DESIGN 87
4.2.2. STUDY SITES 87
4.2.3. STUDY POPULATION 88
4.2.3.1. THE KENYAN COHORT 88
4.2.3.2. THE GERMAN COHORT 88
4.2.4. SAMPLES SIZE 89
4.2.5. SELECTION OF STUDY POPULATION 89
4.2.6. COLLECTION OF PLASMA SAMPLES 90
4.3. MATERIALS AND METHODS 91
4.3.1. STUDY SITE 91
4.3.2. MATERIALS 91
4.3.3. METHODS 92
4.3.3.1. REGISTRATION OF THE ASSAY 92
4.3.3.2. ASSAY PREPARATION 92
4.3.3.3. VALIDATION OF THE ABSOLUTEIDQ P180 KIT PLATE 92
4.3.3.4. PREPARATION OF SAMPLES 93
4.3.3.5. EXTRACTION OF METABOLITES 93
4.3.3.6. ASSAY OF SAMPLES USING THE LIQUID CHROMATOGRAPHY- MASS SPECTROMETER 94
4.3.3.7. INTERPRETATION OF MASS SPECTROMETRIC DATA 95
4.3.3.8. EVALUATION AND EXPORT OF DATA 96
4.3.3.9. MEASUREMENTS FOR QUALITY CONTROL SAMPLES 96
4.3.4. STATISTICAL AND MACHINE LEARNING STRATEGIES 96
4.3.4.1. STATISTICAL ANALYSIS 96
4.3.5. MACHINE LEARNING METHODS 97
4.3.5.1. PARTIAL-LEAST SQUARES DISCRIMINANT ANALYSIS TECHNIQUE 97
4.3.5.2. SIGNIFICANCE ANALYSIS OF MICROARRAY 97
4.3.5.3. PATHWAY MAPPING 98
4.3.6. ETHICS CONSIDERATIONS 99
4.4. RESULTS 99
4.4.1. CHARACTERISTICS OF HIV-INFECTED KENYAN AND GERMAN PATIENTS IN THE STUDY COHORT 99
4.4.2. DETECTED PLASMA METABOLITES 99
4.4.3. SIGNIFICANTLY ALTERED METABOLITES 102
4.4.4. DISTRIBUTION OF SIGNIFICANT METABOLITES IN STUDY POPULATION 105
4.4.5. METABOLIC PATHWAY ANALYSIS 110
4.4.6. POTENTIAL BIOMARKERS FOR POLYNEUROPATHY 110
4.5. DISCUSSION 112
4.5.1. ALTERATIONS IN GLYCEROPHOSPHOLIPIDS 113

4.5.2. CHANGES IN PROPIONYLCARNITINE LEVELS 115
4.5.3. CHANGES IN OTHER METABOLITES 116
4.6. CONCLUSION 118

CHAPTER FIVE: GENERAL DISCUSSION 120
5.1. INTRODUCTION 120
5.2. SAFETY OF LONG-TERM ANTIRETROVIRAL THERAPY 120
5.3. AGING AND LONG-TERM ANTIRETROVIRAL THERAPY 123
5.4. POTENTIAL BIOMARKERS TO ANTIRETROVIRAL TOXICITY 125
5.5. STUDY LIMITATIONS 130

CHAPTER SIX:
CONCLUSIONS AND RECOMMENDATIONS **132**
6.1. CONCLUSIONS 132
6.2. RECOMMENDATIONS 132

REFERENCES **134**
APPENDICES **166**
APPENDIX A: DATA ABSTRACTION INSTRUMENTS 166
APPENDIX B: ETHICS CLEARANCE 179
APPENDIX C: INFORMED CONSENT 180
APPENDIX D: A LIST OF PLASMA METABOLITES MEASURED BY BIOCRATES KIT 185
APPENDIX E: CHARACTERISTICS OF THE METABOLITES MEASURED BY BIOCRATES KIT 188
APPENDIX F: MATERIAL TRANSFER DOCUMENTS 193

LIST OF FIGURES

FIGURE PAGE

1.1: The worldwide distribution of strains of HIV-1 major group……2

1.2: Schematic structure of the HIV……4

1.3: Schematic structure of the HIV……....5

1.4: The CD4+ T cells population versus viral load during HIV-infection……...7

1.5: Reverse transcriptase enzyme……11

1.6: Structures of three domains A, B and C of HIV integrase enzyme……..13

1.7: HIV protease enzyme……..14

1.8: Kinds of neuropathies in HIV-infected patients…….24

1.9: Models of neurotoxicity……..26

2.1: A flow diagram illustrating the recruitment of HIV-infected patients from the SWOP clinics……60

2.2: Prevalence of antiretroviral therapy-related adverse drug reactions in HIV-infected patients on long-term ART at the SWOP clinics……62

2.3: The distribution of antiretroviral therapy-related adverse drug reactions in HIV-infected patients on long-term ART at the SWOP clinics……63

2.4: Duration of the first occurrence of an adverse drug reaction in HIV-infected patients in the SWOP clinics…….66

3.1: A flow diagram illustrating the recruitment of HIV-infected patients with polyneuropathy and those without polyneuropathy at the Pumwani clinic……77

3.2: Percentage of d4T-based regimens during seven visits by HIV-infected patients at the Pumwani clinic……81

3.3: Duration of the development of polyneuropathy in HIV-infected patients at the Pumwani clinic……...82

4.1: A strategy applied to identify plasma metabolites in HIV-infected Kenyan and German patients on long-term TDF/3TC/NVP regimens……...101

4.2: The concentration of plasma metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy…….....103

4.3: Partial least squares discriminant analysis score plots for HIV-infected Kenyan and German patients on long-term ART……...107

4.4: Variable of Importance projection scores for metabolites that distinguished HIV-infected cohorts……109

LIST OF TABLES

TABLE_____PAGE

1.1: Antiretroviral recommended for HIV treatment in Kenya……10

1.2: An outline of the ARV-related adverse drug reactions……..19

1.3: Chemical categories in the Serum Metabolome Database…….....42

2.1: Baseline characteristics of HIV-infected study patients on long-term ART at the SWOP clinics……61

2.2: Types of adverse drug reactions resulting in changes of antiretroviral drugs in HIV-infected patients in SWOP clinics……64

2.3: Factors contributing to changes in antiretroviral drugs in HIV-infected patients (n = 380) in the SWOP clinics……64

2.4: Risk factors for time to the first occurrence of an adverse drug reaction in HIV-infected patients in the study SWOP clinics……..67

3.1: Baseline characteristics of 306 HIV-infected patients with polyneuropathy and those without polyneuropathy at the Pumwani clinic……78

3.2: Risk factors for polyneuropathy in HIV-infected patients with polyneuropathy and those without polyneuropathy at Pumwani clinic……...80

4.1: Characteristics of HIV-infected Kenyan and German patients in the study cohort……100

4.2: Catalog of 26 metabolites in HIV-infected Kenyan and German cohort on long-term antiretroviral therapy……...102

4.3: Significantly elevated levels of metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy……..104

4.4: Significantly reduced levels of metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy……..105

4.5: Modulated metabolic pathways in HIV-infected Kenyan and German patients on long-term ART……110

4.6: Significant metabolites identified by significance analysis of microarray (SAM) in HIV-infected Kenyan and German patients on long-term ART…….....111

4.7: Plasma levels of three potential biomarkers identified in HIV-infected Kenyan and German patients on long-term ART……..112

CHAPTER ONE:

INTRODUCTION AND LITERATURE REVIEW

1.1. Synopsis of Human Immunodeficiency Virus

The Human Immunodeficiency Virus infection (HIV) damages a kind of white blood cells (CD4 + T cells) that fight the infection. The condition of Acquired Immunodeficiency Syndrome (AIDS), which predisposes to "opportunistic infections, is a definitive phase of HIV disease" (Osmond, 1998).

Both "HIV-1 and HIV-2 forms of viruses belong to a family of human retroviruses known as *Retroviridae* and the subfamily known as Lentiviruses" (Robertson *et al.*, 1995). HIV-1 is widely spread and the most virulent type of infection because of its increased ease of transmission. HIV-2 features a higher prognosis than HIV-1 and is particularly localised in "West Africa. The strains of HIV-1 are classified into three categories namely, major group (M), outlier group (O), and new groups (N and P)" (Ananya, 2016). **Figure 1.1** shows "the worldwide distribution of strains of HIV-1 major group"(Lee *et al.*, 2013).

1.1.1. Prevalence of Human Immunodeficiency Virus

Since the year 2000, "HIV has infected 38.1 million individuals and 25.3 million deaths have come about because of AIDS-related infections" (UNAIDS, 2016).

Towards the end of 2014, there were nearly 36.9 million infections of HIV around the world (WHO, 2015).

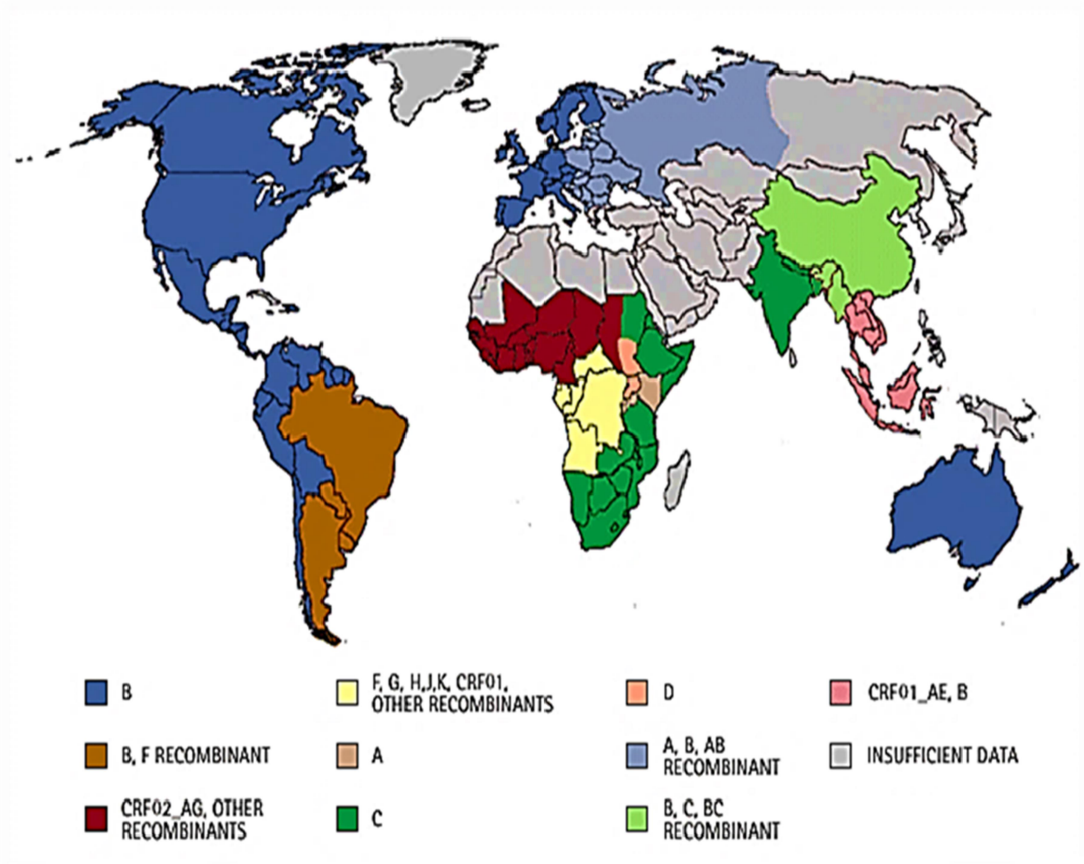

Figure 1.1: The "worldwide distribution of strains of HIV-1 major group" (Jakobsen *et al.*, 2010).

Around 70% of the infected people live in sub-Saharan Africa. Out of all the HIV-infected Kenyan patients (1.6 million), about 59% of them receive ART (UNAIDS, 2016). A report by the National AIDS Control Council showed that Kenya has a mean HIV prevalence rate of 6 % (NACC, 2014a; NACC, 2014b). The HIV-infection has largely affected counties within the western region of Kenya including

Homabay, Siaya, and Kisumu, whereas Wajir, Tana River, and Marsabit are the least affected.

1.1.2. The Structure of Human Immunodeficiency Virus

The HIV has a spherical structure measuring about 120 nm in diameter. It can actually fit into in a red corpuscle 60 times. Its genome comprises of two duplicates of single-stranded ribonucleic acid (RNA), encapsulated by a cone-like capsid made out of the viral protein p24 (**Figure 1.2**). To replicate, HIV utilizes the host cell in transcribing its RNA into deoxyribonucleic acid (DNA).

Reverse transcriptase is "the enzyme that catalyses the transcription of RNA into DNA" (Ferris, *et al.*, 1990; Lu *et al*., 2011). The viral surface has "viral glycoproteins (gp) that include the external glycoprotein 120 (gp120) and the trans-membrane glycoprotein 41 (gp41)" (Zhu *et al.,* 2006). Following the recognition of "CD4 receptors on the surface of the target cells by gp120"(Rajarapu, 2014), "the gp41 mediates the fusion of the host and viral membrane"(Mateu, 2013).

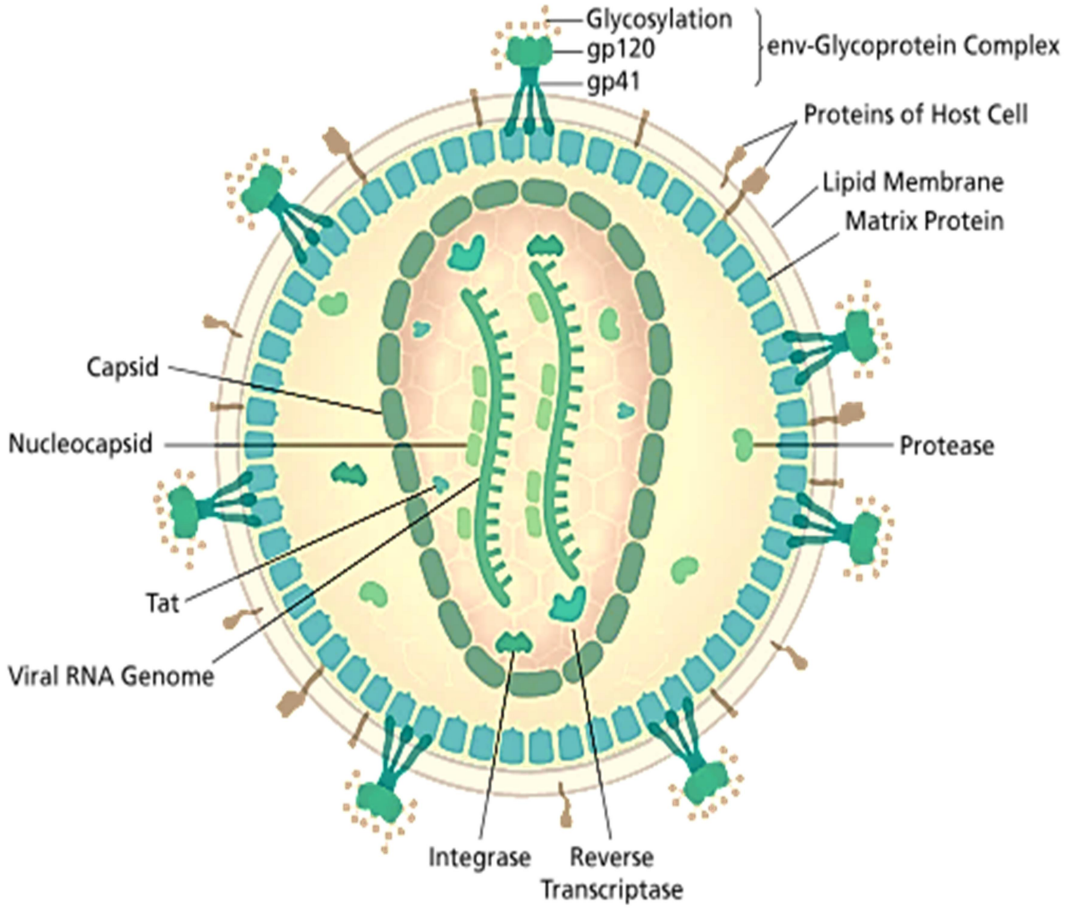

Figure 1.2: Schematic structure of the HIV (Splettstoesser, 2015).

1.1.3. The Life Cycle of Human Immunodeficiency Virus

The HIV disease targets cells expressing the CD4 receptors and chemokines that incorporate "the chemokine co-receptor type-5 (CCR5) and CXC chemokine receptor type-4 (CXCR4)"(Dau and Holodniy, 2008). The "R5 viruses use solely the CCR-5 and X-4 viruses use solely the CXCR4" (Fenyo et al., 2011). These co-receptors additionally facilitate cellular response to the HIV. The "T-tropic HIV strains bind to X4 co-receptor cells, primarily the T-cells whereas the M-tropic strains bind to R5 co-

receptor cells just like the macrophages" (Fenyo *et al.,* 2011). **Figure 1.3** offers a diagram of the life cycle of the HIV inside the CD-4+ cell.

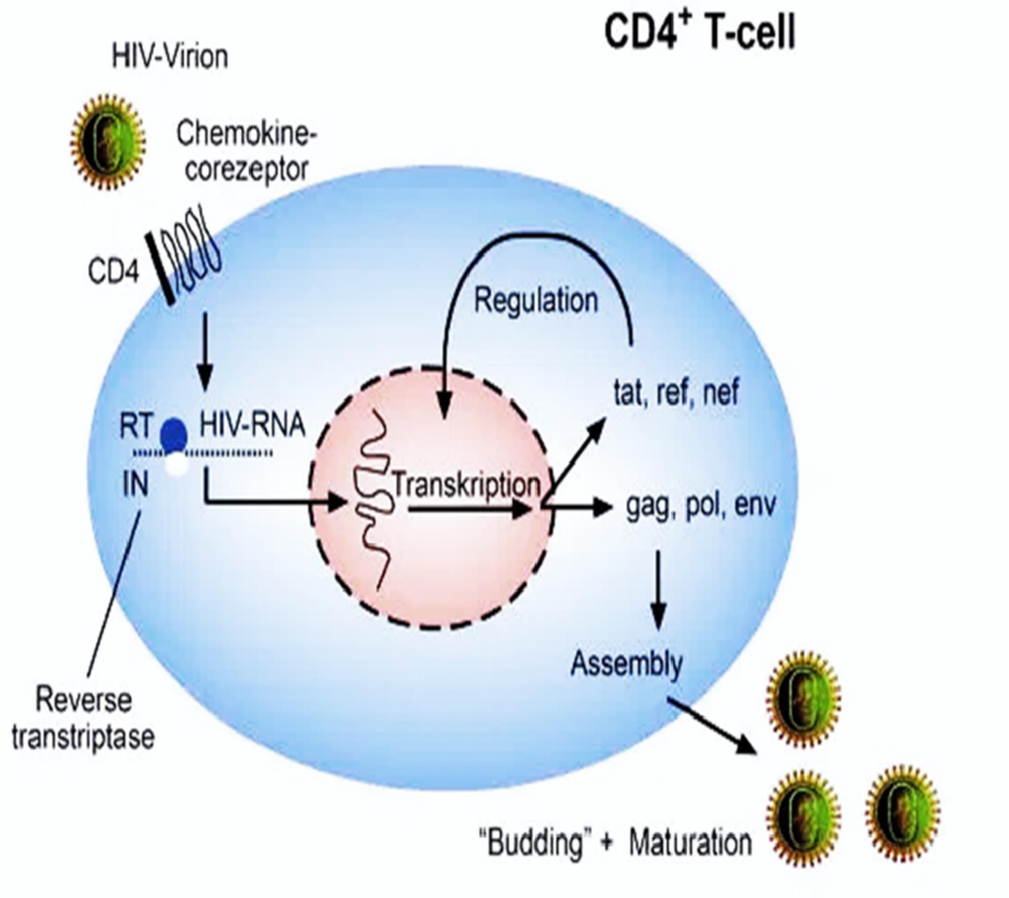

Figure 1.3: Life cycle of HIV inside a host CD4+ T-cell (Rubbert *et al.,* 2011).

The CD4 and CCR5 receptors situated on T-helper cells powerfully attract the viral gp120. Fusion of the outer membranes of the HIV and the T-helper cell happens shortly when HIV envelope protein binds to CD4 and CCR5. At this point, there is the discharge of viral contents into the target cell (Wilen *et al.,* 2012). Following "fusion of HIV cell to the host cell surface, the viral RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell" (Foster, 2014). The process of

reverse transcription leads to the formation of viral DNA, and after transportation across the nucleus, the viral DNA then integrates into the host DNA.

To get to the cell's own particular genes and DNA, the viral DNA then invades the nucleus. The integrase enzyme in the virus splices the strands of the host cell DNA along these lines serving the combination of the virion into the host genome (Zheng *et al.*, 2005). Once integration is complete, the host cell remains infected until death. The integrated viral DNA then becomes a provirus.

The proviral DNA is capable of cellular latency for several years. Throughout "cell division, the proviral DNA transcribes into RNA" (Satyajit, 2010). Viral proteins and polyproteins then undergo translation to create the RNA. The RNA and proteins migrate along "the host cell membrane to assemble into new virions" (Satyajit, 2010). According to Zhang et al. (2015), another life cycle begins when the recently formed virions bud off from the host cell surface then enter into the blood to infect new clean CD4+ cells.

1.1.4. Pathophysiology of HIV Disease

HIV transmission happens during the deposition of the virus on the genital mucosal surface at the time of sexual intercourse (Chan, 2005). Direct transmission happens through the inoculation of the virus into the blood by intravenous (IV) needle sharing among drug users (Panda *et al.*, 2005). In new-born children, transmission

happens "vertically from mother to the child in utero, amid birth, or through breastfeeding" (Coutsoudis *et al.,* 2010). Three noteworthy phases of HIV disease prognosis are an acute phase, clinical latency stage, and symptomatic stage (**Figure 1.4**).

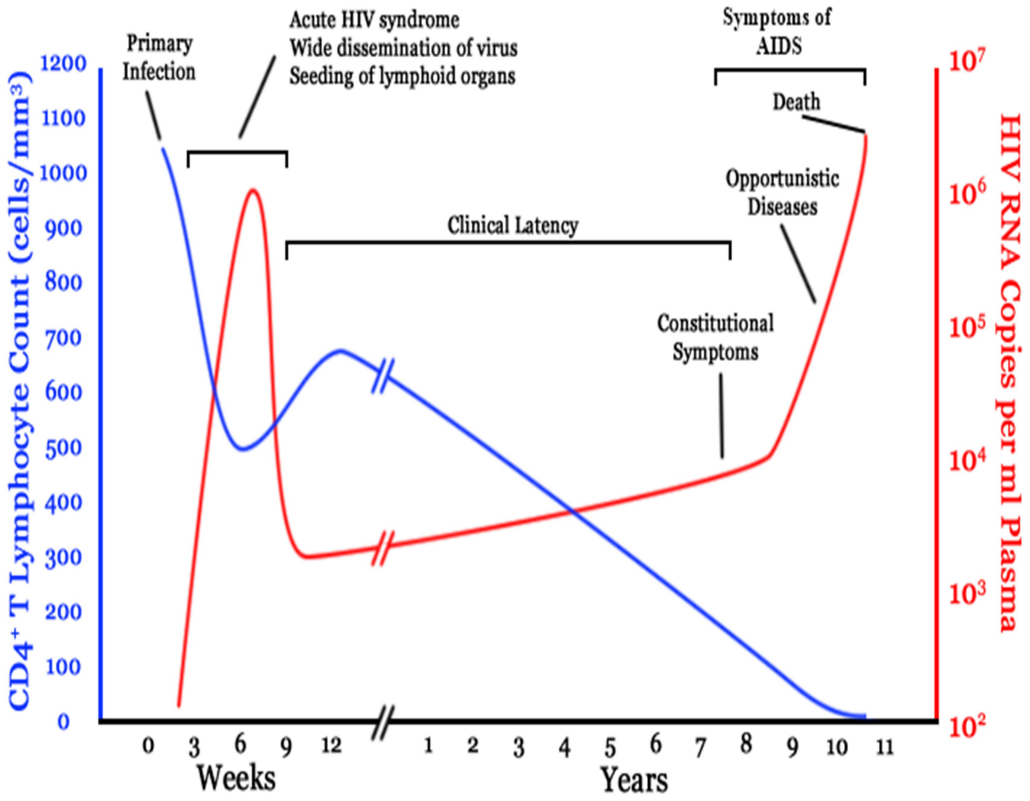

Figure 1.4: The CD4+ T cells population versus viral load during HIV-infection (Fauci *et al.,* 1996).

1.1.4.1. Acute Infection

The acute infection phase presents as an initial burst of viremia and "last up to 6 months after infection. At this stage, anti-HIV antibodies stay undetectable" (NASCOP, 2016). However, HIV, RNA, or p24 antigens are present. In the acute phase, symptoms experienced by about 40 to 90% of patients include fever,

lymphadenopathy, sore throat, skin rash, myalgias, and arthralgias (Daar *et al*., 2001; Hecht *et al.,* 2002). After the initial symptoms, there is "speedy depletion of the memory CD4+ T-cells and high viral load" (Hernandez-Vargas and Middleton, 2013). There is a quick consumption of the cells of the immune system and accordingly the viral replication flourishes. At first, the exhaustion of fringe CD4+ T cells goes uncontrolled (Albrecht *et al.,* 2007). However, with the activation of the opposing HIV-1 CD5+ cytotoxic T cells, there is restricted viral replication (Palmer *et al.*, 2011).

1.1.4.2. Clinical Latency

Clinical latency also named as "asymptomatic HIV infection" or "chronic HIV infection" can last up to ten years. In the absence of ART, most patients in the end progress to AIDS. However, about 5% of patients can still maintain a high level of CD4+ T cells while not using antiretroviral drugs for over five years. This small group of patients comprises of the long-term non-progressors (LTNP) or HIV controllers (Madec *et al.,* 2009). Elite controllers or suppressors representing about 1 in 300 infected people can maintain undetectable viral load without ART (Walker, 2007).

During the symptomatic stage, the immune system is extremely weak. Opportunistic infections and infection-related cancers can strike with ease. In the absence of ART, patients with AIDS typically, survive for about 3 years. Some patients can still

maintain "high CD4 cell counts ranging from 500 to 1, 600 cells/mm^3 (NASCOP, 2016). In any case, the minute astute diseases strike regardless of CD4 tally, the diagnosis for such patients leads to AIDS. The patient advances to AIDS once the CD4+ T cells fall below 200 cells/mm^3 (NASCOP, 2016).

1.2. Chemotherapy of HIV-Infection

Presently, Highly Active Antiretroviral therapy (HAART) forms the standard treatment protocol contained in many guidelines worldwide. The ART recommended "for use as a first-line HIV treatment" (Vitoria *et al.,* 2016) include tenofovir disoproxil fumarate (TDF), "lamivudine (3TC), azidothymidine (AZT), stavudine (d4T), nevirapine (NVP), efavirenz (EFV) and emtricitabine (FTC)"(Susami *et al.,* 2009). The recommended second-line ART regimens include all protease inhibitors (PIs), abacavir sulphate (ABC), and didanosine (ddI). Antiretroviral medication recommended for use as third-line HIV treatment regimen include raltegravir (RAL), darunavir (DRV), and etravirine (ETR). Kenya's guidelines recommend a combination of a minimum of three antiretroviral drugs from different categories (**Table 1.1**).

1.2.1. Reverse Transcriptase Inhibitors

With the assistance of invert transcriptase protein, HIV experiences "turn around" transcription to change over RNA to DNA. Antiretroviral drugs of the nucleoside

reverse transcriptase inhibitors (NRTIs) category inhibit reverse transcription (Wei, *et al.*, 1995; Eron *et al.*, 1995; Das and Arnold, 2013).

Table 1.1: Antiretroviral recommended for HIV treatment in Kenya (NASCOP, 2016)

Age	First-line ART	Second-line ART	Third-line ART
Children	ABC (or AZT) + 3TC + LPV/r ABC+ 3TC + NVP (or RAL) ABC + 3TC + EFV (or RAL) ABC (or AZT) + 3TC + LPV/r AZT + 3TC + EFV (or RAL) TDF (or ABC) + 3TC + EFV (or NVP) TDF (or ABC or AZT) + 3TC + LPV/r (or ATV/r) AZT + 3TC + EFV (or NVP)	AZT + 3TC + LPV/r AZT + 3TC + LPV/r DRT-based 2nd line ABC + 3TC + LPV/r AZT + 3TC + ATV/r (or LPV/r) TDF + 3TC + ATV/r (or LPV/r)	RAL (or DTG) + 3TC + DRV + RTV AZT + RAL (or DTG) + 3TC + DRV + RTV ABC/TDF + RAL (or DTG) + 3TC + DRV + RTV ETV + 3TC + DRV + RTV
Adults	TDF (or ABC) + 3TC + EFV (or NVP) AZT + 3TC + EFV (or NVP) TDF (or ABC or AZT) + 3TC + ATV/r (or LPV/r)	AZT + 3TC + ATV/r TDF + 3TC + ATV/r DRT-based 2nd line	RAL (or DTG) + 3TC + DRV + RTV AZT + RAL (or DTG) + 3TC + DRV + RTV TDF + RAL (or DTG) + 3TC + DRV + RTV ETV + 3TC + DRV/r
Key: Abacavir –ABC, Didanosine – ddI, Emtricitabine – FTC, Lamivudine - 3TC, Efavirenz – EFV, Nevirapine – NVP, Tenofovir disoproxil fumarate – TDF, Delavirdine – DLV, Zalcitabine – ddC, Zidovudine –ZDV or Azidothymidine – AZT, Atazanavir – ATV, Atazanavir/ritonavir - ATV/r, Ritonavir – RTV, Indinavir - IDV, Fosamprenavir – FPV, Nelfinavir – NFV, Lopinavir/Ritonavir - LPV/r, Tipranavir – TPV, Amprenavir – APV, Saquinavir mesylate – SQV, Darunavir – DRV, Darunavir/ritonavir - DRV/r, Dolutegravir – DTG, Raltegravir -RAL.			

The bulk of antiretroviral drugs presently in use belong to nucleoside analogues. The nucleoside analogues, for example, AZT, ddI, ddC, d4T, and 3TC are inhibitors of the enzymatic effects of reverse transcriptase enzyme (Anderson and Mitchell, 2000). The NRTIs terminate the DNA chain, by preventing different nucleosides from being incorporated into the DNA chain (Das and Arnold, 2013). To be effective, NRTIs undergo intracellular phosphorylation. On the other hand, "Non-Nucleoside reverse transcriptase Inhibitors (NNRTIs) such as EFZ and NVP do not need intracellular

phosphorylation" (Ohrngren, 2011). Through this process, these agents inhibit the activities of reverse transcriptase enzymes (Saitoh *et al.,* 2011) (**Figure 1.5**).

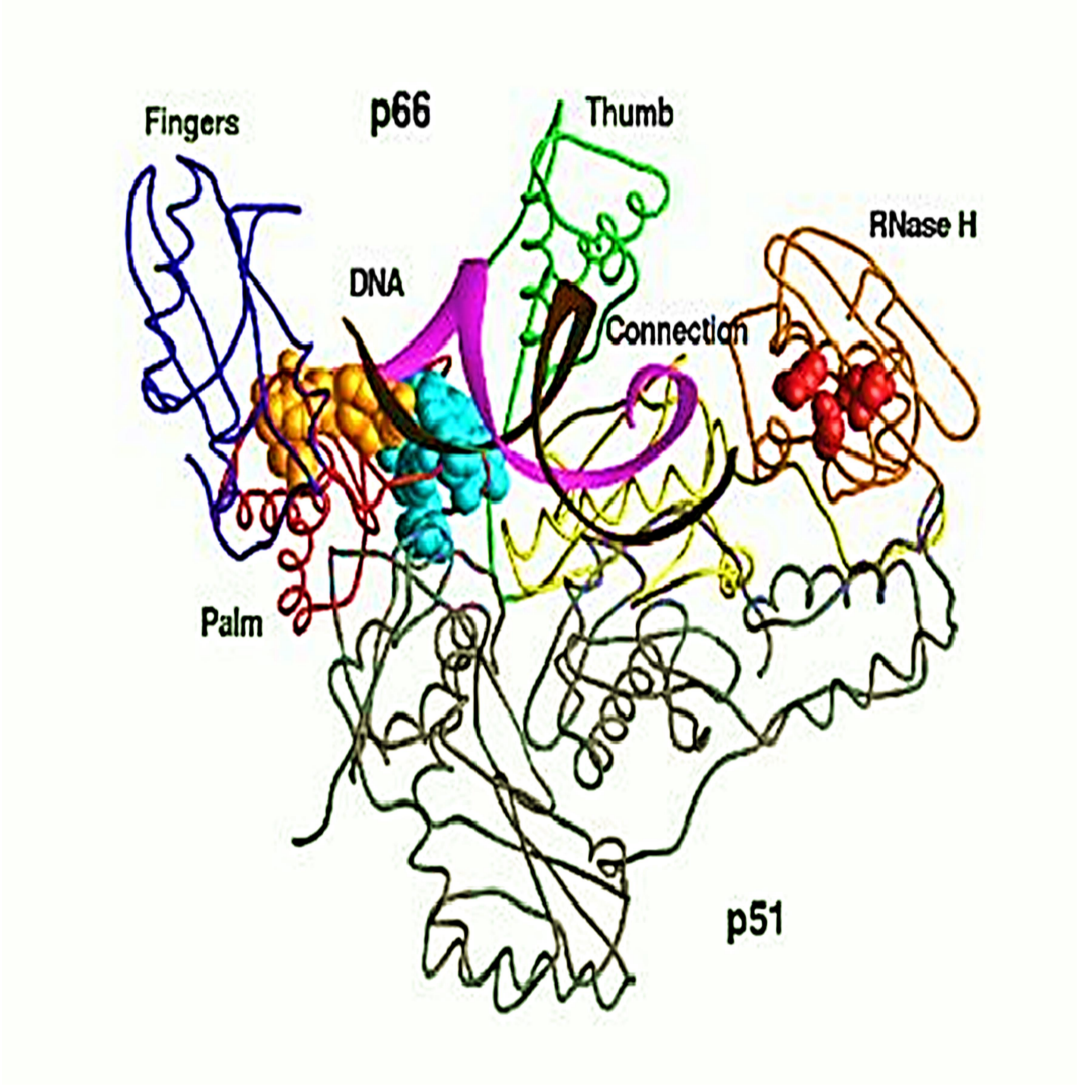

Figure 1.5: Reverse transcriptase enzyme (Racaniello and Despommier, 2010).

1.2.2. Viral Entry Inhibitors

HIV entry starts with the "attachment of the viral gp120 to the CD4 receptor then conformational changes of gp120 takes place"(Racaniello and Despommier, 2010). This exposes structural parts on the V3 loop that binds to the CCR5. Thereupon, the

acceptance of an auxiliary reworking in gp41 that embeds a hydrophobic combination peptide locale into the objective cell membrane brings the virus and cell membrane in close connection to start combination (Agbelusi *et al.,* 2013). Finally, the virus then enters the host target cell" (Racaniello and Despommier, 2010). The viral processes of binding, fusion and entry are the main targets of interference by the entry inhibitors (Biswas *et al.,* 2007). Current entry inhibitors include CCR5 co-receptor antagonist and fusion inhibitors.

Through change of gp120 adaptation and reliance on "tropism, HIV binds to either the CCR5 or the CXCR4 co-receptor" (Moore *et al.,* 2004). Subsequently, the viral gp41 get to be uncovered permitting cell combination to happen. The entry inhibitors, for instance, maraviroc (MVC), bind to "CCR5 and prevent the binding of gp120 to the co-receptor" (Grilo, 2012), in this manner allowing passage of the cell-free virus into the host cell (Grilo, 2012). Fusion inhibitors such as Enfuvirtide (T-20) act on the fringe of "the target helper CD4+ T cell to stop HIV fusion and infection" (Rockstroh *et al.,* 2012). They bind to an envelope protein and block the structural changes essential for the virus fusion with the host helper CD4+ T cell.

1.2.3. Integrase Strand Transfer Inhibitors

Integrase strand transfer inhibitors (INSTIs) inhibit the enzymatic activities of the integrase enzyme that embeds the viral genome into the DNA of the host cell (**Figure 1.6**). The enzyme extracts two nucleotides out of a solitary strand of the DNA, and

afterwards embeds the viral DNA into host cell DNA. This can be the mechanism of action for all INSTIs.

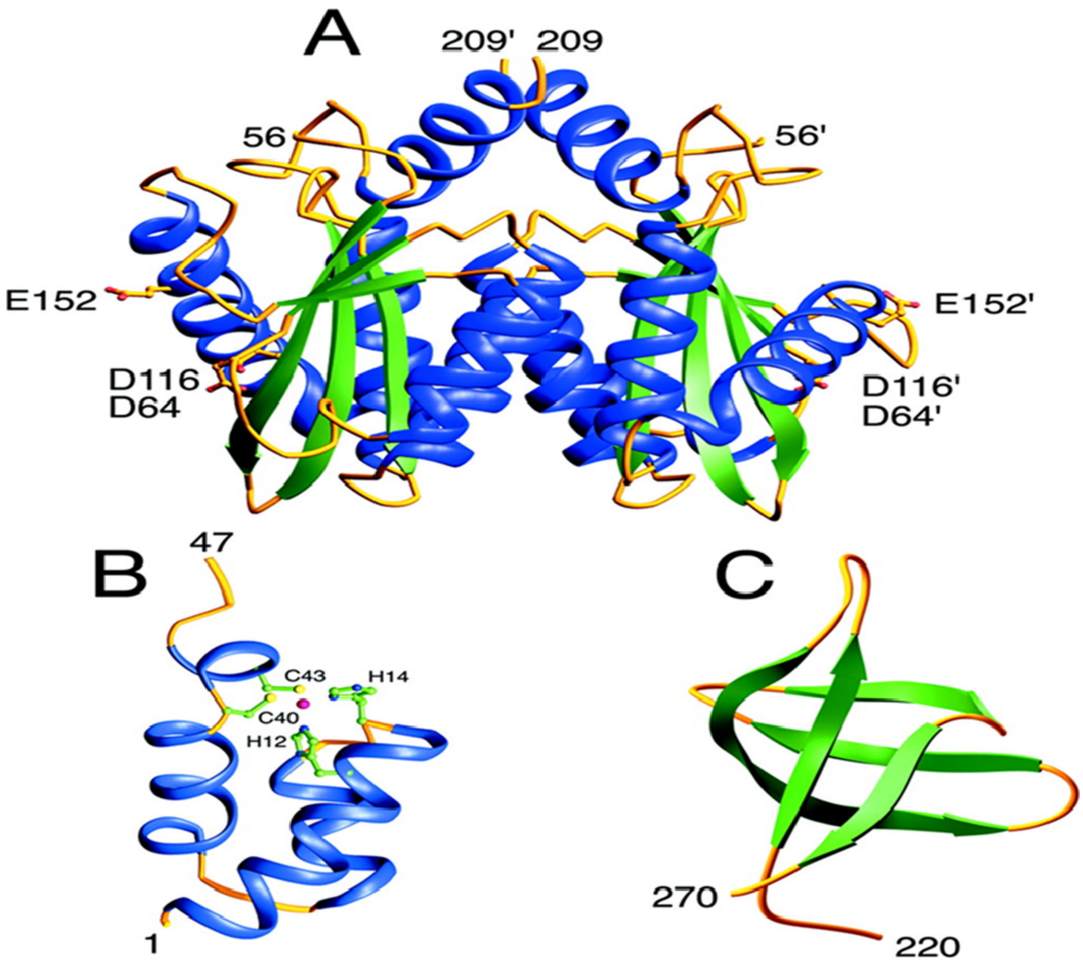

Figure 1.6: Structures of three domains A, B and C of HIV integrase enzyme (Craigie, 2001).

1.2.4. Protease Inhibitors

After effective binding and integration of the viral genetic code, the infected cell is "adapted" to compound new hereditary material and proteins for the HIV. The viral protease enzyme separates the recently translated viral Gag and Gag-Pol polyproteins to functional proteins vital for viral assembly. Protease inhibitors (PIs)

inhibit the protease enzyme by inhibiting the assembly of the new virus. The HIV protease enzyme has two indistinguishable subunits of 99 amino acids (**Figure 1.7**), each of which forms the dynamic site with a reactant aspartate (D25) (Blundell *et al.*, 1998; Dwight, 2013).

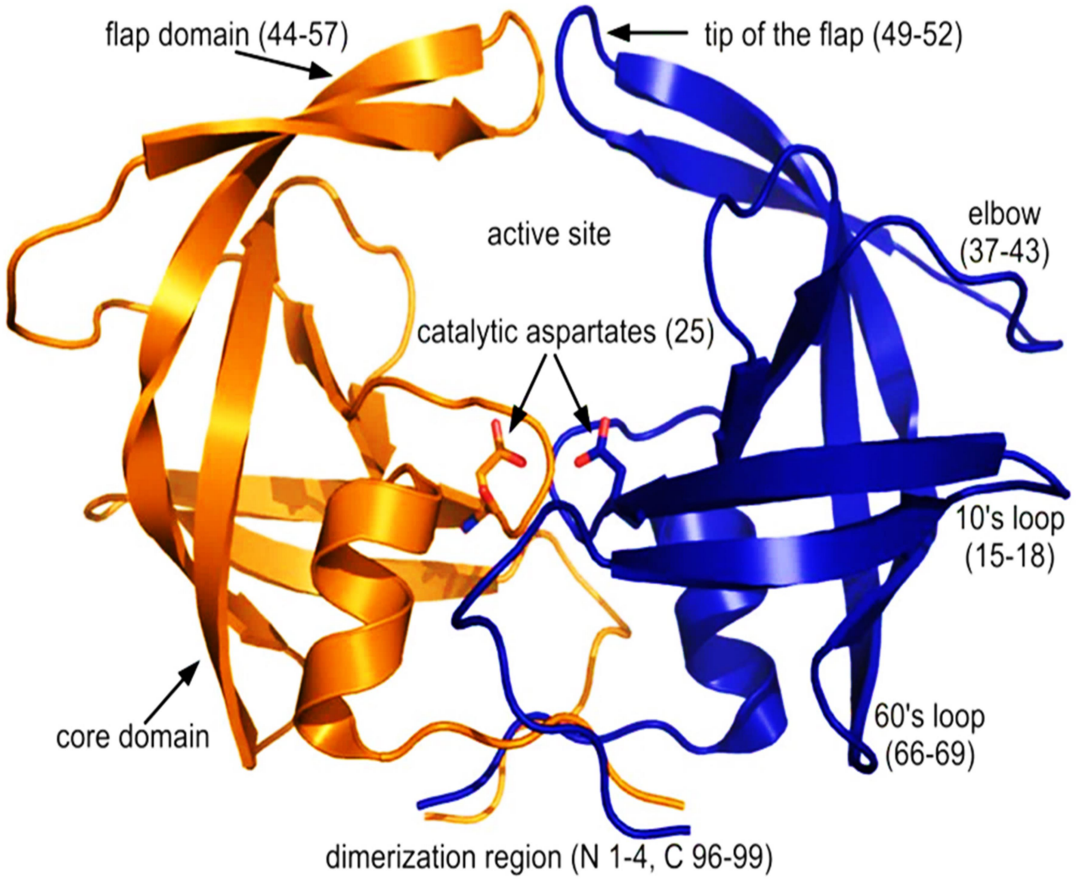

Figure 1.7: HIV protease enzyme. The identical subunits (orange and blue) surround the hydrophobic active site.

Antiretroviral drugs that inhibit HIV protease enzyme strongly bind to the site of the protease enzyme compared to the regular substrates and are considered as favoured substrates. These medications act by "competitively blocking the HIV protease enzyme" (Dalakas, 2001). At last, there is no generation of new virions.

1.2.5. Pharmacokinetic Enhancers

Pharmacokinetic (PK) enhancers like cobicistat increase the effectiveness of HIV therapy. Cobicistat (cobi) increases blood serum concentrations of elvitegravir (INSTI), atazanavir (PI), and darunavir (PI), (Shah *et al.,* 2013).

1.3. Metabolism of Antiretroviral Drugs

Enzymes belonging to cytochrome P450 (CYP) family play an important role in metabolism. Cytochrome P450 enzymes have a heme prosthetic group that contains the iron-porphyrin unit. In the iron-reduced state, the CYP enzymes effectively bind to carbon monoxide (CO) gas leading into CO-bound CYP complex that exhibits strong UV absorbance at 450 nm. The metabolism of ARV medications includes the enzymatic change from lipophilic water-insoluble nonpolar compounds to more water-soluble polar metabolites for excretion (Taxak and Bharatam, 2014). The process of metabolism at times generates metabolites required for drug action besides toxic reactive metabolites generated through the process of bio-activation.

Phase I (functionalization) and phase II (conjugation) reactions occur in drug detoxification reactions. The reactions in each phase might occur (I) sequentially, (II) independently, or (III) simultaneously. Phase I reactions introduce a functional group such as OH, SH, NH2, or COOH. During this stage, there is a modest increment in "hydrophilicity with the exception of few cases like O-methylation and N-acetylation"(Kalgutkar and Didiuk, 2009). Examples of phase I reactions are

oxidation, reduction, hydrolysis, cyclization, and decyclization. These reactions typically occur in the liver and involve mixed function oxidases as well as cytochrome P450 enzymes. Phase II reactions involve the conjugation of the metabolites of phase one reactions to an appropriate moiety like glucuronic acid, glutathione, sulphate and glycine. This results in the formation of "O- and N-glucuronides, sulphate and acetate esters. Hydrophilicity relative to the unconjugated metabolite increases during phase II reactions" (Trontelj, 2012). Transferase enzymes involved in phase II reactions include sulfotransferases, glutathione and uridine diphosphate (UDP)-glucuronosyltransferases. These transferases conjointly exist as a superfamily of enzymes, similar to cytochrome enzymes, however, they metabolise fewer medicines than cytochrome enzymes.

Most drugs including antiretroviral (ARV) drugs are absorbed in the small intestines then transported to the liver, via the portal vein system (Wilkinson, 2005). According to Ingelman-Sundberg and colleagues (Ingelman-Sundberg *et al.,* 1999), once in the liver, the liver microsomal cytochrome enzymes initiate metabolism of the drugs in a two-phase reaction process

1.3.1. Metabolism of Protease Inhibitors

The cytochrome P450 isoenzyme as CYP3A metabolise all protease inhibitors (PIs). Therefore, inducers and inhibitors of CYP3A isoenzyme might alter the metabolic rates of PIs. Co-administration of potent inhibitors of CYP3A with PIs increases

exposure to PIs, which may lead to suboptimal concentration of the PIs (Kempf *et al.*, 1997) and subsequent reduction in their therapeutic effects.

A few PIs have the capacity to induce or inhibit cytochrome isoenzymes. For instance, tipranavir (TPV) is an strong inducer of CYP3A4 isoenzyme (Panel on Antiretroviral Guidelines for Adults and Adolescent, 2015). However, the net effect of ritonavir-boosted tipranavir (TPV/r) might enhance the catalytic effect of CYP3A isoenzyme. This suggests that the concentration of the drug is perhaps going to increase for medicine that is metabolised by CYP3A isoenzyme if the co-administered formulation contains TPV. Co-administering amprenavir, and lopinavir with TPV, considerably decreases their concentration (Kempf *et al.*, 1997). Therefore, substrates of CYP3A isoenzyme with a narrow margin of safety end up accumulating within the body, resulting in drug-related toxicity when administering them with a potent protease inhibitor of CYP3A isoenzyme.

1.3.2. Metabolism of Nucleoside Analogues

The cytochrome P450 isoenzymes metabolize the NNRTIs. Efavirenz (EFV) and nevirapine (NVP) are examples of particular substrates of CYP2B6 isoenzyme. Etravirine (ETR) is a substrate of CYP2C9 and CYP2C19 isoenzymes (Panel on Antiretroviral Guidelines for Adults and Adolescent, 2015). These antiretroviral drugs with the exception of rilpivirine (RPV) are inducers or inhibitors of cytochrome isoenzymes. EFV acts as both an inducer and inhibitor at the same time,

as for NVP, it essentially induces CYP3A and CYP2B6 enzymes (Panel on Antiretroviral Guidelines for Adults and Adolescent, 2015). ETR inhibits CYP2C9 and CYP2C19 enzymes (Piscitelli and Gallicano, 2001).

1.3.3. Metabolism of Integrase Inhibitors

The uridine diphosphate (UDP)-glucuronosyltransferase 1A1 (UGT1A1) enzyme catalyzes the removal of raltegravir (RAL) through glucuronidation (Khan, 2012). Inducers of UGT1A1 enzyme together with rifampicin greatly decreases the concentration of raltegravir (Wenning *et al.,* 2009). Additionally, UGT1A1 primarily mediate the metabolism of dolutegravir (DTG) and to a minor degree by CYP3A enzyme. Any medication that induces or inhibits the CYP3A enzyme can presumably alter the concentration of elvitegravir (EVG) that is why clinicians avoid co-administering EVG/cobi/TDF/FTC in HIV-infected patients.

1.3.4. Metabolism of Entry Inhibitors

Entry inhibitor Maraviroc (MVC) that is a chemokine receptor type 5 (CCR5) blocker is "a substrate of CYP3A isoenzyme and P-glycoprotein (P-gp). There is a significant increase in the concentration of MVC following the administration with strong CYP3A inhibitors like RTV"(Wenning *et al.,* 2009; Panel on Antiretroviral Guidelines for Adults and Adolescent, 2015). Therefore, there is a need to carry out dose adjustment when administering MVC together with these drugs.

1.4. Antiretroviral Drug Toxicity

Adverse drug reactions (ADRs) due to antiretroviral drugs are organ specific and might be acute or chronic (**Table 1.2**). Dermatological reactions and gastrointestinal disorders are the most commonly reported short-term adverse drug reactions (Rudorf and Krikorian, 2005).

Table 1.2: An outline of the ARV-related adverse drug reactions (McNicholl, 2012)

Medication	**Toxicity**
Nucleoside/Nucleotide Analogues	
Didanosine (ddI)	Pancreatitis, peripheral neuropathy, nausea, diarrhea
Abacavir (ABC)	Hypersensitivity, fever, malaise, nausea, anorexia, rash etc.
Emtricitabine (FTC)	Migraine, sleep deprivation, hyperpigmentation
Lamivudine (3TC)	Migraine, dry mouth
Stavudine/d4T	Peripheral neuropathy, pancreatitis, dyslipidemia, diarrhoea
Zidovudine/AZT	Anemia, myopathy, hyperpigmentation of skin and nails
Tenofovir (TDF)	Looseness of the bowels, flatulence, stomach uneasiness, asthenia, renal deficiency, Fanconi disorder
Non-Nucleoside Analogues	
Efavirenz	Irregular dreams, sluggishness, perplexity, mood changes, hyperlipidemia
Nevirapine	Liver complications, hyperlipidemia
Etravirine	Fatigue, hepatitis, nausea, diarrhoea
Delavirdine	Lack of sleep, depression, liver complications
Rilpivirine	
Protease Inhibitors	
Atazanavir	Hyperbilirubinemia and jaundice
Indinavir	Nephrolithiasis, flank pain, hyperbilirubinemia, alopecia, dry skin
Nelfinavir	Diarrhea, nausea, vomiting, and fatigue
Ritonavir	Nausea, vomiting, and diarrhoea
Lopinavir	Diarrhea, nausea, vomiting and dyslipidemia
Fosamprenavir	Diarrhea, nausea, vomiting
Tipranavir	Nausea, vomiting, diarrhoea, increased total cholesterol and triglycerides and rash
Entry Inhibitors	
Enfuvirtide (T20)	Infusion site responses; erythema, pimples, neutropenia, recurrence of pneumonia
CCR-5 inhibitor	
Maraviroc	Loose bowels, queasiness, liver complications, hepatitis, upper respiratory tract diseases, exhaustion, tipsiness, migraine, joint pain, muscle pain
Integrase Inhibitors	
Raltegravir	Sickness, looseness of the bowels, tooting, liver complications, cerebral pain, strange dreams, pruritus, rash, exhaustion, muscle torment
Elvitegravir/cobicistat	Sleep deprivation, irregular dreams, rash
Pharmacokinetic Enhancers	
Cobicistat	Proteinuria, diarrhea, headache

HIV-infected patients develop “a wide range of cutaneous reactions including skin rash, hyperpigmentation, hair loss, hypersensitivity reaction, injection site reaction, urticaria, erythema, Stevens–Johnson syndrome or toxic epidermal necrolysis or” (Khalili *et al.*, 2009).

1.4.1. Long-term Adverse Drug Reactions of ART

Long-term ADRs that present within months or years include hepatotoxicity, metabolic disorders, and mitochondrial toxicity. A third of HIV-infected patients end up with liver toxicity when exposed to ART (Durrieu, *et al.,* 2008). Liver toxicity often relates to the use of NNRTIs.

Metabolic disorders as a rule exhibit in HIV-infected people on ART as "polyneuropathies, sexual dysfunction, hyperlipidemia, bone disorders, lipodystrophy, insulin resistance and diabetes" (Wohl *et al.,* 2006). Grinspoon and Carr found a relationship between "these metabolic variations positively related to the patient's increased danger of cardiovascular manifestations" (Grinspoon and Carr, 2005).

1.4.1.1. Fat Redistribution

The prevalence of fat redistribution or lipodystrophy varies from 20% to 80% in patients on ART” (Wohl *et al.,* 2006). Patients can develop both localized fat collection and fat loss, lipohypertrophy (aggregation of fat), ' buffalo hump' (fat

amassing at the dorsocervical area), visceral adiposity, or enlargement of the breasts" (Nachega *et al.,* 2009). Lipoatrophy (squandering of fat) includes "loss of facial and subcutaneous fat"(Nachega *et al.,* 2009). Lipodystrophy becomes clinically evident when a patient loses 40 to 50% of the fat in the limbs. HIV infection characteristically causes the strange dispersion of body fats in around "1-3% of HIV-infected individuals receiving ART" (Alencastro *et al.,* 2012). The "harmful effects of HIV on adipocytes (fat cells) result at least in part from the infective viral proteins like HIV trans-activating (Tat) protein" (Villarroya *et al.,* 2010).

Exposure to NRTIs notably stavudine causes inhibition of mitochondrial DNA gamma (mtDNAγ), which ends up in mitochondrial dysfunction, a mechanism that leads to abnormalities in the distribution of fat. Mitochondrial dysfunction induces oxidative stress leading to reduced amounts of leptin, adiponectin, and a rise in monocyte chemoattractant Protein-1 (MCP-1) and interleukin-6 (IL-6) production. Additionally, the NRTIs and efavirenz increase "the expression of genes involved in oxidative stress and apoptosis" (Caron-Debarle *et al.,* 2010). This process impairs adipocyte differentiation (Diaz-Delfin *et al.,* 2011). Some PIs induce adipocyte cell death, insulin resistance, and oxidative stress and impair adipokine production leading to the abnormal distribution of fat (Caron-Debarle *et al.,* 2010).

1.4.1.2. Changes in Lipid Metabolism

The HIV infection brings about an elevation in "triglycerides (TG), total cholesterol (TC), and a fall in the levels of high-density lipoproteins (HDL)"(Das, 2010). Atherosclerosis and related disorders result from the build-up of those substances within the plasma.

HIV infection all alone causes insulin resistance and "an increase in cholesterol levels"(Vidal-Puig *et al.,* 2000). Abnormalities of lipid metabolism attributed to HIV itself include changes in triglycerides, total cholesterol, and HDL (El-Sadr *et al*., 2005). ART, on the other hand, increases insulin resistance and dyslipidemia. Most PIs, "except for atazanavir, exhibit a relationship with an increase in total cholesterol, triglycerides, and LDL" (Gazzola *et al.,* 2010). Exposure to NNRTIs increases the amount of "total cholesterol, triglycerides, and LDL" (Nachega *et al.,* 2009). Nevertheless, raised levels of HDL may happen to yield "a net fall in the proportion of total cholesterol to HDL" (Nachega *et al.,* 2009), that occur in the presence of nevirapine. A few stavudine users exhibit a rise in the "total cholesterol, LDL, and triglycerides" (Reiche *et al.,* 2014).

The "recovery of the immune system after HAART often relates to changes in total cholesterol and LDL" (Shelburne *et al.,* 2002). The use of NNRTIs results into a 40% increase in HDL levels (von Eckardstein and Kardassis, 2015). Exposure to thymidine-analogue nucleoside reverse-transcriptase inhibitors (tNRTIs) can cause

mitochondrial dysfunction within the adipocytes followed by depletion of mtDNA and reduced mtDNA expression (Mallon *et al.,* 2008). The death of adipocytes is partially owing to the tNRTIs' ability to stop the replication of mtDNA by inhibiting the activity of DNA polymerase-γ.

1.4.1.3. Peripheral Neuropathy

Polyneuropathy is a neurological disorder characterised as a "burning sensation, numbness, tingling, and pain in the lower extremities. Neurologic examination demonstrates reduced tendon reflexes, notably at the ankles, pinprick sensation, and inflated vibration threshold distally within the lower extremities" (Gonzalez-Duarte *et al.,* 2007). With the involvement of the upper limb, the distribution of this sort of neuropathy is described as glove and stocking (**Figure 1.8**).

According to Gonzalez-Duarte et al. (2006), the most typical variety of neuropathy is distal sensory polyneuropathy (DSP) that affects up to 2/3 of HIV-infected patients. DSP primarily affects patients with the advanced immunological disorder and is secondary to the neurotoxicity of the various ARV drugs. Alternative forms that are less frequent include a mononeuropathy simplex (MNS), a mononeuropathy multiplex (MNM), brachial plexopathy, and inflammatory demyelinating polyneuropathy / Guillain-Barre syndrome (**Figure 1.8**). Clinically, mono-neuropathy presents "as a motor, sensory, and involuntary abnormalities distributed

within the same nerve (simplex) or a non-symmetrical distribution in multiple nerves (multiplex)" (Gonzalez-Duarte *et al.,* 2006).

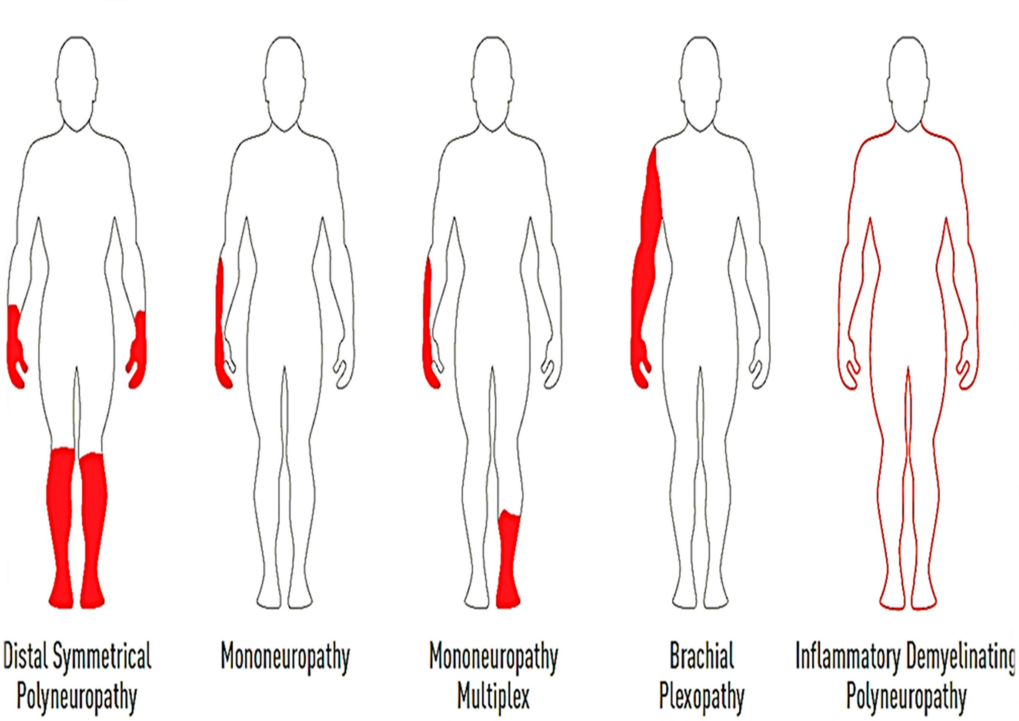

Figure 1.8: Kinds of neuropathies in HIV-infected patients (Gonzalez-Duarte *et al.,* 2006).

These defects may involve nerves of varied types including cutaneous and cranial nerves. The "acute inflammatory demyelinating polyneuropathy (IDP) in most patients is characterised by a rapidly progressive weakness with minor sensory symptoms and areflexia" (Gonzalez-Duarte *et al.,* 2006). Chronic IDP may well be monophasic or relapsing. Plexopathies have no direct association with HIV infection, however, they relate to abscess formation or intravenous drug use.

Both myelinated and unmyelinated fibres are involved in HIV-related polyneuropathy (Ferrari *et al.,* 2006). Axonal degeneration typically described as a 'dying back' pattern starts distally and progresses proximally. HIV does not directly infect neurons. However, instead, it triggers an inflammatory response that finally ends up being neurotoxic. This setting increases mitochondrial dysfunction that causes the death of additional neuronal cells and axonal degeneration. The neurotoxic effects of HIV affect either the axon or soma (Melli *et al.,* 2006). Earlier studies demonstrated direct and indirect models of axonal toxicity (**Figure 1.9a**). During direct axonal toxicity, gp120 attaches itself to the cell surface receptors CXCR4 and CCR5 where it activates an apoptotic caspase pathway within the axon. Indirect axonal toxicity entails exposure of the cell body of the dorsal root ganglion (DRG) to gp120. This triggers apoptosis mediated by Schwann cells and at last the degeneration of the axon (Melli *et al.,* 2006).

The dideoxynucleoside analogues ("d-drugs") including ddI, d4T, and ddC, and a couple of PIs strikingly indinavir cause neurotoxicity (Gao *et al.,* 2011). Models showed the involvement of ddC in subsequent dysfunction in myelination of peripheral nerve fibres and reduced intraepidermal nerve fibre density (IENFD) that was associated with inflated pain intensity in humans (**Figure 1.9b**) (Bhangoo *et al.,* 2007; Zheng *et al.,* 2011). At last, comorbidities frequently connected with HIV-infection including diabetes, hepatitis C, liquor addiction, and vitamin B-12 inadequacy manifest with polyneuropathy (Nachega *et al.,* 2009).

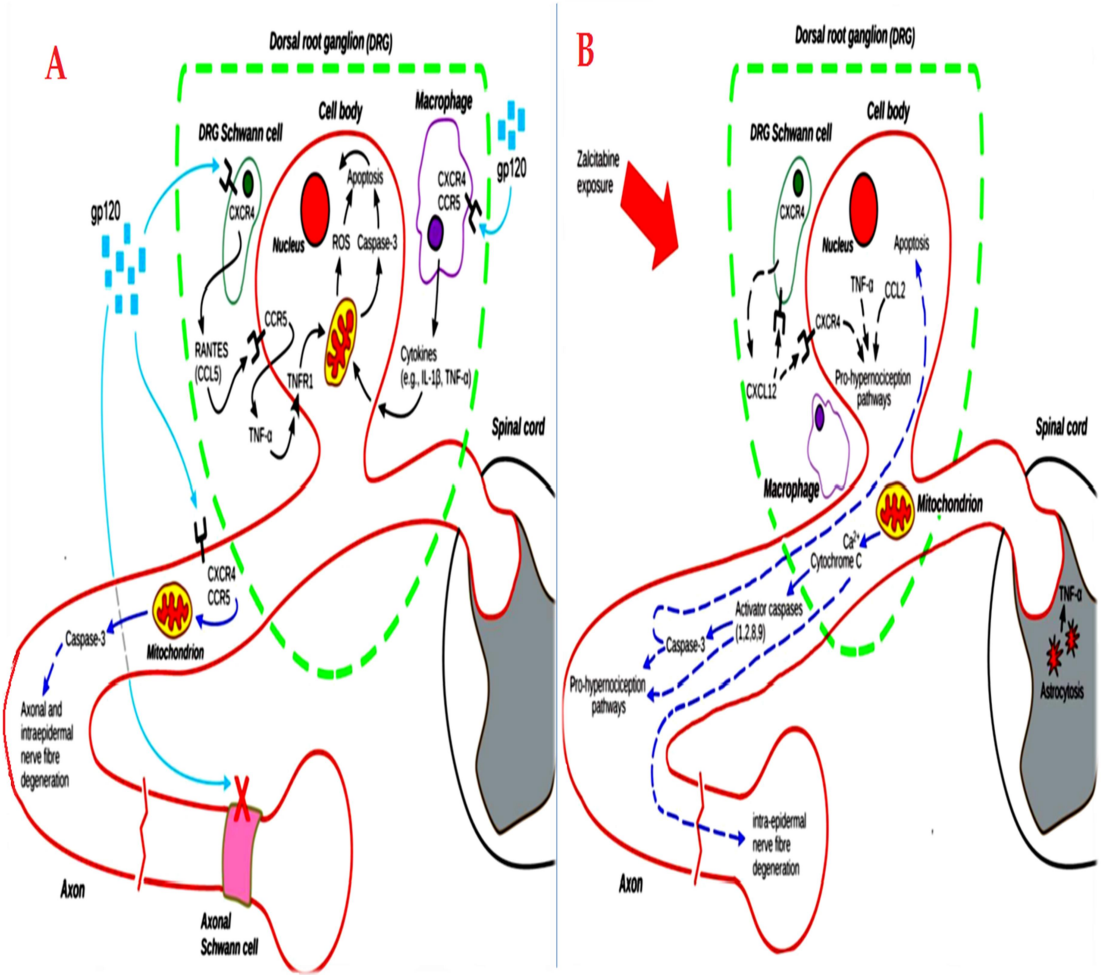

Figure 1.9: Models of neurotoxicity: (A) exposure to HIV-gp120 and (B) exposure to zalcitabine (Kamerman *et al.,* 2012).

1.4.1.4. Disorders of Glycolytic Metabolism

The introduction of ART has increased the occurrence of the disorders of a glycolytic metabolic system including insulin resistance, glucose intolerance, and diabetes, (Brown *et al.,* 2011). The most common abnormality is primarily insulin resistance, which results from the inability of insulin to increase the uptake of glucose by the muscle and forestall hepatic gluconeogenesis. An increase in insulin secretion compensates for a rise in the blood levels of glucose.

Patients using PIs show a greater frequency of directly induced insulin abnormalities. Symptoms of "type 2 diabetes mellitus and insulin resistance manifest in 8-10% of HIV-infected patients on ART" (Barbaro, 2006). Manifestations of hyperglycaemia with or without diabetes mellitus happen in 3 to 17% of patients on ART (Kalra and Agrawal, 2013). Indinavir, lopinavir/ritonavir, d4T, and AZT block glucose transporters in fat and muscle thereby causing mitochondrial toxicity. In patients using PIs, symptoms of increased insulin resistance may present without diabetes mellitus (Nachega *et al.,* 2009). The introduction to indinavir leads to an increase in the levels of glucose and decrease in insulin sensitivity (Kramer *et al.,* 2009).

1.4.1.5. Hyperlactatemia and Lactic Acidosis

Hyperlactatemia or mild lactic acidemia is "defined as a serum lactate level > 2 mmol/1 with normal arterial pH" (Carl *et al.,* 2005). Lactic acidosis is a state of hyperlactatemia where the arterial pH stays below 7.3 or the amount of bicarbonate stays below 20 mmol/l (Falco *et al.,* 2003). Generally, a majority of HIV-infected patients with lactic acidemia do not develop any symptoms but a few patients may end up with lactic acidosis. Patients with hyperlactatemia show a variety of clinical manifestations. If they do not stay symptomless, they present with mild symptoms or develop severe lactic acidosis. This develops at any stage by presenting insidious symptoms including weight loss, fatigue, nausea, regurgitation, and abdominal pain.

Low levels of hyperlactatemia are evident in 8-21% of NRTI users, although majority remain without symptoms (Carr *et al.,* 2000). Incidence rates of lactic acidemia presenting without lactic acidosis vary from 8-10 cases per 1,000 person years. Fatal clinical manifestations unfold during high lactate levels resulting in the failure of multiple organs (Falco *et al.,* 2002). Studies have shown that hyperlactatemia and lactic acidosis as the final consequences of NRTIs-induced mitochondrial toxicity. Damaging more than 70% of mtDNA in cells ends up in a dysfunctional production of cellular energy.

1.4.1.6. Mitochondrial Toxicity

Through the process of oxidative phosphorylation, mitochondria provide the normal cell's energy needs via adenosine triphosphate (ATP) route (Scheffler, 2001). Some 16, 000 bases of mtDNA "code for 22 transfer ribonucleic acids (tRNAs), 13 respiratory chain peptides and a pair of ribosomal ribonucleic acids (rRNAs)" (Van der Watt, 2014). These acids are helpful in the synthesis of mitochondrial proteins involved in the process of oxidative phosphorylation.

NRTIs undergo cellular phosphorylation to inhibit the activity of HIV reverse transcriptase enzyme. They actively compete with endogenous nucleotides by incorporating into the elongating nucleic acid chain and once incorporated, they terminate chain elongation. In addition, NRTIs inhibit mtDNA polymerase γ, causing the depletion of mtDNA (Van der Watt, 2011), which thus cause

mitochondrial dysfunction. Manifestations of mitochondrial dysfunction include lipoatrophy, "lactic acidosis, skeletal myopathy, cardiomyopathy, polyneuropathy, pancreatitis, and nephrotoxicity" (Van der Watt, 2011). Albeit all NRTIs cause mitochondrial dysfunction, didanosine (ddI) and stavudine (d4T) are associated with serious instances of mitochondrial toxicity (Montaner *et al.,* 2003). Likewise, NRTIs' "compete with endogenous nucleosides for phosphorylation by mitochondrial thymidine kinase 2 and for transport into the mitochondria"(Van der Watt, 2014). Past studies demonstrated that introduction to NRTIs increases the "rate of formation of mtDNA mutations" (Van der Watt, 2014), deletions (Maagaard *et al.,* 2006), and a reduction in mitochondrial gene expression (McGee *et al.,* 2012).

1.4.1.7. Oxidative Stress

Following mitochondrial dysfunction, the clinical manifestations that occur relate to the development of oxidative stress. The constant immune activation "in HIV-infected people is characterised by an increased production of reactive oxygen species (ROS), that include hydrogen peroxide (H_2O_2), hydroxyl radicals (OH)' (Liu *et al.,* 2002), superoxide anions (O2−), hypochlorous acid (HOCl), and peroxynitrite (ONOO−) (Gil del Valle *et al*., 2013). Such "excessive production of ROS leads to oxidative modification and, therefore, dysfunctional proteins, nucleic acids, carbohydrates and lipids" According to Gil del Valle and others (Gil del Valle *et al.,* 2013) the state of oxidative stress (OS) occurs when there is an imbalance in redox

reactions sometimes related to oxidative molecular injury that contributes to cellular cell death.

Cells in the body chiefly rely on the mitochondria to generate ROS through oxidative metabolism. Mitochondrial produced ROS moderate cell differentiation, function, and pathological conditions. The "proteins encoded by mtDNA are included in electron transport edifices of oxidative phosphorylation (OXPHOS)" (Gil del Valle *et al.*, 2013). The "inhibition of γ-DNA polymerase by phosphorylated-NRTIs prompts the reduction of mtDNA, modifications in OXPHOS proteins synthesis, energy deficit, and tissue dysfunction" (Gil del Valle *et al.*, 2013). These procedures add to expanded "arrangement of ROS and OS in HIV-infected patients on NRTI" (Haugaard *et al.,* 2005). At last, these patients encounter "OS-mediated cellular damage that relates with mitochondrial toxicity"(Anuurad *et al.,* 2009).

1.4.2. Prevalence of ART-Related Adverse Drug Reactions

Despite gains made in fighting the HIV scourge, adverse drug reactions (ADRs) to antiretroviral drugs remain a significant health burden (WHO, 2006b; Mehta, 2011). World Health Organisation classifies ADRs using a four point scale severity grading (WHO, 2006a). ADRs classified as Grade 1 are "mild" and with no limitation of daily activities; Grade 2 are "moderate" with mild to moderate limitation of activities; Grade 3 are "severe" with marked limitation of activities and Grade 4 as "life

threatening" with the extreme limitation of activities and significant medical intervention.

Documented ART-related ADRs vary depending on the medical setting. In resource-limited developing nations, constraints in getting ART drugs and deferred detection of particular ADRs result from weaknesses within the health systems (Subbaraman *et al.,* 2007). In addition, one study observed that co-morbidities like tuberculosis and malaria and hereditary factors incredibly influence the distribution of ADRs amongst well-off and poor nations (Subbaraman *et al.*, 2007).

1.4.2.1. ART-Related Adverse Drug Reactions in Developed Countries

The Swiss cohort study (Keiser *et al.*, 2007) showed that up to 47% of patients on older ARV drugs had ART-related ADRs. Out of these cases, 25% were rated moderate to severe and 27% had laboratory abnormalities that needed immediate interventions. For the second generation ARV drugs, the same cohort revealed a 44% prevalence rate of clinical ADRs and 23% prevalence rate of laboratory abnormalities (Keiser *et al.*, 2007).

Before the change in regulation, lamivudine and stavudine were the most commonly used NRTI. High rates of "polyneuropathy, pancreatitis, lipoatrophy, and lactic acidosis that resulted from mitochondrial toxicity were strongly related to the use of stavudine" (McComsey and Lonergan, 2004). Studies from the developed countries

show that 10% to 21% of patients who used stavudine had polyneuropathy and about 50%–63% ended up with lipodystrophy. Of all the NRTIs, the use of stavudine strongly related to dyslipidemia and hyperglycaemia (Gallant *et al.,* 2004).

1.4.2.2. ART-Related Adverse Drug Reactions in Sub-Saharan Africa

Genetic variability in the expression and function of CYP450 enzymes contributes to four clinical phenotypes namely poor, intermediate, extensive, and ultra-rapid metabolizers. Poor metabolizers effectively lack a certain enzymatic activity and do not metabolise drugs efficiently (Johansson and Ingelman-Sundberg, 2011). Extensive metabolizers tend to metabolise drugs rapidly and often need higher doses of a drug. Ultra-rapid metabolisers result from gene duplication and that the number of gene copies directly correlates with rapid metabolism of an administered drug. Intermediate metabolisers have a slight decrease in enzyme activity, but typically, they do not need drug dosage adjustment.

Out of all CYP450 enzymes, the CYP3A sub-family, which include CYP3A4, CYP3A5, CYP3A7, and CYP3A43, play an important role in the metabolism of many drugs. CYP3A4 and CYP3A5 play a role in the metabolism of over 50% of all known CYP450 substrates including many of the antiretroviral drugs used in sub-Saharan Africa (Bains, 2013). Many previous studies have shown CYP3A5 as one of the most pharmacologically active drug metabolising enzymes in Africa. CYP3A5 represents at least 50% of the total hepatic and intestinal CYP3A content, which has led to

suggestions that CYP3A5 may be the most important genetic contributor to inter-ethnic and inter-population differences in CYP3A dependent drug clearance (Fröhlich *et al.*, 2004; Bains, 2013).

Several cohorts have provided insight into the distribution of ADRs in sub-Saharan Africa. Polyneuropathy and lipodystrophy are the predominant ADRs observed in Sub-Saharan Africa. A Rwandese study found the prevalence of lipodystrophy of about 34% in patients on stavudine (Mutimura *et al.,* 2007). In many African settings, the prevalence of polyneuropathy varies from 19% to 42% (Evans *et al.,* 2012).

In Cameroon, Luma and colleagues reported the overall prevalence rate of ADR to be 19.5%. Of all the ADRs recorded, polyneuropathy accounted for 21.2% and that 56.1% of the ADRs were related to the use of stavudine (Luma *et al.,* 2012). In Nigeria and Ivory Coast, studies reported the prevalence rates of Zidovudine-induced anaemia to vary from 3% to 12% (Eluwa *et al.,* 2012). Most studies in East Africa reported polyneuropathy prevalence ranging from 20% to 30% after 1 to 3 years of exposure to ART (Forna *et al.,* 2007).

1.4.2.3. ART-Related Adverse Drug Reactions in Kenya

Despite the fact that there are inadequate data on the status of ART-related ADRs in Kenya (WHO, 2010), a handful of past studies reported ART-related toxicity in patients receiving ART (Hawkins *et al.,* 2007; Karara *et al.,* 2010). Polyneuropathy

was the most noteworthy reported toxicity, in the above-stated studies. A second study conducted at the Kenyatta National Hospital reported at least one ADR in 48.6% of the patients undergoing treatment (Mwangangi *et al.,* 2010). In another study, "some patients reported more than one ADR with 1.7% presenting with three ADRs whereas 12.3% had two or additional ADRs" (Mwangangi *et al.,* 2010).

A subsequent study that utilised a more sensitive validated polyneuropathy-screening apparatus reported polyneuropathy in 68% of HIV-infected people in Mombasa, Kenya (Mehta *et al.,* 2010). Fifty-eight percent (58%) of patients determined to have polyneuropathy were on d4T-based treatment. These three studies have affirmed polyneuropathy as the most common ADR in Kenya (Hawkins *et al.*, 2007; Mehta *et al.,* 2010; Mwangangi *et al.,* 2010).

1.5. The Role of Metabolites in the Development of Adverse Drug Reactions

Exposure to ARV drugs can yield a duplex interaction between the drug and the biological system. On one hand, following the administration of medicines, absorption, distribution, and metabolism occurs before excretion from the body. Drugs alter the biological system through two interactions namely drug metabolism and endogenous metabolism. These interactions produce two sets of metabolites namely drug metabolites and endogenous metabolites. The two sets of metabolites play a key role in the development of toxicities.

1.5.1. Drug Related Metabolites

For the most part, metabolism leads to the loss of biological action of the parent compound through the detoxification pathway (Liew *et al.,* 2012). However, some bio-activation pathways generate electrophilic reactive metabolites. These reactive metabolites very often possess a chemically reactive group that causes drug-related toxicity. Medicines capable of forming reactive metabolites of carboxylic acid contain carboxylate functional group and may cause a varied range of toxicities.

Metabolites generated from complex ARV drug interaction greatly contribute to toxicity. Various tissue toxicities and immune-mediated injuries attributable to the buildup of reactive metabolites ensue from the poor elimination of the metabolites from the body (Kalgutkar and Didiuk, 2009).

1.5.1.1. Phosphorylated Metabolites of NRTIs/ NtRTIs' Analogues

Both NRTIs and NtRTIs undergo intracellular phosphorylation to form triphosphate and diphosphate groups that inhibit HIV reverse transcriptase. The active form of NRTIs or NtRTIs is the triphosphorylated state found in the infected lymphocytes. NRTIs go through three intracellular phosphorylation steps to provide biologically active nucleoside triphosphates (Moore *et al.,* 2007). Regularly recommended NRTIs including abacavir (ABC), zidovudine (ZDV), lamivudine (3TC), stavudine (d4T), didanosine (ddI), and emtricitabine (FTC) are 2', 3'- dideoxynucleoside (ddN) analogues and they act through a similar mode (Jitratkosol *et al.,* 2012). The

thymidine analogues (ZDV and d4T) undergo anabolism by thymidine kinase and thymidylate kinase enzymes. The cytidine analogue (3TC) pass through anabolism by "deoxycytidine kinase and deoxycytidine monophosphate kinase enzymes" (Watson, 2014).

The "nucleoside diphosphate kinase" converts all the three NRTI diphosphates into their corresponding active triphosphate forms (Watson, 2014). The resultant NRTI triphosphates competitively inhibit "the activity of HIV reverse transcriptase by incorporating into the proviral DNA, ending in DNA chain termination and interference of viral replication" (Said and Abdelwahab, 2013). Toxicities associated with NRTI's triphosphates have clinical manifestations resembling diseases caused by mitochondrial dysfunction (Kohler and Lewis, 2007).

Unlike "nucleoside analogues, NtRTIs need two phosphorylation steps to become biologically active" (Hurwitz and Schinazi, 2012). The hydrolases (esterases) are incapable of cleaving the phosphonate group in NtRTIs. Once they are incorporated, these compounds become very difficult to split at the 3'-terminal end of the chain. Intracellular metabolism of the commonly used TDF produces tenofovir diphosphate as an active metabolite that competitively inhibits HIV reverse transcriptase. Its phosphorylation into diphosphate form goes through catalysis by the AMP kinase enzyme or phosphoribosyl-1-pyrophosphate (5-PRPP) synthetase

enzyme (Andrade *et al.,* 2011). Studies have suggested that TDF causes renal toxicity through mitochondrial toxicity (Andrade *et al.,* 2011).

1.5.1.2. Genetic Predisposition to NRTIs/ NtRTIs-Related Toxicity

There are suggestions that some HIV-infected patients who experience mitochondrial toxicity have a genetic predisposition to NRTI or NtRTI toxic effects. The exposure of harmful mutations or heteroplasmic mitochondrial DNA mutations to NRTIs may hinder efficient OXPHOS for the production of normal ATP, thence resulting in phenotypic symptoms (Wang *et al.,* 1996). NRTI-related toxicity could also result from "single nucleotide polymorphisms (SNPs) in nuclear genes that are responsible for mtDNA or OXPHOS"(Van Goethem *et al.,* 2001). According to Van Goethem and others, "changes in the DNA pol-γ gene cause mitochondrial sicknesses" (Van Goethem *et al.,* 2001).

Previous studies associated "a common European mitochondrial haplogroup T (7028 C/T, 10398 G/A, and 13368 G/A) and an African mitochondrial sub-haplogroup L1c with polyneuropathy" (Canter *et al.,* 2010; Pavlos and Phillips, 2011). One more study associated mitochondrial haplogroup H with limb lipoatrophy (Hendrickson *et al.,* 2009). Further, "the hemochromatosis gene variant (HFE 187C/G) and polymorphisms in IL-1β 3954 C/T were identified with less fringe lipoatrophy and offered protection against lipoatrophy" (Asensi *et al.,* 2008; Hulgan *et al.,* 2008).

Genetic polymorphisms in the proximal tubule transporters that include the organic anion transporter 1 (OAT1) and organic anion transporter 3 (OAT3) predispose HIV-infected patients to TDF-related tubular toxicity (Hall *et al.,* 2011). Further, adenosine triphosphate (ATP)-binding cassette (ABC) haplotype namely ABCC2 haplotype and ABCC4 3463G variants related well with TDF-related tubular toxicity (Rodríguez-Nóvoa *et al.,* 2009). Polymorphisms including the "uncoupling proteins (UCP) 1 and 2 were associated with diabetic neuropathy in Caucasians with type II diabetes" (Rudofsky *et al.,* 2006). The "minor alleles of the SNPs (- 866G/A UCP2; C-55T UCP3) which previously associated with diabetic neuropathy were also reported to relate to duplicated mRNA expression in various cell lines" (Rudofsky *et al.,* 2006). The "UCP2 and UCP3 play a very important role in the regulation of reactive oxygen species (ROS)"(Vincent *et al.,* 2004). They are also associated with the pathogenesis of "polyneuropathy through mitochondrial dysfunction, oxidative stress, and calcium regulation" (Vincent *et al.,* 2004). Finally, a small risk of polyneuropathy was related to two major HFE gene mutations specifically 845G/A, and 187C/G in patients using d4T/ddI therapy (Kallianpur *et al.,* 2006). Another HFE mutation (187 C/G) relates to lipoatrophy (Hulgan *et al.,* 2008).

1.5.1.3. Hydroxylated Metabolites of NNRTIs' Analogues

Nevirapine (NVP) and efavirenz (EFV) are two common NNRTIs chiefly metabolized by the CYP3A to produce many hydroxylated metabolites. Therefore, CYP3A may be a useful marker for NVP-related toxicity (Walubo *et al.,* 2006). After

glucuronidation, the clearance of resultant soluble conjugates is primarily through the urine. In the case of NVP, the enzyme "CYP3A4 predominantly mediates the formation of derivatives namely 2- and 12-hydroxynevirapine (OH-NVP), whereas CYP2B6 catalyses the formation of 3- and 8-OH-NVP" (Erickson *et al.,* 1999). The production of carboxy-NVP follows the secondary oxidation of 12-OH-NVP. The use of "NVP is related to about 3% incidences of hepatotoxicity and 9% to 11% of cutaneous adverse reactions" (Popovic *et al.,* 2010).

In the case of EFV metabolism, CYP2B6, CYP3A4, and CYP2A6 enzymes are involved (Ogburn *et al.,* 2010). CYP2B6 mediates the "change of EFV to 8-hydroxyefavirenz (OH-EFV) while CYP2A6 mediates the transformation of EFV to 7-OH-EFV" (Habtewold *et al.,* 2016). Other "reactions include hydroxylation of the cyclopropane ring (C-14) of 8-hydroxy-efavirenz, delivering the 8, 14-dihydroxy-efavirenz"(Ogburn *et al.,* 2010; Andrade *et al.,* 2011). Additionally, EFV can undergo direct conjugation to form EFV-N-glucuronide (EFV-G), a major metabolite found in urine. Studies show that variations in EFV metabolite concentrations or central nervous system penetration can cause EFV-related injuries (Arab-Alameddine *et al.,* 2011). Higher EFV plasma levels (> 4 mg/l) was associated with "higher frequency of central nervous system side effects" (Kwara *et al.,* 2009), a situation that might be overcome through dosage reduction (van Luin *et al.,* 2009).

1.5.1.4. Genetic Predisposition to NNRTIs' Toxicity

The most broadly examined single nucleotide polymorphism (SNP) of NVP and EFV metabolism "is CYP2B6 516G/T, that describes the intermediate metabolizing haplotypes with CYP2B6 *6, *7, *9, and *13 alleles" (Pavlos and Phillips, 2011). The rate of efavirenz 8-hydroxylation in these patients is low (Desta *et al.,* 2007), a scenario that is associated with toxicity owing to raised EFV plasma exposure. Further studies found a significantly higher frequency of the allele 516 T in Africans (more than 45%) compared to Hispanics (27.3%), Europeans (21.4%), and Asians (17.4%). The homozygosity of "CYP2B6 516G/T was significantly associated with greater plasma exposure to EFV across a range of ethnicities"(Ribaudo *et al.,* 2010; Pavlos and Phillips, 2011). Also, in East African studies, CYP2B6*6 was significantly related to higher plasma efavirenz concentration in Tanzanian and Ethiopian patients (Ngaimisi, *et al.,* 2013). In Kenya, "CYP2B6 516G/T and CYP2B6 983T/C genotypes were significantly associated with plasma levels of NVP" (Oluka *et al.,* 2015).

The multidrug resistance 1 (MDR1) position "3435C/T allele was associated with a reduced risk of hepatotoxicity in patients on treatment (Srivastava *et al.,* 2010). Later, this "association was confirmed to related well with raised liver transaminases" (Ciccacci *et al.,* 2010). The status of "UGT27*1a gene variant in some individuals was revealed to be an independent indicator for EFV concentration"(Pavlos and Phillips, 2011).

1.5.2. Endogenous Metabolites

The "endogenous metabolites are low sub-atomic weight compounds, intermediates or products of enzyme-mediated biochemical responses" (Jupin *et al.,* 2013). They form the "building blocks for all other biochemical species and structures such as transcripts (nucleotides), proteins (amino acids), genes and cell walls" (Dunn *et al.,* 2011). The interaction between endogenous metabolites and medication metabolites occurs in the blood system. Accordingly, "it is important to characterise the concentration of endogenous metabolites so as to get a better understanding of different ARV medication outcomes" (Kampen, 2009).

Both plasma and serum contain a large range of endogenous metabolites suspended or dissolved in them (**Table 1.3**). The Serum Metabolome database (SMDB) estimates that the human serum contains up to 6,000 endogenous metabolites (Wishart *et al.,* 2009). To date, "the human plasma has more than 300 metabolites" (Lawton *et al.,* 2008; Bicalho *et al.,* 2008). Studies on HIV disease have revealed variations in endogenous metabolites before and after initiating ART. HIV-infected patients showed important alteration in 12 metabolic pathways and 26 metabolites (Munshi, *et al.,* 2013). In particular, there were "raised levels of D-glucose, L-aspartic, methylmalonic acid (MMA), choline and sarcosine in HIV-infected subjects compared to healthy controls" (Kampira *et al.*, 2014).

Table 1.3: Chemical categories "in the Serum Metabolome Database" (Psychogios *et al.,* 2011)

Compound class	Number	Compound class	Number
Acyl glycines	10	Indoles and indole derivatives	12
Acyl phosphates	10	Inorganic ions and gases	20
Alcohol phosphates	2	Keto acids	8
Alcohols and polyols	40	Ketones	6
Aldehydes	3	Leukotrienes	8
Alkanes and alkenes	10	Lipoamides and derivatives	0
Amino acid phosphates	1	Minerals and elements	40
Amino acids	114	Miscellaneous	77
Amino alcohols	14	Nucleosides	24
Amino ketones	14	Nucleotides	24
Aromatic acids	22	Peptides	21
Bile acids	19	Phospholipids	2177
Biotin and derivatives	2	Polyamines	11
Carbohydrates	35	Polyphenols	22
Carnitines	22	Porphyrins	6
Catecholamines and derivatives	21	Prostanoids	23
Cobalamin derivatives	4	Pterins	14
Coenzyme A derivatives	1	Purines and purine derivatives	11
Cyclic amines	9	Pyridoxals and derivatives	7
Dicarboxylic acids	17	Pyrimidines and pyrimidine derivatives	2
Fatty acids	65	Quinones and derivatives	3
Glucuronides	8	Retinoids	11
Glycerolipids	1070	Sphingolipids	3
Glycolipids	15	Steroids and steroid derivatives	109
Hydroxy acids	129	Sugar phosphates	9
		Tricarboxylic acids	2

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There were "lower levels of different metabolites; L-lysine, L-threonine, 5β-cholestanol and acetoacetate in HIV-infected patients before treatment compared to

those on ART" (Munshi *et al.,* 2013). In another study, "35-metabolites that were mapped to lipid, amino acid, and nucleotide metabolism distinguished HIV patients with advanced disease on PI-based ART from controls" (Cassol *et al.,* 2013). The study reported alterations in sulfated steroids, long-chain fatty acids (LCFA), bile acids, acylcarnitines, polyunsaturated fatty acids, and lysophosphocholine (Cassol *et al.,* 2013). HIV-infected patients showed a reduction in "omega-3 and 6, polyunsaturated fatty acids, lysophosphocholine, sulfated steroids and pregnenolone sulfate among others" (Cassol *et al.*, 2013).

1.5.3. Metabolomic Platform for Measuring Drug Toxicity

Metabolomics involves the unbiased "identification and quantification of low molecular weight molecules (< 1000 Da)" (Hufsky and Böcker, 2012), merely known as metabolites. There are "two types of metabolomic platforms namely targeted and untargeted" (Ros Simó, 2013).

1.5.3.1. Targeted Metabolomics

According to Dudley et al., (2010), this technique uses predefined metabolite-specific signals that precisely measure analytical merit as well as relative abundance and concentrations of specific metabolites. Though this approach can only determine a small number of metabolites in a single assay, it has increased sensitivity to detect low concentrations of metabolites in samples. However, this approach overlooks changes associated with other targeted metabolomics besides providing a restricted

scope of the global changes related to biological disruption. Targeted metabolomics is appropriate for the clinical application, pharmaceutical, and toxicological analysis. It complements very well with the well versed genomic and proteomic technologies. AbsoluteIDQ p180 Kit developed by Biocrates Life Sciences from Austria supports targeted metabolomic analysis of biofluids. Using very small amounts of blood (< 10 μl), AbsoluteIDQ p180 Kit can quantify up to 188 metabolites.

The enhanced specificity and reproducibility in "mass spectrometry (MS) and nuclear magnetic resonance (NMR)"(Trent, 2014) have proved advantageous for executing targeted metabolomic studies. However, alternative analytical tools "worth considering include gas chromatography"(Zakrzewicz, 2008; Pasikanti *et al.,* 2008), "high-performance liquid chromatography (HPLC)"(Bjørk *et al.,* 2010), optical spectroscopic techniques and flame ionisation. Bioanalytically, "NMR, and MS are very powerful tools for generating multivariate information in metabolomic investigations" (Sampey *et al.,* 2012).

1.5.3.2. Untargeted Metabolomics

The "untargeted metabolomic techniques are global in nature and aim at the unbiased" (Patti *et al.,* 2013), concurrently full "analysis of all measurable analytes in a sample" (Roberts *et al.,* 2012; Patti *et al.,* 2012). It applies chemometric techniques that compress multiple signals into few specific signals for purposes of searching from a library or database. Since sample preparation and analysis preclude the

investigation of metabolic identification, therefore, the "application of a non-targeted approach offers the advantage of de novo target discovery" (Charve, 2011).

Though either NMR or MS methods perform untargeted metabolomics (Waagsbø *et al.,* 2016), liquid chromatography (LC) coupled with "MS (LC/MS) permits the detection of most metabolites and is usually recommended for global metabolite identification"(Trent, 2014). The LC/MS technique routinely detects thousands of peaks from biological samples. Each of these peaks represents "a metabolite feature that corresponds to a detected ion with an exceptional mass-to-charge ratio and time of retention" (Patti *et al.,* 2012).

Unlike targeted metabolomics, untargeted metabolomics generates exceedingly advanced data sets with huge file sizes "per sample for some new high-resolution MS instrument" (Trent, 2014). To handle these data, the MS instrument has MathDAMP, MetAlign, MZMine, and XCMS software (Lommen, 2009).

1.6. Study Rationale

Kenya has the fourth-largest HIV pandemic on the planet with around 1.6 million adult individuals infected (Kimanga *et al.*, 2014). About 59% of HIV-infected adult patients get ART (UNAIDS, 2016). Even without adequate ART coverage, the population of patients receiving ART is steadily increasing. These data project a rise

in patient population on ART and incidence of long-term ART-related adverse drug reactions.

There are some studies on long-term ART-related ADRs in Kenya. These studies demonstrate increased the prevalence of ADRs, which ranged from 26.5% (Hawkins *et al.,* 2007) to more than 40% (Mwangangi *et al.,* 2010; Nderitu *et al.*, 2013). These studies refer to "polyneuropathy and lipid abnormalities as the most common ADRs in Kenya" (Hawkins *et al*., 2007; Mehta *et al.,* 2010; Mwangangi *et al.,* 2010; Nderitu *et al.,* 2013). Studies conducted in other populations have associated ART with many long-term ADRs including metabolic disorders like disturbances in body fat distribution, dyslipidaemia, mitochondrial toxicity and polyneuropathy (Barbaro, 2006; Margolis *et al.,* 2014). Mitochondrial toxicity on its own contributes to lactic acidosis, dyslipidemia, and polyneuropathy (Margolis *et al.,* 2014). However, in the Kenyan context data on mitochondrial toxicity are insufficient. Moreover, there is a scarcity of adequate information on the underlying mechanisms responsible for the ART-related long-term abnormalities in populations from Sub-Saharan Africa.

In Western countries, evidence exists on the association of ART-related ADRs with genetic polymorphisms of CYP450 genes (Desta *et al.,* 2007), "mitochondrial haplotypes and HFE variants"(Scatena *et al.*, 2012). However, the clinical application of these polymorphisms in monitoring ART-related toxicities particularly in resource-poor settings remains wanting. The association of abacavir hypersensitivity

with “class I MHC allele, HLA-B*5701 is currently in clinical application”(Chaponda and Pirmohamed, 2011). Therefore, “several clinical guidelines and practice in Western countries advocate for a genetic test before the prescription of abacavir” (Chaponda and Pirmohamed, 2011). In the Sub-Saharan settings, genetic testing for abacavir hypersensitivity is not accessible in the most public sector healthcare facilities. This is often because of “a low frequency of HLA-B*5701 mutations in Africans compared to Caucasians”(Mallal *et al.,* 2008). Additionally, there are no sensitive methods of prevention and clinical monitoring of ADRs related to most alternative antiretroviral drugs. Therefore, there is a need to undertake further research using metabolomic techniques.

HIV infection triggers “chronic immune activation characterized by the increased generation of ROS and alterations among the antioxidant defence system”(Valle and Hernandez, 2013). These changes would possibly result in increased oxidative stress, which affects mitochondrial function. Similarly, the use of NRTIs interferes with “mtDNA polymerase γ and cause the depletion of mtDNA” (Van der Watt, 2011). In addition, this situation ends up “in mitochondrial dysfunction, whose clinical manifestations include lipoatrophy, lactic acidosis, skeletal myopathy, cardiomyopathy, polyneuropathy, pancreatitis, and nephrotoxicity” (Van der Watt, 2011). However, it is not clear how metabolomic changes relate to specific clinical manifestations of mitochondrial toxicity.

Numerous studies have identified metabolites related to various disease states suggesting great potential for the use of metabolomic analysis in HIV disease prognostics (Milburn and Lawton, 2013; Menni *et al.,* 2013). Studies carried out using oral biofluids, plasma, sera and urine collected from HIV-infected patients have proved helpful in identifying metabolites that considerably differentiate, HIV-infected patients with toxicities from healthy controls (Emwas *et al.,* 2015). Although these studies primarily detected changes in carbohydrate, lipid, and amino acid metabolism, specifics on metabolite concentrations and their associations with indicators of disease progression were seldom attempted (Sitole *et al.,* 2013). In addition, there are limited data on such studies in most African countries including Kenya. In one such study, "HIV-infected Ugandan patients showed elevated levels of many metabolites including acylcarnitines and triglycerides compared to patients not infected with HIV" (Mody *et al.,* 2014). Therefore, there is a need to conduct additional metabolomics identification studies using populations in African countries.

1.7. Research Question

What are the long-term antiretroviral therapy related adverse drug reactions (ADRs) and corresponding variations in plasma metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy?

1.8. Main Objective

To describe long-term antiretroviral therapy related adverse drug reactions in Kenyan patients and compare variations in plasma metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy.

1.8.1. Specific Objectives

1. To describe the prevalence and determinants of adverse drug reactions associated with long-term antiretroviral therapy in an adult cohort of HIV-infected patients at the Sex Workers Outreach Programme in Kenya.
2. To identify factors influencing the development of polyneuropathy in HIV-infected Kenyan patients undergoing first-line antiretroviral therapy.
3. To describe variations in plasma metabolites in HIV-infected patients on long-term ART including patients with a history of polyneuropathy.
4. To identify potential early biomarkers for polyneuropathy and underlying metabolic processes.

CHAPTER TWO:

THE PREVALENCE AND DETERMINANTS OF ADVERSE DRUG REACTIONS IN HIV-INFECTED PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY

2.1. INTRODUCTION

This chapter presents the retrospective analysis of electronic medical records of HIV-infected patients at the Sex Workers Outreach Programme (SWOP) in Nairobi, Kenya. The study objective was to describe the prevalence and determinants of adverse drug reactions (ADRs) associated with long-term ART in an adult cohort of HIV-infected patients in SWOP facilities. In this regard, the results focused on the prevalence of common ADRs and factors related to long-term ART-related ADRs.

2.2. METHODS

2.2.1. Study Design

The study was a retrospective review of electronic medical records of HIV-infected patients on long-term ART at the Sex Workers Outreach Program (SWOP) facilities in Nairobi, Kenya.

2.2.2. Study Sites

Study sites included Pumwani, Majengo, SWOP city, Babadogo and Kariobangi SWOP health clinics. These are licensed HIV treatment and care facilities. The SWOP

clinics give treatment to HIV-infected sex workers who live in Nairobi slums. The University of Manitoba from Canada and the University of Nairobi conjointly manage the SWOP clinics. Since the start of the SWOP clinics, around 15, 000 HIV-infected individuals have begun ART. The SWOP clinics chosen for the study were located in the same area to facilitate the sampling of patients from the same source population.

2.2.3. Study Population

The study population comprised of a cohort of ART-naïve, both male and female sex workers aged ≥ 18 years, who began the first-line ART between January 2009 and December 2013 and had at least one follow-up visit record. Using WHO's guidelines, the SWOP clinics started ART in people "with severe or advanced HIV clinical disease (WHO clinical stage III or IV) and in people with CD4 count ≤ 350 cells/mm^3" (WHO, 2013; NASCOP, 2016). Before 2008, the first-line ART regimens comprised of either stavudine (d4T) or azidothymidine (AZT) combined with lamivudine (3TC) and efavirenz (EFV) or nevirapine (NVP). However, in 2010, patients already on AZT or d4T containing regimens continued using them unless toxicity developed. All patients had their follow-up visits after two weeks on starting ART, monthly after stabilising and every quarter for monitoring of treatment outcomes. Longitudinal clinical data routinely collected and reviewed included quarterly the CD4 cell counts, haematological, hepatological and renal function tests in patients who had stabilised on ART. The SWOP facilities did not regularly perform viral load

tests due to high cost. In addition, throughout every visit, research clinicians collected the patients' details on standardised forms to support data transcription in the Kenyan AIDS management database.

2.2.4. Inclusion and Exclusion Criteria

Patients were included into the study in the event that they were HIV-infected, ≥ 18 years, ART-naive, and introduced to the "first-line regimen of stavudine (d4T) or zidovudine (AZT) or tenofovir disoproxil fumarate (TDF) with lamivudine (3TC) and either efavirenz (EFV) or nevirapine (NVP)" (WHO, 2013; NASCOP, 2016) between January 2009 and December 2013. The study excluded patients with confirmed or suspected active tuberculosis, disorders related to the central nervous system, current, or history of alcohol intake, diabetes, hepatitis, cirrhosis of the liver, vitamin B-12 deficiency, hypothyroidism, renal failure, cancer and all patients with suspected adverse drug reactions that developed prior to ART.

2.2.5. Sample Size

The calculation of sample size entailed the application of the single population proportional formula, with the assumption that the prevalence of ADRs was 50%, and 5% margin of error at 95% confidence interval. The sample size was calculated using the following formula.

$$n = \frac{X^2 * N * P * (1-P)}{(ME^2 * (N-1)) + (X^2 * P * (1-P))}$$

Where:

n = sample size

X^2 = Chi – square for the specified confidence level at 1 degree of freedom

N = Population Size

P = population proportion

ME = desired Margin of Error (expressed as a proportion)

Since inception, the SWOP clinics have diagnosed about 25 000 HIV-infected patients. Using this as a source population, 1 500 medical records that met the eligibility criteria were selected. These records were selected from the source population by systematic random sampling. The sampling interval (k) was determined by dividing the total source population by the sample size (25 000/ 1 500 = 16.6). The first medical record was selected randomly from the first sixteen records in the sampling frame and then every sixteenth record was included in the study.

2.2.6. Data Collection

The study used electronic medical records collected from SWOP clinics and maintained in the Kenya AIDS Control Project database (KACP). This database is an electronic system that maintains electronic data of medical records of HIV-infected patients receiving ART. It has structured clinical records containing demographic characteristics, clinical notes from physicians, information on ART regimens and corresponding dates of use, and laboratory diagnostic information for every patient. These data were captured using ARV initiation review form, medical review form, adult clinical staging form and ART review form (**Appendix A**) The data obtained

from KACP database assisted in the description of ADRs and factors that influence their presentation in HIV-infected sex workers. Before extracting electronic files, there was an intensive examination of the information on the medical records. Hard copies of medical records accessed from the SWOP facilities formed the reference for the extraction of any missing information for the analysis.

Data collected included demographic and anthropometric characteristics including age, sex, weight (kg) height (m) and body mass index (BMI). Clinical parameters collected in the study included ADRs, CD4 T cell counts, pregnancy status, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and haemoglobin (Hb); treatment variables, including the types of ART regimens, ART initiation and regimen change dates, reasons for change, new ART regimens, treatment outcomes and dates of outcomes.

The research clinicians performed all anthropometric measurements using graduated scales. Measurements of current weight (kg) and height (cm) were duplicates based on World Health Organisation's guidelines (WHO, 1995). To calculate body mass index (BMI), the analysis used the arithmetic mean obtained from the two measurements to divide the weight (kilograms) by the square of the height (metres).

2.2.7. Study Variables

The predictor variables in the study included demographic variables such as age at ART initiation and sex, laboratory parameters such as CD4 cell counts, Hb, creatinine, ALT, and AST, treatment variables, including types of ART regimens, ART initiation and regimen change dates, reasons for change, new ART regimens, treatment outcomes and dates of outcomes.

The primary outcome during this analysis was the first incident of an adverse drug reaction (ADR) defined by the Kenya National Clinical Manual as any severe and critical effect of antiretroviral (ARV) drugs presented at normal doses (NASCOP, 2007). Censoring occurred at the time of the first incident of an ADR.

Another outcome was drug changes that covered either an individual drug substitution from d4T, ZDV and TDF because of ADRs specifically associated with that drug or a regimen switch from first-line to second-line treatment in the event of virological failure.

2.2.8. Definition of Terms

First-line ART:

First-line ART consisted of the initial regimen prescribed for a patient who fulfilled national clinical and laboratory criteria to start ART (NASCOP, 2007; NACC, 2014a). Kenyan guidelines recommended first-line ARV drugs at the time of the study

included two categories of the initial treatment that were two nucleoside / nucleotide reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI).

Second-line ART:

Second-line ART included the next regimen used in sequence immediately after the failure of the first-line therapy. Kenyan treatment guidelines at the time of the study recommended second-line ART to comprise of a protease inhibitor (PI) (NASCOP, 2016).

Drug substitutions

Antiretroviral drug substitutions (usually within the same class) referred to the replacement of an individual drug due toxicity, drug-drug interaction, or intolerance; and did not signal the initiation of a second-line regimen.

Regimen Switch

This involved the replacement of first-line treatment with the second-line regimen because of the development of virological failure.

2.2.9. Types of Adverse Drug Reactions Evaluated in the Study

Polyneuropathy

Polyneuropathy consisted of symptoms that included tingling, burning, pain, numbness, weakness in limbs, paraesthesia, muscle weakness, and inability to walk.

Lipodystrophy

Signs characterizing lipodystrophy included a hump on the back, loss of facial fat, diminishing or squandering of the limbs, and additionally aggregation of fat in the abdomen.

Hepatotoxicity

The study considered hepatotoxicity based on the levels of liver enzymes detected in the blood. Patients with alanine transaminase (ALT) or aspartate transaminase (AST) levels above the upper limit of normal (ULN, > 40 IU/L) were described as having hepatotoxicity.

Anaemia

Patients with anaemia had <11 g/dl of haemoglobin (Hb), without a history of anaemia or a > 1 g / dl drop in Hb after starting ART (van der Klauw *et al.,* 1999).

Skin Rash

Patients with a skin rash had erythema, pruritus, and serious mucous membrane involvement (Stevens-Johnson disorder).

Renal Toxicity

Patients with renal toxicity consisted of cases with > 150 μmol / l of serum creatinine after starting ART.

Baseline characteristics included measurements of demographic parameters such as age and sex; anthropometric variables including weight, height, and body mass

index (BMI); laboratory parameters such as CD4 cells, Hb, ALT, AST, and creatinine; and clinical history collected closest to ART starting date.

2.2.10. Statistical Analysis

Data analysis was performed using SPSS software version 17.0. Chicago: SPSS Inc. (USA). All the collected data were subjected to descriptive analysis. The Shapiro-Wilk test was used to determine which continuous variables were normally distributed. Further, continuous variables, which were not normally distributed, were reported as medians and interquartile ranges (IQR) while those with normal distribution as means. All categorical variables were presented as proportions. The calculation of the prevalence rate was done as a proportion of people with specific ADRs.

Estimation of survival time to the initial occurrence of an adverse drug reaction was conducted through Kaplan-Meier survival analysis (Kaplan and Meier, 1958). Censoring of patients targeted the time of the first occurrence of an adverse drug reaction. Modeling for the risk factors associated with occurrence of ADRs used the Cox Proportional Hazards analysis. The model covered the subsequent variables: age, sex at ART initiation, height, weight, BMI, Hb, CD4 cell count, diastolic and systolic blood pressure, AST, ALT, creatinine, types of ART regimens and calendar year of ART initiation. The analysis considered significant factors with a p - value of less than 0.05. To avoid over-adjustment, the multivariate model did not adjust for

height and weight. To introduce BMI, Hb, AST and ALT as continuous variables in the Cox model, the analysis used standardised residuals to check for linearity before transforming the data by Log_{10} transformation.

2.2.11. Ethical Considerations

The study received approval from the Kenyatta National Hospital / University of Nairobi Ethics and Research Committee (**Appendix B**). The Sex Workers Outreach programme (SWOP) administration granted permission for the use of electronic and hard copies of medical files in the clinics.

2.3. RESULTS

The total number of patients that met the eligibility criteria was 1 500. However, after exclusion of patient records with duplicate data sets, 1 450 patient medical records were analysed (**Figure 2.1**). These represented 7 250 clinical visits with a mean of five visits per patient.

Out of 1 450 medical records selected 36% were from Babadogo, 34% from Pumwani 17% from Majengo, 9% from SWOP city and 4% from Kariobangi clinic.

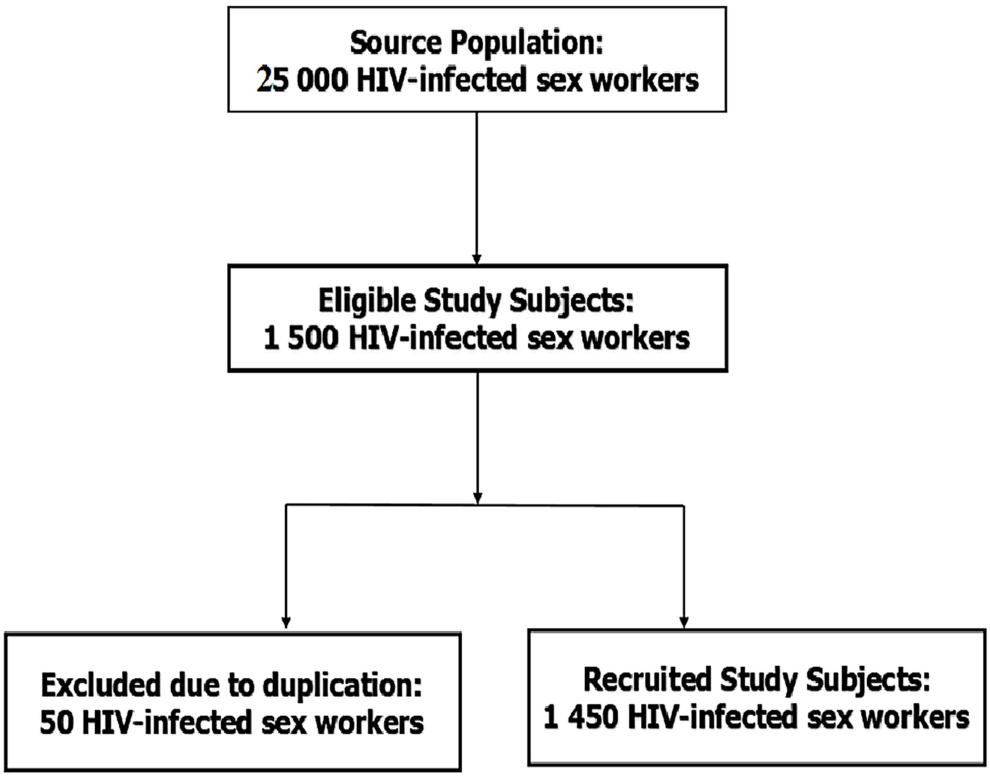

Figure 2.1: A flow diagram illustrating the recruitment of HIV-infected patients from the SWOP clinics

2.3.1. Baseline Characteristics

The median age of the study patients was 39 (33-45) and the percentages of patients in age groups ≤ 30, 31-40, 41-50 and ≥ 50 were 10.3% (150), 42.8% (620), 35.9% (520), and 11.1% (160) respectively (**Table 2.1**). The demographic characteristics of the 1 450 study patients showed that the majority were female [1 128 (77.8%)] and a minority being male [322 (22.2%)]. About 507 (35%) of the study patients recorded CD4 cell count of < 200 cells/μl, 757 (52.5%) of patients had CD4 cell counts of between 200-350 cells/μl, and 186 (13%) had > 350 cells/μl. About 522 (36%) of the

HIV-infected patients had been initiated on AZT-based regimens, 680 (47%) on d4T-based regimens, and 250 (17%) on TDF-based regimens.

Table 2.1: Baseline characteristics of HIV-infected study patients on long-term ART at the SWOP clinics

Baseline Characteristics		**Patients without ADRs n=944 (65.1%)**	**Patients with ADRs n= 506 (34.9%)**
Age (years)	Mean	36.8±8	39±8
Gender			
Female	n = 1 128	743(78.7%)	385(76.1%)
Male	n = 322	201(21.3%)	121(23.9%)
Height (m)	Median (IQR)	1.6(1.4-1.8)	1.6(1.4-1.9)
BMI (kg/m^2)	Median (IQR)	23.2(15.5-39.8)	23.7(15.9-36.5)
CD4 count (cells/mm^3)	Median (IQR)	248(9-566)	233(35-500)
Haemoglobin (g/dL)	Median (IQR)	13(11-14)	13(11-14)
Alkaline phosphatase (IU/L)	Median (IQR)	226(66-463)	246(68-488)
Aspartate aminotransferase (IU/L)	Median (IQR)	21(7-85)	23(9-86)
Alanine aminotransferase (IU/L)	Median (IQR)	19(3-150)	23(6-195)
ART-initiated			
AZT-Based	n = 520	380(26%)	140(9.6%)
d4T-Based	n = 680	392(27%)	288(19.9%)
TDF-Based	n = 250	172(12%)	78(5.4%)
ART-duration (years)	Median (IQR)	4.3(0.8-5.8)	4.3(1.7-5.3)

2.3.2. The Prevalence of Adverse Drug Reactions in the Patient Population

This study reports an overall prevalence of ADRs of 34.9%. Lipodystrophy, polyneuropathy, and anaemia were the most frequently encountered ADRs in the patient population (**Figure 2.2**).

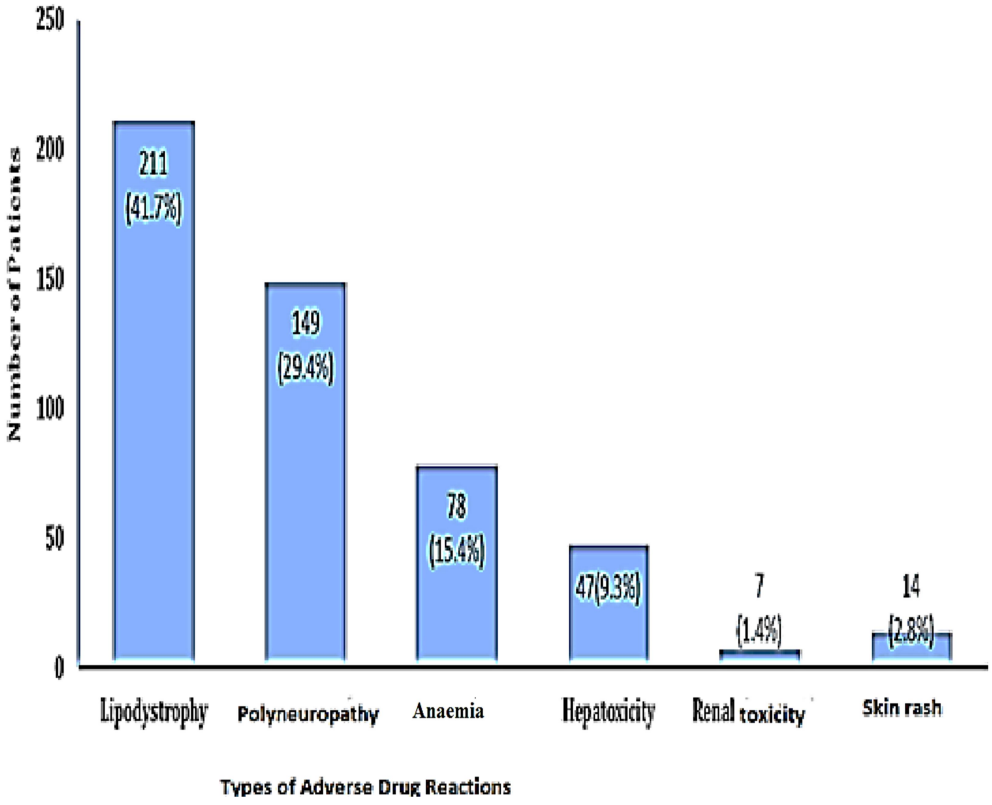

Figure 2.2: Prevalence of antiretroviral therapy-related adverse drug reactions in HIV-infected patients on long-term ART at the SWOP clinics.

Figure 2.3 shows the distribution of antiretroviral-related ADRs in HIV-infected patients in SWOP clinics over a period of 5 years. The ADRs distribution by age group < 30, 30-40, 40-50 and above 50 years were 40 (2.8%), 196 (13.5%), 220 (15.2%) and 50 (3.4%) respectively.

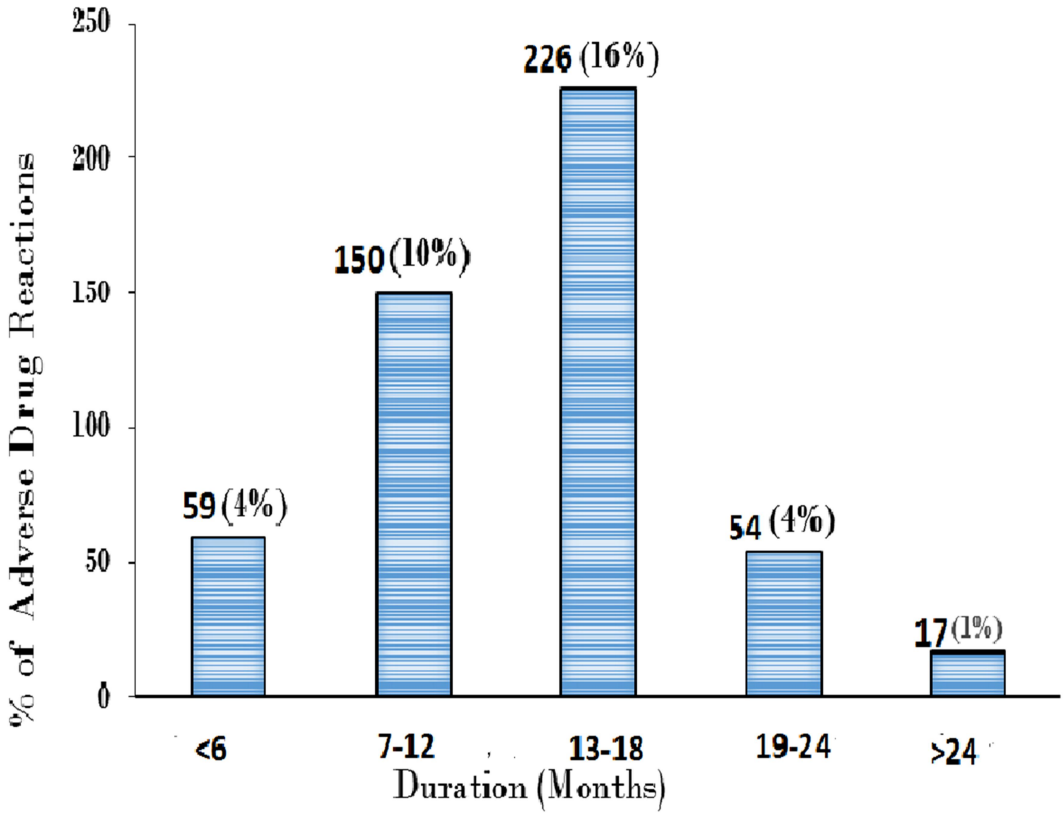

Figure 2.3: The distribution of antiretroviral therapy – related adverse drug reactions in HIV-infected patients on long-term ART at the SWOP clinics

2.3.3. Antiretroviral Therapy Drug Changes

Most patients changed their first-line ART regimens after experiencing lipodystrophy and polyneuropathy (**Table 2.2**). The results showed that 78 people had anaemia of which 10 (12.8%) of patients had drug substitutions.

Table 2.2: Types of adverse drug reactions resulting in changes of antiretroviral drugs in HIV-infected patients in SWOP clinics

Types of ADRs	Zidovudine (n=15)	Tenofovir (n=4)	Stavudine (n=268)
Lipodystrophy	4 (26.7%)	3 (75%)	204 (76.1%)
Polyneuropathy	2 (13.3%)	0 (0%)	54 (20.1%)
Anaemia	7 (46.7%)	0 (0%)	3 (1.1%)
Renal toxicity	0 (0%)	0 (0%)	0 (0%)
Hepatotoxicity	0 (0%)	1 (25%)	6 (2.2%)
Skin rash	2 (13.3%)	0 (0%)	1 (0.4%)

ADRs-adverse drug reactions; AZT-zidovudine; d4T-stavudine; TDF-tenofovir disoproxil fumarate; ART-antiretroviral therapy

Table 2.3 shows the factors related to ART drug changes. In general, most patients had a drug change owing to a single factor. However, a small proportion of patients changed drugs owing to many factors. The main factor for a drug change was ADRs, accounting for 75.5% of the drug changes (**Table 2.3**). Drug changes associated with tuberculosis infection were rare among AZT, d4T, and TDF users.

Table 2.3: Factors contributing to changes in antiretroviral drugs in HIV-infected patients (n = 380) in the SWOP clinics

Factors	Zidovudine (n=26)	Tenofovir (n=16)	Stavudine (n=338)
Adverse Drug Reactions	15 (57.7%)	4 (25%)	268 (79.3%)
Treatment failure	1 (3.8%)	2 (8%)	2 (0.6%)
Immunological failure	0	1 (6.3%)	7 (2.1%)
Defaulted treatment	1 (3.8%)	1 (6.3%)	7 (2.1%)
Tuberculosis	2 (7.7%)	7 (43.8%)	8 (2.4%)
Pregnancy	1 (3.8%)	1 (6.3%)	14 (4.1%)
Drug stock-out	0	0	5 (1.5%)
Reason not stated	6 (23.1%)	0 (0%)	27 (8%)

ADRs-adverse drug reactions; ART-antiretroviral therapy; AZT-zidovudine; TDF- tenofovir disoproxil fumarate; d4T-stavudine

Individual drug substitutions from one first-line to a different first-line regimen were the key drivers of ART drug changes owing to ADRs. However, other factors contributing to the switch of ARV drugs from one first-line ART regimen to a second-line ART regimen included immunological failure, treatment failure, and poor adherence to ART.

2.3.4. Risk Factors Associated with Adverse Drug Reactions

A Kaplan-Meier plot presented in Figure 2.4 illustrates the time to the development of the first occurrence of an ADR in the patient population (**Figure 2.4**). The risk of patients experiencing an ADR during 24 months after initiation to either AZT or TDF or d4T based regimens was statistically significant (Log-rank test $p = 0.044$).

Further, univariate and multivariate analyses of time to the first occurrence of an ADR were performed. In a univariate analysis, an increased hazard of an adverse drug reaction was significantly related to HIV-infected patients aged ≥ 40 years, height, creatinine, and in HIV-infected patients who were initiated on ART in 2010 (**Table 2.4**).

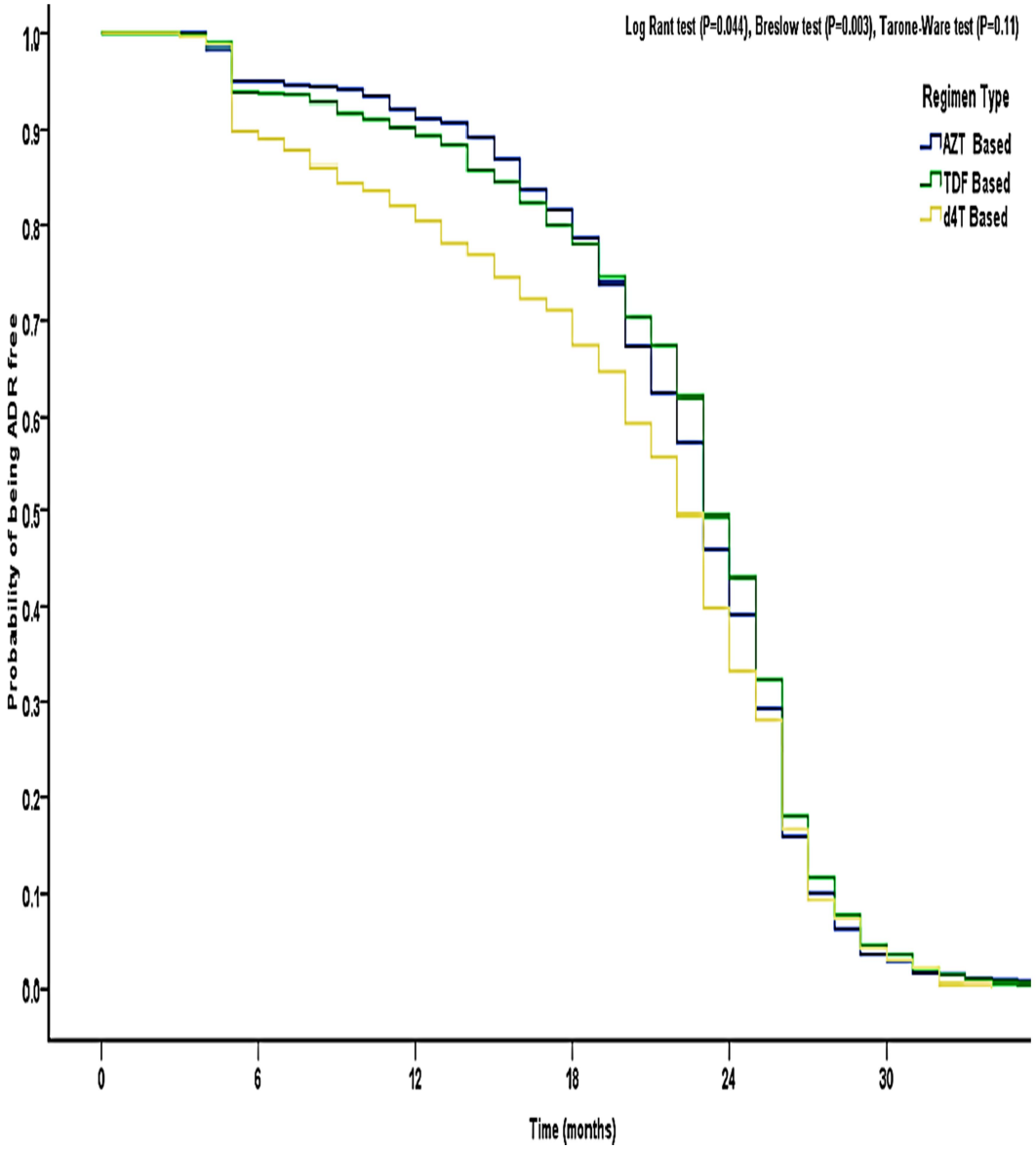

Figure 2.4: Duration to the first occurrence of an adverse drug reaction in HIV-infected patients in the SWOP clinics.

The multivariate analysis identified an increased risk for experiencing an ADR in older HIV-infected patients (≥ 40 years) [hazards ratio 1.0; 95% CI: 1.0-1.1] and reduced risk of experiencing an ADR in HIV-infected patients on TDF [hazards ratio 0.50; 95% CI: 0.3-0.8].

Table 2.4: Risk factors for time to the first occurrence of an adverse drug reaction in HIV-infected patients in the study SWOP clinics

Variable	Univariate Analysis			Multivariate Analysis		
	Hazard ratio	95% CI	*P* -value	Hazard ratio	95% CI	*P* - value
Age (years)						
Age <40	**Reference**					
Age ≥40	1.0	1.0-1.6	**0.004***	1.0	1.0-1.1	**0.002***
Sex						
Male	**Reference**					
Female	0.5	0.30-0.80	**<0.001**	1.4	0.9-2.2	0.116
Baseline CD4 cell count	**Reference**					
<200						
≥ 200	1.0	1.0-1.0	0.84	1.00	1.00-1.01	0.68
Height (cm)	2.6	1.8-3.9	**0.04**	1.1	0.30-3.30	0.98
Baseline BMI	1.00	1.00-1.00	0.43	3.31	0.54-21.01	0.19
Baseline Hb	0.99	0.95-1.02	0.48	0.87	0.29-2.56	0.79
Baseline AST	1.00	0.99-1.00	0.82	1.34	0.70-5.57	0.37
Baseline ALT	1.00	1.00-1.00	0.44	1.17	0.66-2.56	0.60
Baseline creatinine	1.01	1.00-1.01	**0.001**	1.01	1.00-1.01	0.23
ART Regimen						
Zidovudine	**Reference**					
Stavudine	1.10	0.70-1.60	**0.71**	1.1	0.70-1.70	0.57
Tenofovir	0.50	0.30-0.80	**0.01***	0.50	0.30-0.80	**0.01***
Year of Initiation						
2009	**Reference**					
2010	6.24	2.30-16.9	**0.00**	1.23	0.44-3.39	0.71
2011	1.61	0.54-4.77	0.39	0.86	0.29-2.57	0.78
2012	1.19	0.89-3.66	0.76	0.57	0.18-1.77	0.33

ART- antiretroviral therapy; ALT-alanine transaminase; AST-aspartate aminotransferase; BMI-Body Mass Index; CD4-Cluster of Differentiation 4; HB-haemoglobin; SWOP-sex workers outreach programme.

2.4. DISCUSSION

Data on the prevalence of adverse drug reactions (ADRs) and the risk factors related to their presentation in HIV-infected patients are deficient in the Kenyan context. The objective of this study was to describe the prevalence and determinants of ADRs related to long-term ART in an adult cohort of HIV-infected patients at the Sex Workers Outreach Programme (SWOP) in Nairobi, Kenya. The findings revealed

that the overall prevalence of ADRs was 34.9%. Lipodystrophy (41.7%) and polyneuropathy (29.4%) were the most commonly encountered ADRs in the patient population, accounting for 75.5% of reported antiretroviral drug changes. Whereas older age (≥ 40 years) was significantly related to an increased hazard of developing ADRs [hazards ratio 1.0; 95% CI: 1.0-1.1], the use of TDF was significantly related to a reduced risk of experiencing ADRs [hazards ratio 0.50; 95% CI: 0.3-0.8].

Owing to the application of various study methodologies, this study estimated a lower prevalence of ADRs of 34.9% compared to regional studies, which reported higher prevalence rates of ADRs of 48% in Kenya (Mwangangi *et al.*, 2010) and 40% in Uganda (Forna *et al.,* 2007). The observed variations in prevalence rates of ADRs between studies may be because of bias ensuing from two sources of data. Patients are likely to contribute to bias during self-reporting and on the other hand; clinicians capturing patients' data at the facility level may contribute to recording bias.

Although commonly reported types of ADRs vary from one study to another, the findings in this study support regional studies that reported lipid abnormality and polyneuropathy are the most commonly encountered ADRs (Hawkins *et al.,* 2007; Mwangangi *et al.,* 2010). Nonetheless, comparisons between studies remain problematic as a result of variations in ADR reporting procedures (Menezes de Pádua *et al.,* 2007) and the possible influence of concurrent medication (Westreich *et al.,* 2009). In this study, patients who were receiving concomitant treatments

including cotrimoxazole, antituberculosis drugs, anti-cancer drugs, multivitamins and other viral agents were not eligible. This is because evidence shows that such medication may influence the development of different types of ADRs experienced by HIV-infected patients (Jha *et al.*, 2015). Dermatological toxicities are common adverse drug reactions in HIV-infected patients on ART. For that reason, other commonly prescribed concomitant drugs in SWOP clinics, which may have influenced the development of dermatological ADRs, include sulfonamides and amoxicillin. Studies have associated sulfonamides and amoxicillin with the development of skin rashes including Stevens-Johnson syndrome (Jha *et al.*, 2015).

Before the WHO ban on the use of d4T (WHO, 2010), this regimen was common in the SWOP facilities compared to AZT and TDF. During the study period, HIV-infected patients on TDF-based regimens experienced fewer drug changes and these regimens were well tolerated. This observation is supported by previous studies conducted in Southern Africa (Chi *et al.*, 2010; Boyle *et al.,* 2012; Brennan *et al.*, 2013). Though these studies suggest that the use of TDF is well-tolerated, other studies have linked TDF to short-term renal effects (Bygrave *et al.,* 2011; Chua *et al.*, 2012; Mugomeri *et al.,* 2014). However, in the case of this study, no patient discontinued treatment due to renal adverse reactions. The present findings additionally attest that ADRs are the main reason for the ART drug change as reported in a variety of resource-poor settings (Maggiolo *et al.,* 2007; Hawkins *et al.,* 2007; Inzaule *et al.,* 2014). In Uganda and Swaziland, the main causes of d4T-based drug changes

included lipodystrophy and polyneuropathy, whilst anaemia caused most of the AZT-based drug substitutions (Castelnuovo *et al.,* 2011; Evans *et al.*, 2012). Similarly, the present study had a significantly higher proportion of patients who changed d4T to TDF. Most of these changes were in line with the WHO's recommendations of 2010 (WHO, 2010).

Studies have related older age to the development polyneuropathy, lipodystrophy and renal toxicity (WHO, 2010; Prosperi *et al.,* 2012). This is in agreement with the current study where older patients frequently changed ART due to lipodystrophy and polyneuropathy. Evidence from an Italian study "showed higher rates of ADRs or personal choice discontinuation among patients over 50 years of age" (Orlando *et al.,* 2010). To the contrary, some studies reported that "older patients stayed significantly longer on first-line ART regimen" (Kowalska *et al.,* 2016). This study lacked information on patients who did not change to second-line ART due to loss to follow-up, therefore the general ART drug changes reported in the current study were underestimated.

Lastly, the duration of treatment also influences the incidence of ADRs in HIV-infected patients. According to Eluwa et al. (2012), the longer a patient stays on ART the less likely they would experience ADRs; possibly as a result of stability in ARV regimen, coming after many changes and eventually settling on an acceptable regimen (Eluwa *et al.*, 2012). That explains why in this study the risk of HIV-infected

patients' experiencing an ADR during the initial 24 months after initiation of ART was statistically significant.

2.5. CONCLUSIONS

The findings of this study suggest a moderate prevalence of adverse drug reactions among sex workers in Nairobi, Kenya. Lipodystrophy and polyneuropathy were the most often encountered kinds of adverse drug reactions that accounted for most ART drug changes in this cohort. Patients with advanced age (≥ 40 years) were at a higher risk of experiencing adverse drug reactions and hence more likely to change ART regimen.

CHAPTER THREE:

FACTORS INFLUENCING DEVELOPMENT OF POLYNEUROPATHY IN HIV-INFECTED PATIENTS IN THE SEX WORKERS OUT-REACH PROGRAMME IN KENYA

3.1. INTRODUCTION

The results given in chapter two depicts polyneuropathy as a typical adverse drug reaction (ADR) among HIV-infected patients in the study population. However, Kenya has limited data on factors related to the development of polyneuropathy. Additionally, findings from studies performed in other African countries and rich nations have produced conflicting evidence on factors affecting the development of polyneuropathy in HIV-infected people. In view of this, chapter three presents the analysis of a nested case-control cohort of HIV-infected patients at the Sex Workers Outreach Programme (SWOP) clinic in Nairobi, Kenya. The study objective was to identify independent factors influencing polyneuropathy development in HIV-infected Kenyans undertaking first-line antiretroviral therapy (ART).

3.2. METHODS

3.2.1. Study Design

This was a nested matched case-control study involving HIV-infected patients with polyneuropathy (cases) and those without polyneuropathy (controls) on long-term ART.

3.2.2. Study Site

The sampling of HIV-infected patients took place at Pumwani clinic, because, it is a well-established cohort of sex workers (Fowke *et al.*, 1996). Pumwani clinic is part of the SWOP facilities managed by both the University of Manitoba from Canada and the University of Nairobi, from Kenya. A clinician examined all ART-eligible patients before initiating first-line ART according to Kenyan ART guidelines.

3.2.3. Study Population

Pumwani clinic is one of the SWOP clinics located in Nairobi, Kenya. Briefly, Pumwani sex workers cohort started in 1985 as an observational cohort of sexually transmitted infections (STIs) (Plummer *et al.*, 1985). Being an open prospective cohort, monitoring of enrolled patients is biannual. Moreover, patients receive services related to HIV and STI prevention and care, alongside consultation, provision of condoms, and treatment of other infections. Pumwani cohort has an overall HIV prevalence of around 70% and has an annual incidence of HIV infection among seronegative women of four per 100 person-years (PY). By the end of 2013, close to 6,202 sex workers had registered in the Kenya AIDS control Project database at the Pumwani clinic.

3.2.4. Inclusion and Exclusion Criteria

Eligible patients were HIV-infected, ≥18 years, ART-naive, and introduced to the first-line ART regimen of d4T/AZT/TDF with 3TC and either EFV or NVP between

January 2009 and December 2013. Patients with suspected or confirmed active tuberculosis, disorders associated with the central nervous system, current, or history of diabetes, hepatitis, cirrhosis of the liver, vitamin B12 deficiency, renal disorder, cancer, hypothyroidism, and history of alcohol consumption were not included in the analysis.

3.2.5. Sample Size

The study projected three controls per case using Dupont's power calculations for matched case-control studies (Dupont, 1988). Therefore, the analysis approximated that 104 cases and 312 controls were necessary to attain 80% power to detect an odds ratio of two. The following formula was used to calculate the sample size.

$$n = \left(\frac{r+1}{r}\right)\frac{(\overline{p})(1-\overline{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

Where n = Sample size in the case group
r = Ratio of controls to cases
$\overline{p}$ = A measure of variability (similar to standard deviation)
$(P_1 - P_2)^2$ = Effect Size (the difference in proportions)
$Z\beta$ = Represents the desired power (typically .84 for 80% power)
$Z\alpha$ = Represents the desired level of statistical significance (typically 1.96)

3.2.6. Selection of Cases with Polyneuropathy

To diagnose polyneuropathy the clinicians used their own judgment and presence of "a minimum of one of the lower limb neuropathic clinical symptoms namely pain or aching, dysesthesia, stabbing, burning sensation, pins, and needles" (Cherry *et al.*,

2008; Evans *et al.*, 2012). Clinicians in SWOP clinics had undergone intensive training in clinical presentation of polyneuropathy and diagnosis polyneuropathy.

3.2.7. Selection of Controls

Controls consisted of HIV-infected patients from the same population as cases, who had no history of polyneuropathy. Controls were matched to the cases based on the period of ART, gender and type of regimen. At the beginning, controls were assigned a date corresponding to the date when polyneuropathy cases were diagnosed. This date had an identical number of days after the start of ART as matched cases with polyneuropathy. Following retrieval and confirmation of the information based on the eligibility criteria, the controls were recruited if found eligible.

3.2.8. Data Collection

Using the Kenya AIDS Control Project database, HIV-infected patients with polyneuropathy were identified from a cohort of 6 202 commercial sex workers who started ART between January 2009 and December 2013 period. The diagnosis of polyneuropathy was confirmed in the identified cases during the next routine visit. The date of ART initiation formed the baseline. The study then used the Kenya AIDS Control Project database to abstract data pertaining to the patient's demographic and clinical characteristics. The abstraction period covered up to seven follow-up visits, which totalled to about 5 to 6 months.

3.2.8.1. Anthropometric Measurements

Using calibrated scales, weighing of patients was free of any heavy items, executed by removal of shoes. Measurements of current weight (kg) and height (cm) were taken in duplicate as per the recommendations by World Health Organisation (WHO, 1995). Calculations of Body Mass Index (BMI) used the arithmetic means obtained from the duplicate weight and height measurements. To calculate BMI, weight (kg) was divided by the square of the height (m).

3.2.9. Data Analysis

Data were analysed using SPSS software, version 20 (IBM, SPSS. USA). All the data were subjected to descriptive data analysis. Continuous variables were analysed by use of means and medians. Categorical variables were analysed and presented as percentages. The student t test was used to analyse pairs of continuous variables while χ^2 tests were used to analyse variables that fit a normal distribution.

3.2.10. Ethics Considerations

The study received approval from the Kenyatta National Hospital / University of Nairobi Ethics and Research Committee (**Appendix B**). The Sex Workers Outreach programme (SWOP) administration granted permission to the electronic and hard copies of medical files in the clinics. HIV-infected patients who were recruited to donate their blood signed an informed consent (**Appendix C**).

3.3. RESULTS

After exclusion of patients with complete lack of laboratory data during the ART initiation, patients with duplicate data sets, and unconfirmed signs and symptoms for polyneuropathy, 320 patients (n = 212 without polyneuropathy, n = 14 pre-ART polyneuropathy, n = 94 post-ART polyneuropathy) were identified (**Figure 3.1**). The 14 were excluded from the study because they exhibited 14 pre-ART polyneuropathy.

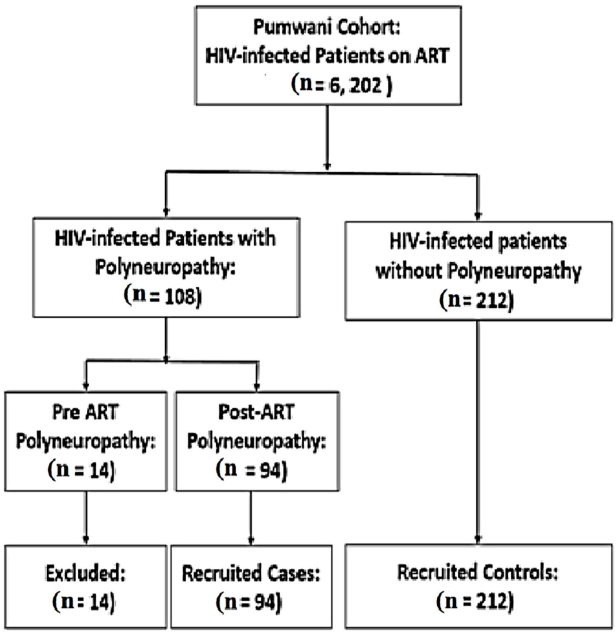

Figure 3.1: A flow diagram illustrating the recruitment of HIV-infected patients with polyneuropathy (cases) and those without polyneuropathy (controls) at the Pumwani SWOP clinic.

3.3.1. Baseline Characteristics of patients

At baseline, more than half of all patients (50.7%) were on d4T (**Table 3.1**). However, patients who received d4T and the controls showed no significant variations (p = 0.593) (**Table 3.2**). Additionally, 16.7 % of these patients ended up developing

polyneuropathy. HIV-infected patients with polyneuropathy were older (mean age = 39±8) compared to patients without polyneuropathy who had a mean age of 36.8±8 (p = 0.017) (**Table 3.2**).

Table 3.1: Baseline characteristics of HIV-infected patients with polyneuropathy and those without polyneuropathy at the Pumwani SWOP clinic (n=306).

Baseline characteristics		**Polyneuropathy Free** n = 212 (69.3%)	**Polyneuropathy After ART** n = 94 (30.7%)
Age (years)	Mean ±	36.8±8	39±8
Gender	Female	148(48.4%)	82(26.8%)
Height (m)	Median (IQR)	1.6(1.4-1.8)	1.6(1.4-1.9)
BMI (kg/m^2)	Median (IQR)	23.2(15.5-39.8)	23.7(15.9-36.5)
CD4 count (cells/mm^3)	Median (IQR)	248(9-566)	233(35-500)
Haemoglobin (g/dL)	Median (IQR)	13(11-14)	13(11-14)
Alkaline phosphatase (IU/L)	Median (IQR)	226(66-463)	246(68-488)
Aspartate aminotransferase (IU/L)	Median (IQR)	21(7-85)	23(9-86)
Alanine aminotransferase (IU/L)	Median (IQR)	19(3-150)	23(6-195)
ART-Regimen initiated	AZT-Based d4T-Based TDF-Based	91(29.7%) 104(33.9%) 17(5.5%)	38(12.4%) 51(16.7%) 19(6.2%)
ART-current	AZT-Based d4T-Based TDF-Based	104(33.9%) 26(8.5%) 82(26.8%)	44(14.4%) 11(3.6%) 52(17%)
ART-duration (years)	Median (IQR)	4.3(0.8-5.8)	4.3(1.7-5.3)

3.3.2. Risk Factors for Polyneuropathy

Patients who developed polyneuropathy during treatment did not significantly differ at baseline in terms of gender, height, body mass index (BMI), heart rate, blood pressure, CD4 cell count, haemoglobin, white corpuscle count, platelet count,

and CD4/CD8 ratio from patients without polyneuropathy (**Table 3.2**). Similarly, there was no significant difference in the initial ART (except TDF), time to initial change of first-line ART regimen and ART duration between the two study patients' groups. Patients with polyneuropathy were considerably older ($p = 0.017$) and had a higher systolic blood pressure ($p = 0.025$) than those who did not develop polyneuropathy.

There was a significant difference in first-line ART regimen, with many patients on TDF-based ART in the polyneuropathy cohort following ART initiation ($p = 0.017$). Other first-line ART regimens showed no significant difference (**Table 3.2**). Regarding gender and ART duration matched patients, and with the exclusion of different known factors of polyneuropathy, height was not significantly different in patients who developed polyneuropathy.

Table 3.2: Risk factors for polyneuropathy in HIV-infected patients with polyneuropathy and those without polyneuropathy at Pumwani SWOP clinic

Baseline Characteristics [Mean (SD)]	**No PN (n = 212)**	**PN-After ART (n = 94)**	**Total (n = 306)**	**Differences No PN vs. PN after ART**
Age (years)	36.8±7.9	39.2±8.0	37.6±8.2	**t =-2.40 p=.017**
Gender (Female) [n(%)]	148 (69.8%)	68(72.3%)	230(71.9%)	χ^2 =0.2 p=0.685
Height (cm)	163.0±8.8	162.1±8.1	162.6±8.7	**t** =.83 p=.405
BMI (kg/m^2)	24.1±4.3	24.4±4.4	24.3±4.3	t =-.51, p=.608
Heart rate (bpm)	83.4 ±13.6	83.4±12.7	83.3±13.2	t =-.01 p=.990
Blood pressure systolic (mmHg)	115.6±13.6	120.1±18.2	117.0±16.2	**t =-2.25 p=.025**
Blood pressure diastolic (mmHg)	69.0±10.3	70.8±11.0	69.5±10.6	t =-1.44 p=.150
CD4 count (cells/mm^3)	240.0±102.4 (n=125)	235.4±103.4 (n=64)	239.9±103.2 (n=196)	t =.30 p=.766
Hemoglobin (g/dL)	12.7±2.7 (n=125)	12.4±2.4 (n=64)	12.6±2.6 (n=196)	t =.61 p=.583
White blood cell count (tsd. cells/µl)	5.4±2.5 (n=125)	5.6±2.6 (n=64)	5.5±2.5 (n=196)	t =-.55 p=.583
Platelet count (tsd. cells/µl)	294.4±100.2 (n=118)	280.9±101.4 (n=57)	291.9±100.2 (n=182)	t =.83 p=.407
CD4/CD8 ratio	0.28±0.17 (n=97)	0.29±0.18 (n=53)	0.3±0.2 (n=157)	t =-.28 p=.778
ART-initiated				
• AZT - Based	91(42.9%)	34(36.2%)	125(40.3%)	χ^2 =1.23 p=0.314
• d4T - Based	104(49.1%)	43(45.7%)	147(48.4%)	χ^2 =0.29 p=0.593
• TDF - Based	17(8%)	17(18.1%)	34(11.3%)	**χ^2 =6.68 p=0.017**
• 3TC - Based	212(100%)	94(100%)	306(100%)	
• NVP - Based	182(85.8%)	76(80.9%)	258(84.4%)	χ^2 =1.23 p=0.307
• EFV - Based	30(14.2%)	18(19.1%)	38(15.6%)	χ^2=1.23 p=0.307
Time to first change of ART (months)	37.7±13.8	34.7±17.0	36.8±14.9	t =1.67 p=.097
ART-duration (months)	47.6±10.4	48.4±10.0	47.9±10.2	t =-.64 p=.525

SD-standard deviation; cm-centimeter; n-number; kg/m2-kilogram per square meter; BMI-body mass index; bpm-beats per minute; mmHg-millimeters of mercury; CD4-cluster of differentiation-4; CD8-cluster of differentiation-8; g/dL-grams per deciliter; tsd cells/µl-thousand cells per microliter; AZT-zidovudine; d4T- stavudine; 3TC-lamivudine; TDF-tenoforvir disoproxil fumerate; NVP-nevirapine; EFV-efavirenz; ART-antiretroviral therapy; PN-polyneuropathy.

Changing from d4T was more frequent in HIV-infected patients who developed polyneuropathy than in HIV-infected patients who did not develop polyneuropathy (**Figure 3.2**).

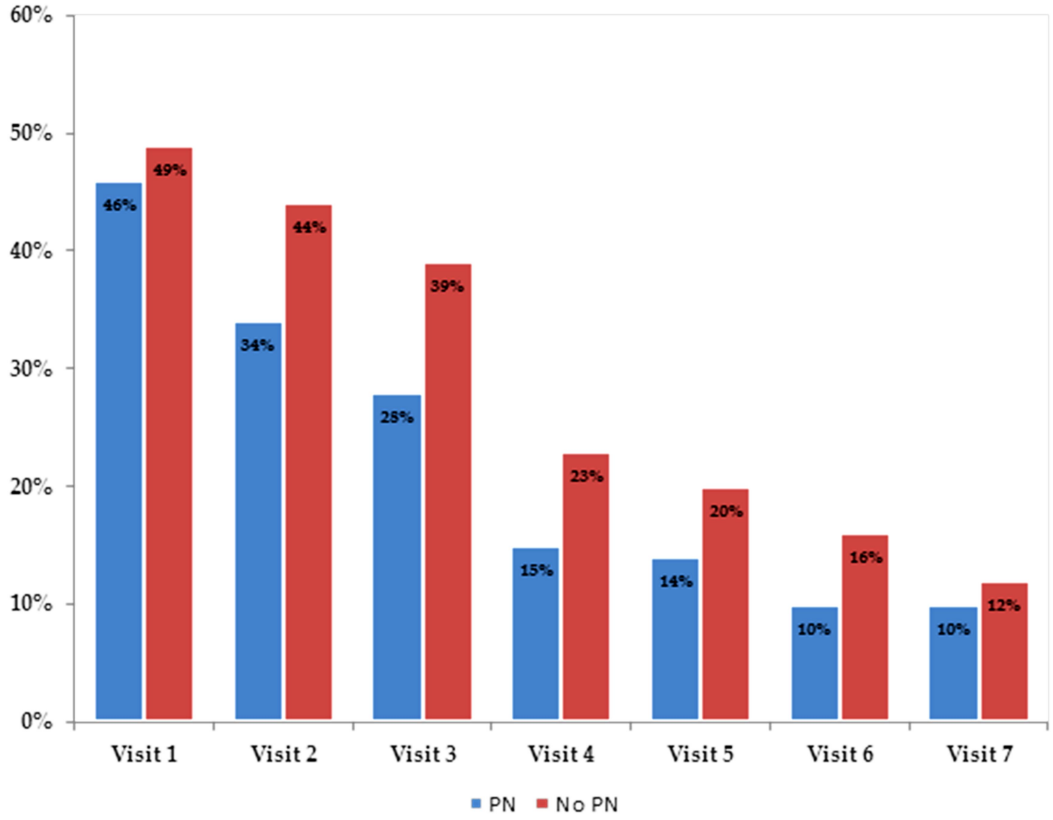

Figure 3.2: Percentage of d4T-based regimens during seven visits by HIV-infected patients at the Pumwani SWOP clinic.

3.3.3. Time to Development of Polyneuropathy

The mean time to development of polyneuropathy was 13.1 (±11.4) months after initiation of ART (**Figure 3.3**). The mean follow-up period was 4.0 years for all the patient groups.

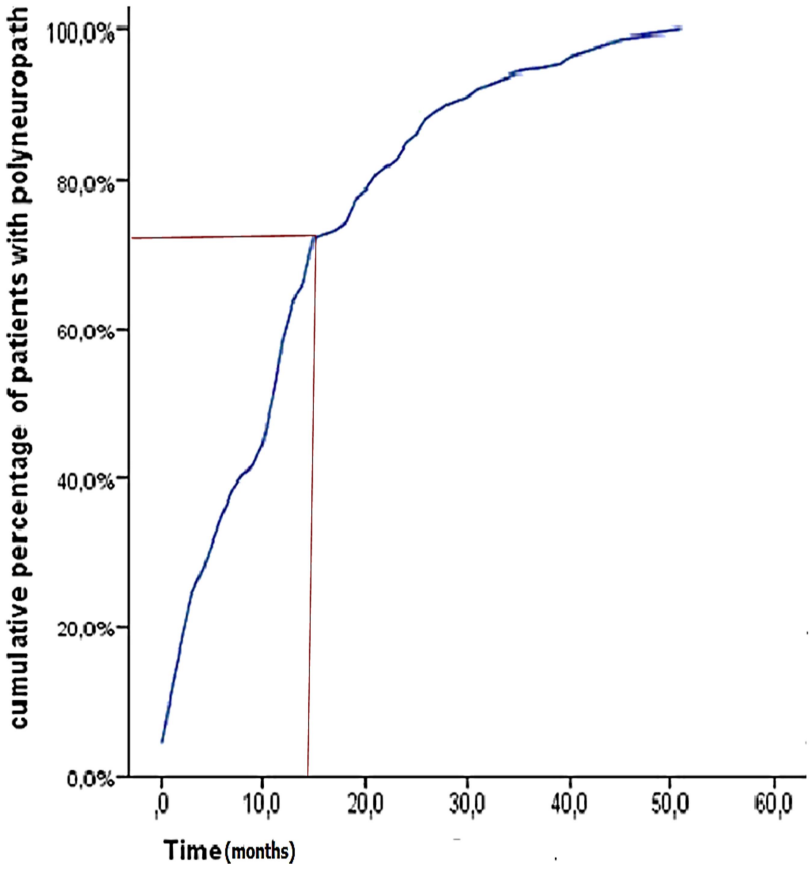

Figure 3.3: Time to the development of polyneuropathy in HIV-infected patients at the Pumwani SWOP clinic.

3.4. DISCUSSIONS

The study aimed to identify risk factors for polyneuropathy in HIV-infected Kenyan patients receiving first-line antiretroviral therapy. By controlling some risk factors such as height, other risk factors for polyneuropathy were investigated. The present findings confirmed that polyneuropathy independently relates to older age. Apparently, a significant number of patients on TDF ended up with polyneuropathy. This suggests that patients, who developed polyneuropathy while on TDF, had a previous exposure to d4T before changing to TDF. With the mean

time to the development of polyneuropathy of 13.1±11.4 months upon initiating ART, changing from d4T was more frequent in patients who had polyneuropathy than in those who did not. The study reports a major distinction in first-line ART regimens with more patients on TDF in the post-ART group. Though more patients were using d4T (48.4%) at baseline, there was no significant distinction between patients with polyneuropathy and the controls. Whereas d4T strongly relates to polyneuropathy, these findings did not confirm this relationship. Furthermore, some patients with no history of d4T usage ended up developing polyneuropathy. Therefore, polyneuropathy remains a burden even after the WHO's ban on the use of d4T.

The overall prevalence of polyneuropathy (29.4%) reported in Kenyan sex workers is comparable to other Kenyan studies (Mehta *et al.,* 2010; Cettomai *et al.,* 2013). Generally, the prevalence of polyneuropathy varies greatly in studies done in similar resource-limited settings. The prevalence rate recorded in SWOP facilities was lower compared to prevalence rates found in earlier studies (Luma *et al.,* 2012; Cettomai *et al.,* 2013) but higher (29.4%) when compared to a prevalence rate of 22% reported in a recent study (Arenas-Pinto *et al.,* 2016). Observation of lower prevalence rates is likely to result from a combination of factors including variations in the diagnosis of polyneuropathy. Further, the restriction of the diagnosis of polyneuropathy to clinical signs and symptoms as was performed in the current study is likely to result in fewer cases of polyneuropathy hence contributing to the underestimation of the

overall prevalence rate. Studies that "use tests such as nerve conduction with laboratory evaluations for vitamin B12 and diabetes have ended up reporting high prevalence rates of polyneuropathy" (Callaghan *et al.,* 2012).

Previous studies from "sub-Saharan Africa have demonstrated that the use of stavudine and older age were the main factors that contribute to the development of polyneuropathy" (Kiwuwa-muyingo *et al.,* 2014). Although the current study found no association between the use of stavudine and the development of polyneuropathy, older age was significantly associated with polyneuropathy.

Earlier studies "have shown consistent associations between older age and the development of polyneuropathy" (Cherry *et al.,* 2009; Oshinaike *et al.,* 2012). In the current analysis, age at baseline was considerably different in patients who developed polyneuropathy versus the controls. These findings support one Kenyan study that identified age as a risk factor for developing polyneuropathy in HIV (Mehta *et al.,* 2010). According to some authors, "metabolically stressed, lengthy and large peripheral nerves are doubtless notably susceptible to toxicity and harm caused by antiretroviral drugs with increased age" (Evans *et al.,* 2011; Margolis *et al.,* 2014). So far, age stands out as the most notable and consistent risk factor for polyneuropathy. With increased international access to ART and the rapidly ageing HIV population on effective treatment, ageing as a risk factor for polyneuropathy portends an ongoing burden in HIV-infected populations on long-term ART.

More than 50% of the patients who developed polyneuropathy in the patient population never had any d4T exposure. Additionally, TDF users who developed polyneuropathy were considerably different from TDF users without polyneuropathy. Even after excluding known risk factors for polyneuropathy, a number of patients still developed polyneuropathy. Although these findings support other studies that reported the continued high prevalence of polyneuropathy in the absence of d4T exposure (Cornblath and Hoke, 2006; Ellis *et al.,* 2010), a handful of studies have consistently reported strong association between polyneuropathy and the use of stavudine (Maritz *et al.,* 2010; Mateo *et al.,* 2013). Whereas some authors suggested that lack of association was due to the limited proportion of d4T users (Banerjee *et al.,* 2011; Luma *et al.,* 2012), others have implicated unknown risk factors like variations in metabolites which could play a role in the development of polyneuropathy (Banerjee *et al.,* 2011).

3.5. CONCLUSION

HIV-associated polyneuropathy is a common disorder among HIV-infected Kenyan sex workers at Pumwani SWOP clinic in Kenya. Although the present study identified older age and systolic blood pressure as risk factors for polyneuropathy, these results do not support the postulated increased risk by height when matching for gender and ART duration. Although d4T positively associates with toxic polyneuropathy, many patients in the current study who had no previous exposure to d4T developed polyneuropathy. This means there could be involvement of other

unknown risk factors like variations in metabolites in the development of polyneuropathy. Therefore, there is a need for further research on the role of genetic variation and metabolite changes as risk factors of PN in HIV-infected populations.

CHAPTER FOUR:

VARIATIONS IN METABOLOMIC PROFILES IN HIV-INFECTED PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY

4.1. INTRODUCTION

This chapter covers the analysis of the exploratory cohort that consisted of HIV-infected Kenyan and German patients on long-term ART. The primary objective was to describe variations in plasma metabolomic profiles in HIV-infected patients on long-term ART including study patients who developed polyneuropathy. The second objective was to identify potential biomarkers for polyneuropathy and the underlying metabolic processes.

4.2. METHODS

4.2.1. Study Design

This was an exploratory cohort study. The selection of study patients included in the exploratory cohort was from two independent cohorts, namely the Pumwani nested case-control cohort and the Hannover medical school cohort.

4.2.2. Study Sites

The selection of study patients was from two study sites, namely Pumwani Sex Workers Outreach Programme (SWOP) facility in Nairobi, Kenya and the HIV clinic at the Hannover medical school in Germany.

4.2.3. Study Population

HIV-infected patients aged ≥ 18 years were eligible for the study if they were presently receiving TDF/3TC/NVP. The selection of study subjects was from the nested case-control Pumwani cohort from Kenya and from the Hannover medical school HIV clinic cohort from Hannover in Germany.

4.2.3.1. The Kenyan Cohort

The Kenyan cohort from Pumwani SWOP clinic consisted of HIV-infected patients aged ≥ 18 years who had a history of using d4T/3TC/NVP regimen, however, their current regimen was TDF/3TC/NVP.

The exclusion criteria targeted study subjects with suspected or confirmed active tuberculosis, disorders associated with the central nervous system, current, or history of vitamin B12 deficiency, renal failure, diabetes, viral hepatitis, cancer, hypothyroidism and cirrhosis of the liver. The study also excluded patients with a history of alcohol and drug intake, current gestation or lactation, history of pregnancy during ART and those currently not using TDF/3TC/NVP.

4.2.3.2. The German Cohort

The German cohort from the HIV clinic at the Hannover Medical School comprised of HIV-infected Germans on standard medical care at the Hannover Medical School, in Germany. Patients aged ≥ 18 years were eligible if they had no history of

polyneuropathy, were on long-term TDF/3TC/NVP regimen for a median duration of 8.1 years (6.7-9.6) and had never changed to another regimen.

The exclusion criteria targeted study subjects with suspected or confirmed active tuberculosis, disorders of the central nervous system, current, or history of vitamin B12 deficiency, renal failure, cancer, diabetes, viral hepatitis, hypothyroidism and cirrhosis of the liver. Additionally excluded were patients with a history of alcohol and drug intake, history of pregnancy during ART and those currently not using TDF/3TC/NVP.

4.2.4. Samples Size

Sixty-five (65) HIV-infected patients of Kenyan and German origin who met the eligibility criteria were selected to form an exploratory cohort. Out of the 65 HIV-infected patients, 36 patients were Kenyan and 29 were Germans.

4.2.5. Selection of Study Population

HIV-infected Kenyans who were recruited to take part in this study were sourced from a nested matched case control cohort that consisted of 306 patients (n= 94 post-ART polyneuropathy, n = 212 without polyneuropathy). Out of the Kenyan study patients who met the eligibility criteria, the study selected 18 female patients with polyneuropathy as cases. The controls were patients without polyneuropathy, selected by matching every case by gender and the type of regimen. The study

selected 18 HIV-infected Kenyan patients without polyneuropathy as controls. Patients who consented to participate in the study signed informed consent forms (**Appendix C**).

In total 29 German patients with no history of polyneuropathy met the eligibility criteria. Out of these, 14 patients were females and 15 were males. Matching for these study patients was based on age and ART regimen type. Concerning the 29 eligible German patients, the analysis used stored plasma samples collected before and after exposure to ART. Patients were informed and had consented that these samples may be used for research.

4.2.6. Collection of Plasma Samples

In the case of the Kenyan cohort, 3 millilitres of blood was collected from consenting patients into vacutainers containing ethylenediaminetetraacetic acid (EDTA). The tubes were then gently but thoroughly shaken, stored in a cold box and transported to the research laboratory at UNITID within two hours. Once in the research laboratory, plasma preparation was done by centrifuging at 20-24 °C for 10 minutes at 2500 g. The separated plasma was transferred using disposable pipette tips, into pre-cooled Falcon collection vials, labeled and storage at - 80°C until shipment to Hannover, Germany in accordance with shipment protocol.

Concerning the German cohort, plasma samples from patients who met the eligibility criteria were identified using details from the electronic database in the HIV clinic at the Hannover Medical School. After identification, the plasma samples were obtained from stored specimens in the laboratory. The Clinic for Rheumatology and Immunology at the Hannover medical school donated all eligible samples to be analysed in the study.

4.3. Materials and Methods

4.3.1. Study Site

Preparation of samples and metabolomic analysis took place at the Hannover Medical School in Germany in the Pharmacology Department.

4.3.2. Materials

Plasma samples were analysed using "a Waters' ACQUITY high-performance liquid chromatography (HPLC) system (Waters Corporation, USA) (Zhuang *et al.,* 2016), coupled with a triple-quadrupole mass spectrometer (Waters, Corporation, USA) and "the AbsoluteIDQ p180 Kit (Biocrates Life Sciences, Austria)" (Bouatra *et al.,* 2013). The "AbsoluteIDQ p180 Kit contains a 96-deep-well plate that facilitates the quantification of up to 186 metabolites" (Walsh *et al.,* 2012)**.** A detailed list of metabolites that can be analysed by AbsoluteIDQ p180 Kit is in **Appendix D**.

AbsoluteIDQ p180 Kit has an automated sample preparation workflow assimilated with sensitive MS methods including software solution (MetIQ Software). The software had four separate modules particularly the Laboratory Information Management System Module (MetLIMS), MetConc, MetVal, and MetStat. These modules facilitated the metabolomic analysis steps.

4.3.3. Methods

4.3.3.1. Registration of the Assay

The Metabolomic Laboratory Information Management System Module (MetLIMS) enabled the registration of all samples and generated a 96-well plate overview to guide subsequent processes.

4.3.3.2. Assay Preparation

The preparation of samples for the assay followed a procedure specified by the kit's user manual. The "AbsoluteIDQ p180 kit which has a 96 deep-well plate with a filter plate fitted with sealing tape, kit reagents, and solvents" (Walsh *et al.,* 2012) was used. Standards and quality control were pipetted go into the first 14 wells of the kit.

4.3.3.3. Validation of the AbsoluteIDQ p180 Kit Plate

The MetVal module of the software system assisted in determining if values obtained for the internal standards and quality control samples were within the ranges set in the standard operating procedure (SOP) technique. Presentation of

results was in the form of numerous graphical layouts. Similarly, the software system presented graphs to represent the data on values that fell outside of the outlined ranges before their inclusion in the results table.

4.3.3.4. Preparation of Samples

The preparation of samples started with the thawing of previously stored plasma on ice before further processing. The first step involved measurement of 10 µl of thawed plasma and being dropped onto the centre of the filter paper found on top of the kit. The filter spots were then dried in a stream of nitrogen for 30 minutes. Later, amino acids and biogenic amines were derivatized by addition of 50 µL of 5% phenylisothiocyanate (green solution), followed by 20 minutes of incubation and drying for 30 minutes beneath nitrogen flow. After incubation, the filter spots dried for the second time in an evaporator.

4.3.3.5. Extraction of Metabolites

Extraction of the metabolites was through a single step achieved by adding 300-µl of methanol containing 5-millilitres ammonium acetate and shaking for a half-hour at 450 rpm (Walsh *et al.,* 2012). Precisely, centrifuging for two minutes at 500, times of gravity ensured the transfer of the extracts into the lower part of the wells. The extracts were then diluted by 600 µl of running solvent provided with the AbsoluteIDQ p180 Kit.

The kit plate was additionally loaded with a single blank sample (without an internal standard and sample), three phosphates buffered saline samples (water-based zero samples), and three quality control samples. Verification of assay and mass spectrometer performance was through quality control samples that comprised of human plasma and metabolites of varying concentrations.

4.3.3.6. Assay of Samples Using the Liquid Chromatography- Mass Spectrometer

The analysis of the extracts "using liquid chromatography-mass spectrometry technique" was done according to the method previously reported by Walsh et al. (2012). In this study, a 4000 QTRAP® system (Applied Biosystems /MDS Sciex) was used for the analysis. The system included a hybrid triple quadrupole LIT (linear ion trap) and mass spectrometer designed specifically for quantitative and qualitative applications. The Q3 region was operated either as a standard quadrupole mass spectrometer or as a linear ion trap mass spectrometer.

For analysis, 20 μL of extracted plasma sample was injected via an auto sampler. The autosamplers which were controlled by the computer software performed the injection of the extracted samples for flow injection analysis–electrospray ionization–tandem mass spectrometry (FIA-ESI-MS/MS) technique (Applied Biosystems, 2004). Briefly, glycerophospholipids, sphingolipids, and acylcarnitines were analysed using tandem quadrupole mass spectrometers by flow injection analysis (FIA) in positive mode. Later, the analysis of hexose was through the FIA acquisition in negative

mode. The analysis of amino acids and biogenic amines was through the HPLC system coupled with tandem MS in positive mode.

In the FIA-MS/MS technique, the analysis of every extracted sample lasted 3 minutes to run in both negative and positive mode. First, the analysis in the positive mode involved 175 multiple reaction monitoring (MRM) pairs. Subsequent analysis in negative ion mode lasted two MRM pairs and lastly, 2 × 20 μL injection at a flow rate of 30 μL/min. Therefore, it took 7 minutes per sample to complete the analysis including the injection step. For that reason, data collection for a full plate of 96 wells was performed overnight. The Biosystems software generated the calculation of analyte concentration from a mass spectrometer.

4.3.3.7. Interpretation of Mass Spectrometric Data

Metabolites were "identified and quantified using internal standards and Multiple Reactions monitoring (MRM) detection" as previously reported by Psychogios et al. (2011). The use of the absolute IDQ p180 kit and MetIQ software system resulted into high turnout analysis of the metabolites. Data analysis and calculation of the metabolites concentrations from FIA was automated using MetIQ software package.

The TargetLynx Application Manager analysed peaks obtained by HPLC before the importation of results into MetIQ software system for further processing and statistical analysis.

4.3.3.8. Evaluation and Export of Data

Finally, the MetStat module summarized the results in different tables, sorting them by metabolite category, concentration values, or intensities. Following the transfer of validated data to the results tables, the exportation of data for bioinformatics analysis was done in the form of comma-separated value (CSV) files.

4.3.3.9. Measurements for Quality Control Samples

For quality control purposes, a metabolite was included for further analysis as long as it had a mean concentration of ≥ 0.1 μL in all samples or if 50% of the samples had their concentrations over the limit of detection (LOD). After applying the quality control, a total, of 134 metabolites were identified (**Appendix E**).

4.3.4. Statistical and Machine Learning Strategies

4.3.4.1. Statistical Analysis

A web-based comprehensive tool referred to as MetaboAnalyst, that has varied statistical tools and machine-learning algorithms was used for analysis. The comma-separated value (CSV) files generated from intensities of the collected spectra were uploaded into the MetaboAnalyst.

Univariate analysis was applied in order to generate an overall summary of the information or rough ranking of potentially important metabolites before further analysis. To examine each individual variable while at the same time considering the

effect of the multiple comparisons, the MetaboAnalyst performed analysis of variance (ANOVA). Further, multivariate analyses based on projection strategies were used to test the effect of various predictors such as gender and the race between study patient groups (Trygg *et al.,* 2007).

4.3.5. Machine Learning Methods

4.3.5.1. Partial-Least Squares Discriminant Analysis Technique

Partial-Least Squares Discriminant Analysis (PLS-DA) which "projects the relation between matrix X and vector Y" (Lorber *et al.,* 1987), was used to "visualise the data by plotting the scores of the first two elements against one another" (Lorber *et al.,* 1987). The "feature of importance measure utilized was the Variable Importance in Projection (VIP) score" (Xia *et al.,* 2009). The "quantity of explained Y-variance in every dimension based on the weights that are a function of a decrease of the sums of squares traversing the amount of PLS elements" (Xia *et al.,* 2009). The second feature of importance measure used was "the weighted sum of PLS-regression coefficients" (Xia *et al.,* 2009). The "VIP indicates the importance of the variable to the whole model and it is a weighted sum of squares of the PLS weight" (Xia *et al.,* 2009). The analysis, therefore, used a VIP threshold of 1.0.

4.3.5.2. Significance Analysis of Microarray

The MetaboAnalyst performed the "Significance Analysis of Microarray (SAM) to select significant metabolites that distinguished between patient study groups"

(Efron *et al.,* 2001). While "running multiple tests on high-dimensional data" (Xia *et al.,* 2009), SAM deals with the problem of False Discovery Rate (FDR). It begins by assigning scores of significance to each variable based on its change relative to the standard deviation of repetitive measurements. Next, there is the selection of variables with scores larger than an adjustable threshold and compared with their relative distinction to the distribution calculable by random permutations of the category labels (Xia *et al.,* 2009). For every threshold, a definite quantity of the variables in the permutation set is also significant by chance. This range then calculates the FDR. The delta plots visualised in the table generated by SAM contained the estimated FDR and the number of identified metabolites for a set of delta values. In this study, the analysis used a default delta value of 0.5 that has an FDR of 12%, to identify about 10 significant compounds higher than this threshold.

4.3.5.3. Pathway Mapping

Names of significantly altered metabolites identified after the analyses were used to search for affected metabolic pathways. The compound names of the identified metabolites were entered into the pathway library of the Human Metabolome database (HMDB), thereby disclosing the identity of the corresponding pathway as well as detailed information regarding the metabolite (Wishart *et al.,* 2009).

4.3.6. Ethics Considerations

The Kenyatta National Hospital / University of Nairobi Ethics and Research Committee approved this study (**Appendix B**). HIV-infected patients who participated signed an informed consent before donating their blood samples (**Appendix C**). The Ministry of Health granted approval for the shipment of the plasma samples from Kenya to Germany for advanced metabolomic analysis (**Appendix F**).

Archived samples collected from German patients were donated from remaining backup samples obtained during routine care for HIV-RNA measurements. Patients were informed and had consented that these samples may be used for research.

4.4. RESULTS

4.4.1. Characteristics of HIV-infected Kenyan and German Patients in the Study Cohort

All study patients in the Kenyan cohort were female whereas 53% of the German patients were male (**Table 4.1**). The average number of years the Kenyan and German cohorts had been on ART was 4.4 years and 8.1 years respectively.

4.4.2. Detected Plasma Metabolites

Overall, targeted metabolomic identification of plasma collected from HIV-infected Kenyan and German patients detected 184 metabolites. During the analysis, there

was pre-processing to exclude metabolites that had 50% of measured concentrations below the limit of detection (LOD).

Table 4.1: Characteristics of HIV-infected Kenyan and German patients in the study cohort

Characteristic	**Kenyan Cohort (n=36)**	**German Cohort (n=29)**
Mean age (Years)	35 (31-40)	40 (30-51)
Gender (Female) (%)	36 (100%)	14 (48%)
Initiated Regimen	d4T/3TC/NVP	TDF/3TC/NVP
Current Regimen	TDF/3TC/NVP	TDF/3TC/NVP
ART Duration (Years)	4.4 (3.0-4.7)	8.1 (6.7-9.7)
Nadir CD4-T cell count (cells/ul)	248 (177-319)	158 (112-221)

Through this approach, 134 metabolites met the threshold criteria and were further analysed using univariate data analysis (**Figure 4.1**). One-way analysis of variance (ANOVA) identified elevation in 26 important metabolites (**Figure 4.1**).

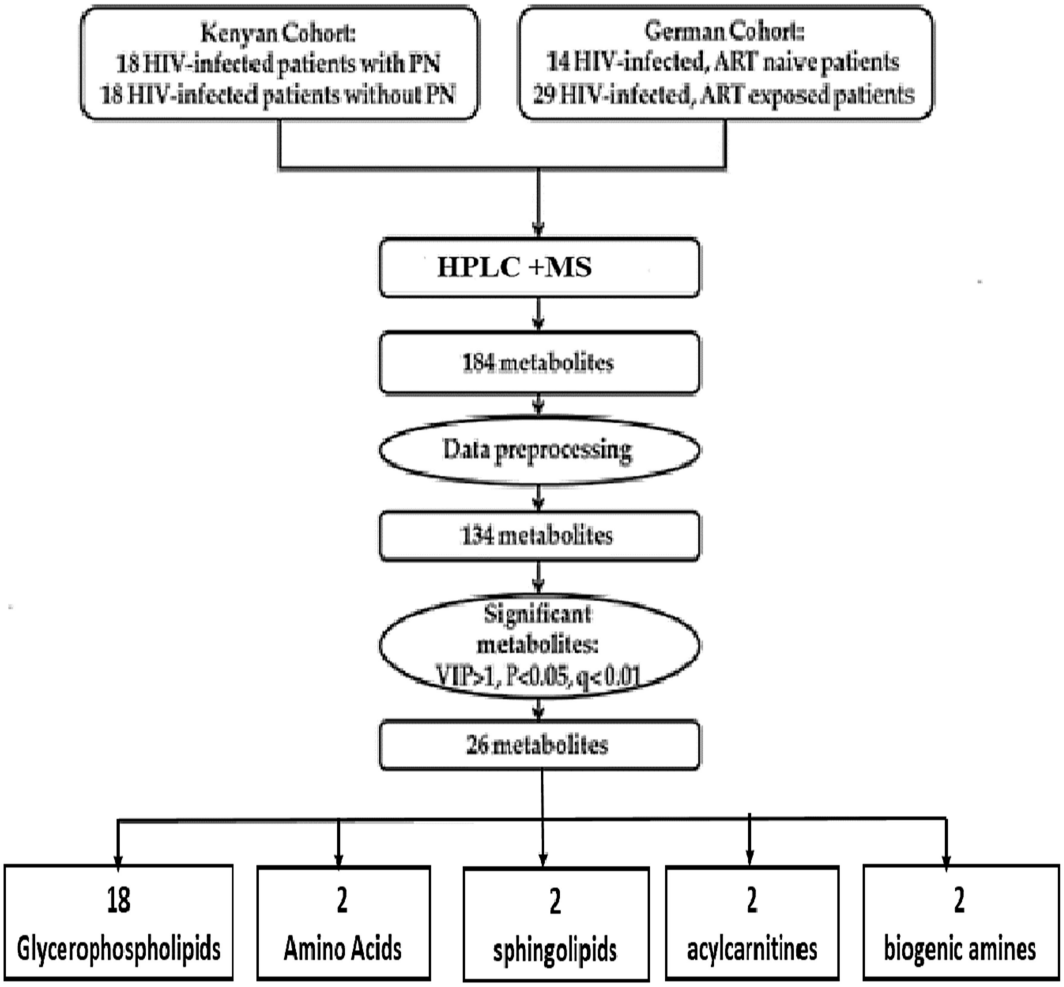

Figure 4.1: A strategy applied to identify plasma metabolites in HIV-infected Kenyan and German patients on long-term TDF/3TC/NVP regimens.

The 26 important metabolites included 2 amino acids, 18 glycerophospholipids, 2 sphingolipids, 2 biogenic amines and 2 acylcarnitines (**Table 4.2**). The concentrations of important metabolites selected by one-way ANOVA are shown in Figure 4.2.

Table 4.2: Catalog of 26 metabolites in HIV-infected Kenyan and German cohort on long-term antiretroviral therapy

Metabolite	**Abbreviation**	***p*. value**
Threonine	Thr	0.001
Phosphatidylcholine acyl-akyl C36:2	PC ae C36:2	0.001
LysoPhosphatidylcholine acyl C28:0	LysoPC a C28:0	0.002
Sphingomyeline C18:1	SM C18:1	0.002
Phosphatidylcholine diacyl C40:4	PC aa C40:4	0.002
Phosphatidylcholine diacyl C42:0	PC aa C42:0	0.003
Phosphatidylcholine diacyl C36:5	PC aa C36:5	0.003
Phosphatidylcholine diacyl C32:1	PC aa C32:1	0.004
Phosphatidylcholine diacyl C34:4	PC aa C34:4	0.004
Sphingomyeline C24:0	SM C24:0	0.004
Phosphatidylcholine acyl-akyl C34:0	PC ae C34:0	0.007
Creatinine	Creatinine	0.007
Acetylcarnitine	C2	0.008
LysoPhosphatidylcholine acyl C18:2	LysoPC a C18:2	0.009
Symmetric Dimethyl arginine	SDMA	0.011
Phosphatidylcholine acyl-akyl C32:2	PC ae C32:2	0.011
Phosphatidylcholine acyl-akyl C32:1	PC ae C32:1	0.012
Phosphatidylcholine acyl-akyl C44:4	PC ae C44:4	0.020
Propionylcarnitine	C3	0.020
Phosphatidylcholine diacyl C40:5	PC aa C40:5	0.027
LysoPhosphatidylcholine acyl C28:1	lysoPC a C28:1	0.030
Phosphatidylcholine acyl-akyl C44:3	PC ae C44:3	0.032
LysoPhosphatidylcholine acyl C17:0	LysoPC a C17: 0	0.035
Phosphatidylcholine diacyl C28:1	PC aa C28:1	0.036
Isoleucine	Ile	0.048
Phosphatidylcholine acyl-akyl C44:6	PC ae C44:6	0.049

4.4.3. Significantly Altered Metabolites

The multivariate analysis used the supervised partial least squares discriminant analysis (PLS-DA) method to explain the utmost separation between pre-defined sample-classes.

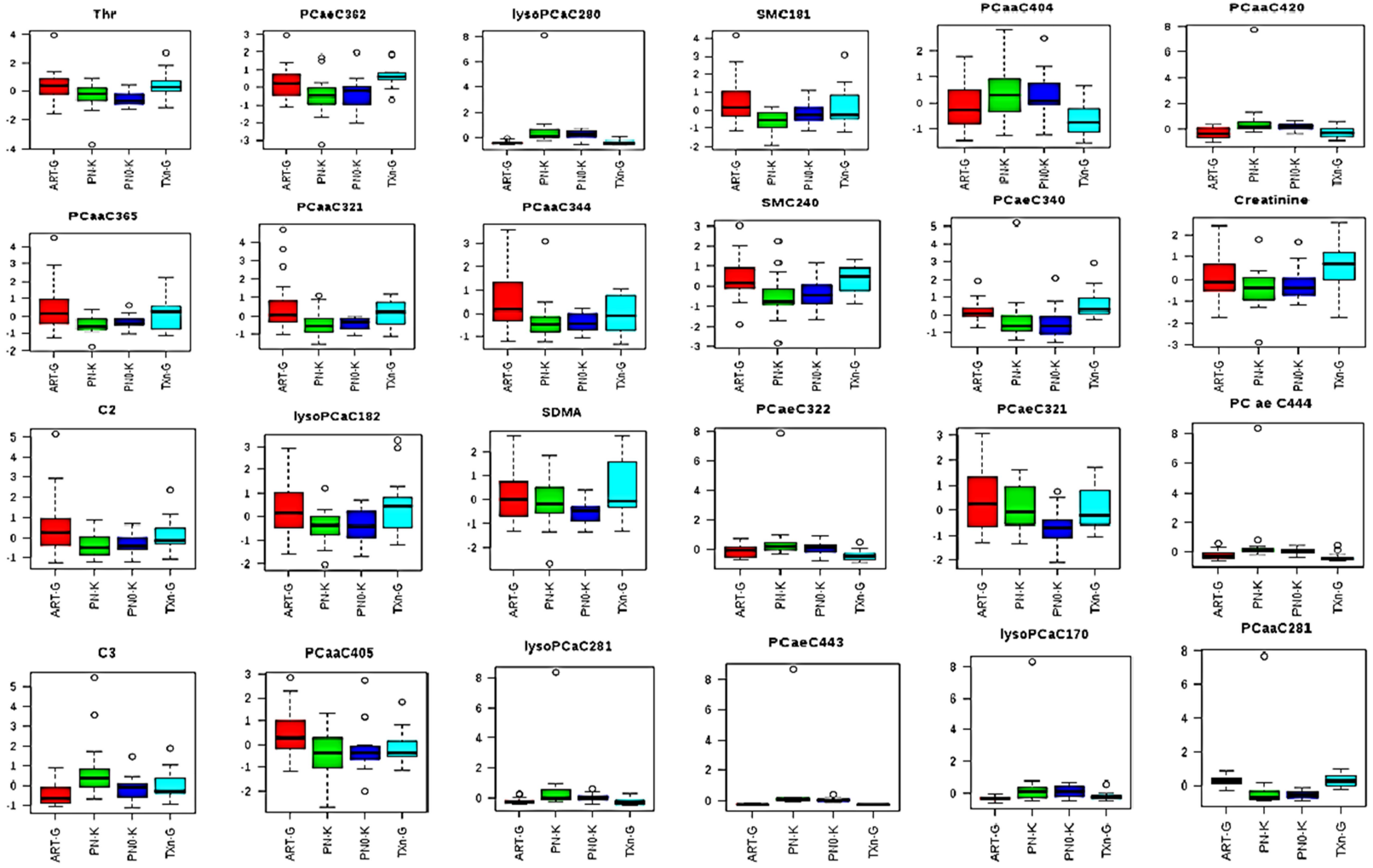

Figure 4.2: The concentration of plasma metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy. German patients on ART (ART-G), Kenyan patients with polyneuropathy (PN-K), Kenyan patients without polyneuropathy (PNO-K), and treatment naive German patients (TXn-G). The y-axis shows the normalized concentrations of the metabolites.

First, this method categorized every sample into one of the four study groups' namely HIV-infected German patients on ART (ART-G), Kenyan patients with polyneuropathy (PN-K), Kenyan patients without polyneuropathy (PNO-K) and treatment naive German patients (TXn-G). The software sent this information as a Y-table for the PLS-DA. The increased level of metabolites selected using a variable importance in projection (VIP) with a threshold of 1.0 is presented in **Table 4.3.** Similarly, the reduced level of metabolites selected using a variable importance in projection (VIP) with a threshold of 1.0 is shown in **Table 4.4.**

Table 4.3: Significantly elevated levels of metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy

Metabolite	**HIV-infected Study Groups**				
	Mean Concentration (µM)	**PN-Kenyan (n = 18)**	**PN-free Kenyan (n = 18)**	**ART-Naive Germans (n = 14)**	**ART-exposed Germans (n = 29)**
Thr	290.6±29.1	276.8±72.7	280.6±30	286±16.5	**298.5±32.5***
PC ae C36:2	11.2±3.38	10.2±3.6	**11.4±3.2***	**11.3±3.9***	**11.2±3.7***
PC aa C42:0	0.7±0.25	**0.8±0.3***	**0.7±0.15***	0.5±0.2	0.5±0.2
PC aa C34:4	1.5± 0.65	1.2±0.5	1.3±0.4	1.3±0.7	**1.8±0.8***
SM C24:0	16.9±4.00	15.8±5.0	**17.7±4.1**	16±4.0	**17.1±4.4***
PC ae C34:0	1.33±0.53	1.1±0.5	1.2±0.7	**1.5±0.6***	**1.4±0.4***
Creatinine	224.6±62.77	202±79.7	**228.8±61.9***	**236.5±54.8***	22.7±67.3
LysoPC a C18:2	21.2±7.64	18.1±6.7	**20.5±6.0***	**22.2±7.8***	**22.3±±9.6***
SDMA	17.5±4.94	**17.7±6.3***	15.7±3.5	**18.3±5.4***	17.4±5.5
C3	0.27±0.11	**0.3±0.1***	0.2±0.1	**0.28±0.1***	0.2±0.1
LysoPC a C17: 0	2.04±0.87	**2.3±0.9***	**2.1±0.9***	1.6±0.5	1.5±0.5
Ile	239.2±81.5	228±78	225±56.8	**247±77.8***	**243.3±108.6***

The selection of metabolites was based on a variable importance in projection with a threshold of 1.0 (p = 0.05). Study groups included Kenyan with polyneuropathy (PN); Kenyans free of PN; antiretroviral therapy (ART) naïve Germans; and Germans on ART.

After a regression analysis, the PLS-DA score plot showed a good distinction between the study groups (**Figure 4.3**). These results indicate that the model generated from changes in plasma metabolomic profiles was good.

Table 4.4: Significantly reduced levels of metabolites in HIV-infected Kenyan and German patients on long-term antiretroviral therapy

Metabolite	**HIV-infected Study Groups**				
	Mean Concentration (μM)	**PN-Kenyan (n = 18)**	**PN-free Kenyan (n = 18)**	**ART-Naive Germans (n = 14)**	**ART-exposed Germans (n = 29)**
Thr	290.6±29.1	**276.8±72.7***	**280.6±30***	**286±16.5***	298.5±32.5
PC ae C36:2	11.2±3.38	**10.2±3.6***	11.4±3.2	11.3±3.9	11.2±3.7
PC aa C42:0	0.7±0.25	0.8±0.3	0.7±0.15	**0.5±0.2***	**0.5±0.2***
PC aa C34:4	1.5± 0.65	**1.2±0.5***	**1.3±0.4***	**1.3±0.7***	1.8±0.8
SM C24:0	16.9±4.00	**15.8±5.0***	17.7±4.1	**16±4.0***	17.1±4.4
PC ae C34:0	1.33±0.53	**1.1±0.5***	**1.2±0.7***	1.5±0.6	1.4±0.4
Creatinine	224.6±62.77	**202±79.7***	228.8±61.9	236.5±54.8	**22.7±67.3***
LysoPC a C18:2	21.2±7.64	**18.1±6.7**	20.5±6.0	22.2±7.8	22.3±±9.6
SDMA	17.5±4.94	17.7±6.3	**15.7±3.5***	18.3±5.4	**17.4±5.5***
C3	0.27±0.11	0.3±0.1	**0.2±0.1***	0.28±0.1	**0.2±0.1***
LysoPC a C17: 0	2.04±0.87	2.3±0.9	2.1±0.9	**1.6±0.5**	**1.5±0.5**
Ile	239.2±81.5	**228±78***	**225±56.8***	247±77.8	243.3±108.6
The selection of metabolites was based on a variable importance in projection with a threshold of 1.0 (p = 0.05). Study groups included Kenyan with polyneuropathy (PN); Kenyans free of PN; antiretroviral therapy (ART) naïve Germans; and Germans on ART.					

4.4.4. Distribution of Significant Metabolites in Study Population

Using a variable importance in projection (VIP) threshold of 1.0, PLS-DA selected 12 significant metabolites out of the 26 important metabolites previously selected by

ANOVA. Table 4.3 and 4.4 shows the distribution of the 12 significant metabolites in the plasma of HIV-infected Kenyan and German patients using long-term ART.

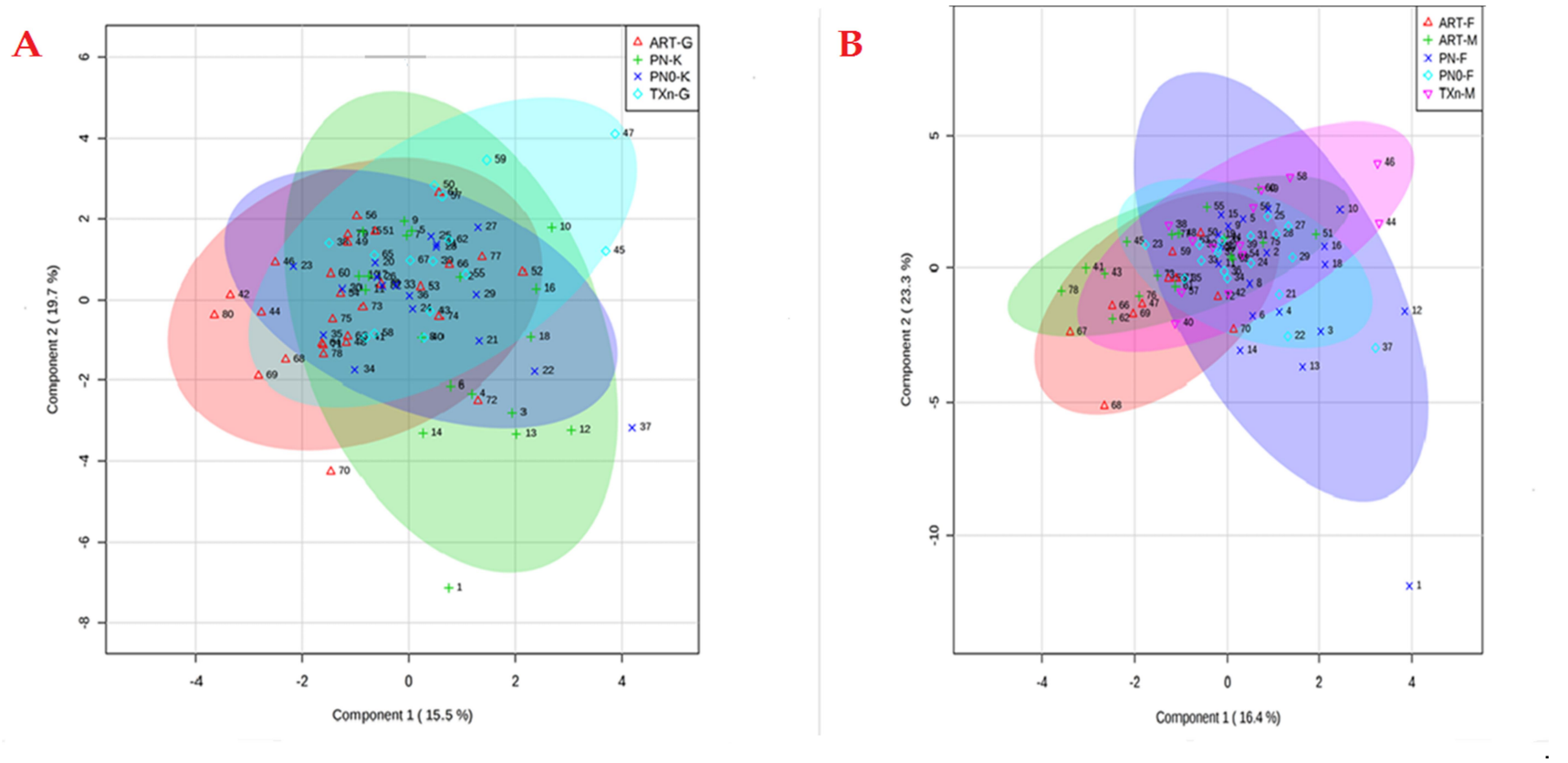

Figure 4.3: Partial least squares discriminant analysis score plots for HIV-infected Kenyan and German patients on long-term ART. A: a two-dimensional score plots developed for metabolite concentration of HIV-infected German patients on ART (ART-G), Kenyan patients with polyneuropathy (PN-K), Kenyan patients without polyneuropathy (PN0-K) and treatment naive German patients (TXn-G). B: a two-dimensional score plots developed for metabolite concentration of HIV-infected German female (ART-F) and male (ART-M) patients, Kenyan female patients with polyneuropathy (PN-F) and without polyneuropathy (PN0-F), and treatment naive German-male patients (TXn-M).

Compared to different study groups, HIV-infected ART-naive German (TXn-G) patients showed significantly elevated levels of creatinine, PC ae C36:2, LysoPC a C18:2, PC ae C34:0, SDMA and isoleucine (**Figure 4.4**). HIV-infected German patients on ART (ART-G) had significantly elevated levels of PC aa C34:4 compared to the HIV-infected patients with polyneuropathy and those with no polyneuropathy (VIP > 1; $p < 0.05$). HIV-infected Kenyan patients previously diagnosed with polyneuropathy (PN-K) during ART, had significantly elevated levels of SMC 24:0, PC aa C42:0, LysoPC a C17:0, C3 and Threonine. HIV-infected Kenyan patients who had no history of polyneuropathy (PN0-K) showed significantly decreased levels of PC ae C34:0, SDMA and Thr (VIP > 1; $p < 0.05$).

In comparisons involving gender, PLS-DA selected nine significant metabolites. HIV-infected ART-naive German male patients (TXn-M) had significant elevations in creatinine, LysoPC a C18:2, PC ae C36:2 and SDMA (**Figure 4.4**). HIV-infected German female patients (ART-F) showed significant elevations in PC aa C34:4 and PC aa C36:5 compared to the other study groups. HIV-infected Kenyan female patients previously diagnosed with polyneuropathy (PN-F) had significantly elevated levels of PC aa C42:0, LysoPC a C17:0 and C3 (VIP > 1; $p < 0.05$) compared to HIV-infected Kenyan and German patients with no history of polyneuropathy.

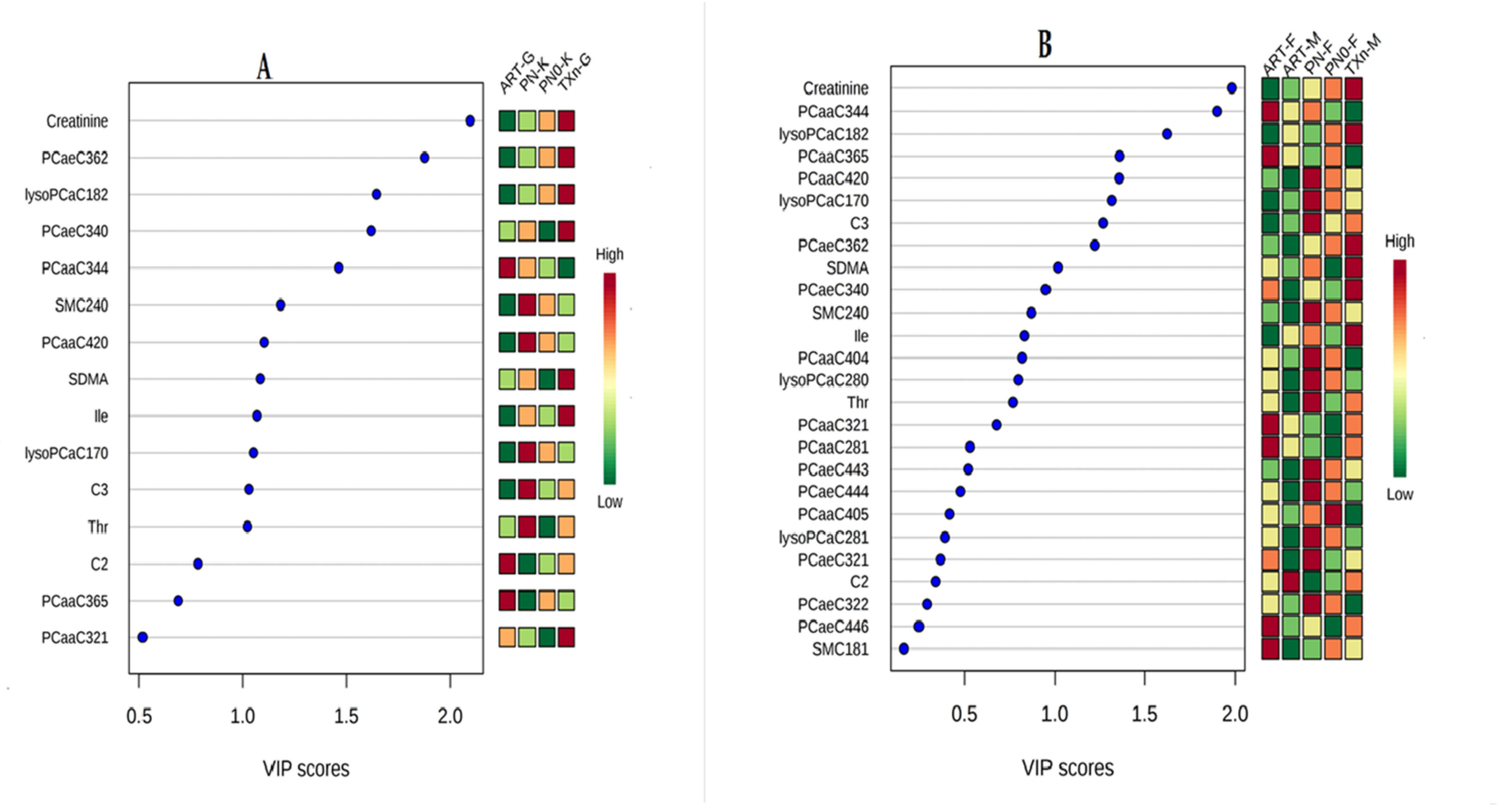

Figure 4.4: Variable of Importance projection scores for metabolites that distinguished HIV-infected cohorts.

The construction of the top VIP scores from PLS-DA models used 26 metabolites from the following:
A: HIV-infected German patients on ART (ART-G), Kenyan patients with polyneuropathy (PN-K), Kenyan patients without polyneuropathy (PN0-K) and treatment naive German patients (TXn-G).
B: HIV-infected German female (ART-F) and male (ART-M) patients on antiretroviral therapy, Kenyan female patients with polyneuropathy (PN-F) and without polyneuropathy (PN0-F), and treatment naive German-male patients (TXn-M). Red and green indicate increased and decreased concentration levels of the metabolites, respectively.

4.4.5. Metabolic Pathway Analysis

After subjecting the metabolites to pathway analysis, three metabolic pathways had a statistically significant modulation ($p < 0.05$) among HIV-infected study patients (**Table 4.5**). Outstanding modulations occurred in the metabolism of fatty acids and glycerophospholipids, suggesting the occurrence of disruptive metabolic changes during HIV infection and after long-term ART.

Table 4.5: Modulated metabolic pathways in HIV-infected Kenyan and German patients on long-term ART

Pathway Name	Total Metabolites	Raw *p* - value	Number of Hits	Name of Metabolite Hits
Glycine, serine and threonine metabolism	48	0.019942	1	L-Threonine
Glycerophospholipid metabolism	39	0.016203	13	LysoPC(18:0), PC(18:0/24:0), PC(o-16:0/18:0), PC(14:1(9Z)/14:0), PC(16:0/16:1(9Z)), LysoPC(18:2(9Z,12Z)), "PC(o-16:1(9Z)/16:1(9Z)), PC(o-18:0/18:2(9Z,12Z)), PC(14:0/20:4(5Z,8Z,11Z,14Z)), PC(o-22:0/22:3(10Z,13Z,16Z)), PC(18:0/22:4(7Z,10Z,13Z,16Z)), PC(16:0/20:5(5Z,8Z,11Z,14Z,17Z)), PC(o-22:1(13Z)/22:3(10Z,13Z,16Z))"
Oxidation of fatty acids metabolism	14	0.017	2	L-Acetylcarnitine, Propionylcarnitine
The term "raw p" refers to the raw *p*-values from regular *t*-tests				

4.4.6. Potential Biomarkers for Polyneuropathy

To distinguish potential biomarkers related to polyneuropathy, 12 significant metabolites selected by the PLS-DA (VIP > 1; $p < 0.05$) were subjected to Significance

Analysis of Microarray (SAM). By applying SAM, the analysis addressed the problem of False Discovery Rate (FDR) leading to the distinction between HIV-infected study groups.

Using a delta value of 0.5, FDR of 12%, and a p value of less than 0.005, the SAM identified 10 significant metabolites that included nine glycerophospholipids and one acylcarnitine (**Table 4.6**).

Table 4.6: Significant metabolites identified by significance analysis of microarray (SAM) in HIV-infected Kenyan and German patients on long-term ART (delta = 0.5; p < 0.05)

Compound	**Abbreviation**	**d. value**	**Standard deviation**	**raw *p***	**q. value**
Phosphatidylcholine diacyl C40:4	PC aa C40:4	5.520	0.671	0.001	0
Phosphatidylcholine diacyl C42:0	PC aa C42:0	5.259	0.686	0.001	0
Phosphatidylcholine acyl-akyl C32:2	PC ae C32:2	4.466	0.731	0.001	0
Lysophosphatidylcholine acyl C28:0	LysoPC a C28:0	4.065	0.755	0.001	0
Phosphatidylcholine acyl-akyl C44:4	PC ae C44:4	3.580	0.785	0.001	0
Lysophosphatidylcholine acyl C17:0	LysoPC a C17:0	2.991	0.822	0.001	0
Propionylcarnitine	C3	2.718	0.840	0.001	0.001
Lysophosphatidylcholine acyl C28:1	LysoPC a C28:1	2.543	0.852	0.001	0.001
Phosphatidylcholine acyl-akyl C44:3	PC ae C44:3	2.335	0.866	0.002	0.002
Phosphatidylcholine acyl-akyl C32:1	PC ae C32:1	1.220	0.945	0.056	0.067
The term "raw p" refers to the raw *p*-values from regular t-tests					

However, when the three analyses were considered, three metabolites were found to be systematically significant (p < 0.05; VIP > 1; d < 0.05). These metabolites including PC aa C42:0, LysoPC a C17:0 and C3 seemed to be potential biomarkers related to

polyneuropathy in HIV-infected Kenyan patients. Table 4.7 presents plasma levels of the three metabolites in respective population.

Table 4.7: Plasma levels of three potential biomarkers identified in HIV-infected Kenyan and German patients on long-term ART

Metabolite	**HIV-infected Study Groups**						
	Normal Conc. (μM)	**Mean Conc. (μM)**	**PN-Kenyan (n = 18)**	**PN-free Kenyan (n = 18)**	**ART-Naive Germans (n = 14)**	**ART- exposed Germans (n = 29)**	**Raw *p*-value**
PC aa C42:0	unknown	0.7±0.3	0.8±0.3	0.7±0.2	0.5±0.2	0.5±0.2	0.001
C3	0.31±0.2	0.27±0.1	0.3±0.1	0.2±0.1	0.28±0.1	0.2±0.1	0.001
LysoPC a C17:0	2.5±1.1	2.04±0.9	2.3±0.9	2.1±0.9	1.6±0.5	1.5±0.5	0.001

The selection of metabolites was based on Analysis of variance (ANOVA) ($p < 0.05$), Partial Least Squares Discriminant Analysis (PLS-DA) (VIP > 1; $p < 0.05$) and Significant Analysis of Microarray (SAM) (delta = 0.5; $p < 0.05$). Study groups included Kenyan with polyneuropathy (PN); Kenyans free of PN; antiretroviral therapy (ART) naïve Germans; Germans on ART; Conc. – Concentration and ART-Antiretroviral Therapy.

4.5. DISCUSSION

The first objective of the study was to describe variations in metabolomic profiles in HIV-infected Kenyan and German patients on long-term ART including patients with a history of polyneuropathy. The second objective was to identify potential early biomarkers for polyneuropathy and underlying metabolic processes. The multivariate analysis identified 12 significant metabolites namely two amino acids (threonine and isoleucine), six glycerophospholipids (PC ae C36:2, PC aa C42:0, PC aa C34:4, PC ae C34:0, LysoPC a C18:2 and LysoPC a C17:0), one acylcarnitine (C3), one sphingolipid (SM C24:2) and two biogenic amine (creatinine and SDMA). However, after subjecting these metabolites to Significant Analysis of Microarray (SAM) to assess their potential as biomarkers, three metabolites namely PC aa C42:0,

LysoPC a C17:0 and C3 remained significant. These results showed significant up-regulation in the levels of PC aa C42:0, LysoPC a C17:0 and C3 ($p < 0.05$; VIP > 1; False Discovery Rate < 0.5), that were mapped to the modulation in the oxidation of fatty acids and glycerophospholipid metabolism. This seems to suggest that significant changes occur in metabolic processes during HIV infection and in the course of long-term exposure to ART. There was a significant elevation in levels of LysoPC a C17:0 and PC aa C42:0 in HIV-infected Kenyan patients with a history of polyneuropathy compared to the German patients who had no history of polyneuropathy. Additionally, this study shows elevated levels of C3 in HIV-infected Kenyan patients with a history of polyneuropathy compared to Kenyan and German HIV-infected patients without a history of polyneuropathy.

4.5.1. Alterations in Glycerophospholipids

Most of the significant metabolites in this study consisted of glycerophospholipids. However, in Significance Analysis of Microarray (SAM) analysis, only two glycerophospholipids and a single short-chain acylcarnitine remained significant. Glycerophospholipids regulate many cellular activities, which include metabolic, inflammatory and immune function. Any "disturbance in the lipid signalling pathways may end up causing disorders such as insulin resistance and cardiovascular disease (CAD)" (Wellen and Hotamisligil, 2005; Bensinger and Tontonoz, 2008; Cassol *et al.,* 2013). Therefore, changes in the concentration of plasma metabolites can generate data that might point to the involvement of genetic

factors in metabolic pathways in HIV-infected patients on long-term ART. Additionally, this may change the development of therapeutic strategies that may minimize negative outcomes related to chronic exposure to ART in the ageing HIV-infected populations. In the current study, targeted metabolomic profiling revealed 12 vital plasma metabolites indicative of modulation in glycerophospholipids (PC ae C36:2, PC ae C34:0, PC aa C34:4, PC aa C42:0, LysoPC a C18:2 and LysoPC a C17:0), acylcarnitine (C3), amino acids (threonine and isoleucine), biogenic amines (SDMA, and creatinine) and sphingolipid (SMC 24:0). However, only three metabolites (PC aa C42:0, LysoPC a 17:0 and C3) significantly distinguished HIV-infected Kenyan patients with and without polyneuropathy as well as HIV-infected German patients before and after exposure to ART.

Metabolomic studies have reported either up-regulation of down-regulation of metabolites in HIV-patients on long-term ART. An earlier study negatively associated many lipid categories including lysophosphatidylcholines (LPC, lysoPC) with HIV infection (Wong *et al.,* 2014). Elevated "levels of LPC16:0 and LPC20:4, and diminished levels of LPC 18:0, LPC 18:1 and LPC 20:4 associated with various disorders in HIV-infected patients on ART" (Fernandez *et al.,* 2013; Cassol *et al.,* 2013). The authors suggested that the alterations in the HIV plasma metabolome are indicative of dysregulated lysoPC metabolism. In the present study, LysoPC a C17:0 and PC aa C42:0 had considerably elevations in HIV-infected Kenyan patients with a history of polyneuropathy compared to Kenyan patients without a history of

polyneuropathy and HIV-infected German patients. HIV-infected treatment-naive German patients had considerably altered levels of PC ae C36:2, PC ae C34:0 and LysoPC a C18:2 compared to the Kenyan patients with and without a history of polyneuropathy. HIV-infected German patients receiving ART showed considerably elevated levels of PC aa C34:4 compared to other study groups. This study, therefore, supports the suggestion that alterations in the HIV plasma metabolome are indicative of "dysregulated glycerophospholipid metabolism" (Cassol *et al.* 2013). A separate study showed a positive relationship between the levels of LysoPC (16:0) and those of other LysoPCs species (Kim *et al.,* 2014). This positive relationship reflected an alternative source of LysoPC (16:0) production in addition to oxidatively damaged lipoprotein (ox-LDL) (Kim *et al.,* 2014).

4.5.2. Changes in Propionylcarnitine Levels

Acylcarnitines are significant "very valuable biomarkers because they are by-products of fat, glucose, and amino acids mitochondrial oxidation" (Sampey *et al.,* 2012). The findings presented in the current study showed higher levels of propionyl-carnitine (C3) in Kenyan patients with HIV, who had a previous history of exposure to d4T and polyneuropathy. Previous studies associated L-carnitine deficiency in HIV-infection to the effects of dysfunctional mitochondria and oxidant stress (Valcour *et al.,* 2009; Benedini *et al.,* 2009). The use of stavudine and "didanosine plays a role in L-carnitine deficiency in patients who had ART-induced polyneuropathy and lipoatrophy" (Herzmann *et al.,* 2005; Waagsbø *et al.,* 2016).

Additionally, "advanced HIV infection and rapid disease progression associated with low levels of short-chain (propionyl-carnitine) and medium-chain (octanoyl-carnitine) acylcarnitines" (Waagsbø *et al.,* 2016). These authors have proposed dietary changes and defects in carnitine production as additional factors that determine the level of plasma acylcarnitines.

Whereas acyltransferases, which are principally located in peroxisomes and microsomes, catalyse "acylcarnitines of short- and medium-chain acyl groups, carnitine palmitoyltransferase I and II located within the mitochondrial membranes catalyse long-chain acylcarnitines" (Huang *et al.,* 2016; Waagsbø *et al.,* 2016). The findings of this study may reflect selectively disturbed metabolism involving short- and medium-chain acylcarnitines in the peroxisomes and liver microsomes of HIV-infected Kenyan patients with a history of polyneuropathy. However, there is a need further studies to assist establish the mechanisms for high levels of acylcarnitines (C3) in HIV-infected patients.

4.5.3. Changes in other metabolites

In HIV-infected patients, the study observed alterations in plasma levels of amino acids (threonine and isoleucine), biogenic amines (SDMA and creatinine), and a sphingolipid (SM C24:0). On one hand, threonine, "which is an essential amino acid that plays a crucial role in the nervous system and fat metabolism"(Dutta *et al.,* 2013), was considerably elevated in HIV-infected Kenyan patients with a history of

polyneuropathy compared to controls and German patients. To the contrast, previous studies reported reduced levels of threonine in HIV-infected patients (Di Rocco *et al.,* 2002; Munshi *et al.,* 2013). On the opposite hand, isoleucine, which is a vital amino acid concerned in detoxification of nitrogenous wastes and stimulating the immune function, was considerably elevated in HIV-infected German patients before the introduction of ART. This finding does not support previous findings that found reduced levels of isoleucine in HIV-infected patients who were ART naive (Munshi *et al.,* 2013). As essential amino acids, threonine and isoleucine are available from the diet, so this means that the plasma alteration of these metabolites depends on dietary intake.

Plasma creatinine, which is an indicator of kidney function, was considerably high in ART-naïve HIV-infected Germans compared to patients receiving ART. A previous study reported a rise in serum creatinine and subsequent decline in creatinine clearance in HIV-infected patients on TDF-based ART (Sax *et al.,* 2014). Sources of creatinine meat, therefore, the observed rise in creatinine levels in HIV-infected German patients is indicative of increased dietary intake. Since plasma levels of symmetric dimethylarginine (SDMA) inversely related to glomerular filtration rate, a previous finding projected its use as an endogenous marker of renal function (Kielstein *et al.,* 2006). The finding of this study support previous findings that reported increased plasma levels of SDMA in HIV-infected patients, not ART (Kurz *et al.,* 2009; Jang *et al.,* 2011). In another study, Kurz et al. (2012) observed that the

concentration of SDMA and arginine decreased in parallel with decreasing HIV-RNA levels in HIV-infected patients on ART (Kurz *et al.,* 2012).

Previous studies reported abnormalities in sphingolipids metabolism in the "brains and cerebrospinal fluid of HIV-infected patients with dementia" (Haughey *et al.,* 2004; Bandaru *et al.,* 2007). These patients showed significant increases in sphingomyelin "C16:0, C18:0, C20:0, C22:0 and C24:0 compared to HIV-infected patients with no dementia or patients with HIV-active dementia" (Bandaru *et al.,* 2007). In the present study, the levels of SM C24:0 were considerably up-regulated in HIV-infected Kenyan patients with a history of polyneuropathy compared to patients without polyneuropathy. This means that these patients had a dysfunctional sphingolipids metabolism.

Pathway analysis revealed varying concentrations of many plasma metabolites in the glycerophospholipid and fatty acid oxidation metabolism between HIV-infected Kenyan patients with a history of polyneuropathy, those without a history of polyneuropathy and HIV-infected German patients without a history polyneuropathy.

4.6. CONCLUSION

These findings suggest differentially regulated glycerophospholipids and fatty acid oxidative metabolism in HIV-infected patients receiving long-term ART. The up-

regulation of glycerophospholipids (PC aa C42:0 and LysoPC a C17:0) and short-chain acylcarnitine (C3) was related to HIV-infected Kenyan patients with a history of polyneuropathy suggesting an increase in the levels of these metabolites after stopping the offensive ART.

CHAPTER FIVE: GENERAL DISCUSSION

5.1. INTRODUCTION

The overall objective of the thesis was to describe long-term antiretroviral therapy (ART)-related adverse drug reactions (ADRs) and corresponding variations in plasma metabolites in HIV-infected Kenyan and German patients on long-term ART. The findings of the study revealed that HIV-infected patients with advanced age (≥ 40 years) and on long-term ART duration were at a higher risk of developing ADRs particularly lipodystrophy and polyneuropathy. Several patients without previous exposure to d4T significantly developed polyneuropathy suggesting the involvement of other factors such as metabolites in the development of polyneuropathy. The up-regulation of glycerophospholipids (PC aa C42:0 and LysoPC a C17:0) and propionylcarnitine (C3) was related to HIV-infected Kenyan patients with a history of polyneuropathy signifying an increase in the levels of these metabolites after stopping the offensive d4T containing ART.

5.2. Safety of Long-term Antiretroviral Therapy

There is a paucity of data concerning the prevalence of ADRs and the related regimen changes in resource-limited settings. The results of this study report an overall ADR prevalence of 35%. The specific prevalence rates for lipodystrophy and polyneuropathy were 41.7% and 29.4% respectively. Although the overall prevalence rates of ADRs and specific prevalence rates of types of ADRs vary from one study to the other, the rate reported in this study were lower compared to studies previously

conducted in Kenya and Uganda (Forna *et al.,* 2007; Hawkins *et al.,* 2007; Mwangangi *et al.,* 2010). This could possibly be due to the availability of better-tolerated ART regimens.

Despite the improvement in antiretroviral therapy, as reflected by the excellent treatment outcome of the German cohort observed in this study, some HIV-infected Kenyan patients ended up changing their first-line therapy due to a number of reasons. The risk of developing an ADR during the first 24 months of treatment was high for patients on d4T-based regimens compared to other regimens. Patients with lipodystrophy and polyneuropathy were more likely to change their first-line treatment compared to other ADRs. Many studies have reported ADRs, as the main reason for changing first-line ART in several resource-poor settings (Maggiolo *et al.,* 2007; Limaverde Lima *et al.*, 2012; Cesar *et al.,* 2015). In the Kenyan context, these findings support two Kenyan studies that cited ART-related toxicity as the main reason for changing treatment (Hawkins *et al.,* 2007; Inzaule *et al.,* 2014). Although comparing these studies remain difficult because of a number of limitations (Menezes de Pádua *et al.,* 2007; Westreich *et al.,* 2009), it is clear that specific ADRs such as lipodystrophy and polyneuropathy strongly influence the safety of long-term ART.

During the study period, the use of d4T-based regimens was common at the Sex Workers Outreach Programme (SWOP) facilities. However, patients discontinued

the use d4T because of the development of ADRs (lipodystrophy and polyneuropathy) or intolerance or clinicians proactively changed d4T to a well-tolerated option such as TDF. In addition, sex workers may disproportionately consume alcohol and drugs abuse. If the medical reviews do not capture the information, these factors may end up contributing to the overall prevalence of ADRs in the SWOP settings. Compared to AZT and d4T, these findings support studies that showed TDF as a well-tolerated drug regimen with fewer drug changes (Chi *et al.*, 2010; Boyle *et al.,* 2012; Njuguna *et al.,* 2013; Brennan *et al.,* 2013). The change of d4T to a safer option such as TDF aimed at managing the development of lipodystrophy and polyneuropathy.

Timely change of ART regimen "in order to minimise the risk of developing ADRs and improve the long-term safety of ART may be useful in supporting adherence and improve long-term viral suppression" (Arribas *et al.,* 2008; Mercadé *et al.,* 2012). Similar to studies conducted in Uganda and Swaziland (Castelnuovo *et al.,* 2011; Evans *et al.,* 2012), the present study had a significantly higher proportion of patients who needed to change d4T to TDF due to lipodystrophy and polyneuropathy. Additionally, from 2010 onwards, d4T was mostly changed in line with the WHO's recommendations of 2010 (WHO, 2010). Though some studies associated lower rates of TDF substitutions with renal impairment (Cassetti *et al.,* 2006; Bygrave *et al.,* 2011; Kalemeera *et al.,* 2015), patients in the current study did not change the use of TDF

because of renal impairment. However, there is a need for further investigation to determine the clinical reasons for changing TDF-based regimens.

In patients who developed polyneuropathy nevirapine (NVP) was preferentially used as a third ART drug in 81% of patients compared to 19% of patients who used efavirenz (EFV). Although "EFV is a well-tolerated regimen that demonstrates durable viral suppression" (Mercadé *et al.,* 2012), the neurological toxicity associated with its use may have influenced the preferential use of NVP as a safer option for long-term ART in the Kenyan study population. Moreover, considering that the Kenyan study population largely consisted of female sex workers, due to pregnancy concern and co-infection with tuberculosis, NVP was a valid alternative to EFV in this setting (Mercadé *et al.,* 2012; Bhatt *et al.,* 2015). Faced with the problem of limited treatment options in this settings, it appears that most of the Kenyan study patients had to bear the risk of hypersensitivity reaction to NVP (Sanne *et al.,* 2005).

5.3. Aging and Long-term Antiretroviral Therapy

More than 50% of the patients in this study, including 36% of AZT users and 18% of TDF users, who developed polyneuropathy, had never used any d4T. Moreover, not all d4T-exposed patients developed polyneuropathy. Even when excluding renowned risk factors for polyneuropathy such as a number of patients, as well as those exposed to TDF still, developed polyneuropathy. On one hand, this may "implicate the role of other risk factors including age, genetic variation and changes

in metabolites in the development of polyneuropathy" (Banerjee *et al.*, 2011; Ndakala *et al.,* 2013; Ndakala *et al.,* 2016). On the other hand, considering that d4T is no longer first-line treatment, it means polyneuropathy continues to be an ongoing burden in HIV-infected populations. To improve the safety of current long-term ART for the ageing HIV-infected populations, there is a need for further studies on risk factors associated with ART-related polyneuropathy.

The "older patients are more likely to succumb to polyneuropathy related to diabetes, paraproteinaemia-associated malignancies and chronic inflammatory neuropathies"(Siyum & Medcalf, 2012). Age has consistently demonstrated to be an independent risk factor in the development of neuropathy in diabetic patients (Popescu *et al.,* 2016). Similarly, studies conducted in the sub-Saharan Africa have reported older age as an independent risk factor in patients who develop polyneuropathy (WHO, 2010; Jakait *et al.*, 2012; Arenas-Pinto *et al.,* 2016). In the current study, people of older age (≥ 40 years) were at an increased risk of developing ART-related adverse drug reactions.

In addition, the current study observed an increased risk for developing polyneuropathy with older patients aged 40 years and above. These findings concur with previous studies that identified "age as a risk factor for developing polyneuropathy in HIV-infected people" (Mehta *et al.,* 2010; Evans *et al.,* 2011; Evans *et al.,* 2012; Kamerman *et al.,* 2012; Arenas-Pinto *et al.,* 2016). So far, age stands out as

"the most notable and consistent risk factor for polyneuropathy" (Evans *et al.*, 2011). With enhanced global access to ART and the rapidly ageing HIV population on effective treatment, ageing as a risk factor for polyneuropathy portends an "ongoing burden in the HIV-infected population receiving long-term ART treatment" (Evans, *et al.,* 2011). According to Evans et al. (Evans *et al.,* 2011), peripheral nerves are long, substantial, and metabolically disturbed cells as they age and for that reason, they are likely to succumb to toxicity and damage caused by ART drugs.

In other studies, the exposure to d4T has proved to be another consistent risk factor related to polyneuropathy (Hawkins *et al.,* 2007; Millogo *et al.,* 2008; Maritz *et al.,* 2010; Mateo *et al.,* 2013). However, this study did not realize a similar association. There are plenty of studies in the literature that did not identify the use of d4T as a risk factor for the development of polyneuropathy (Luma *et al.,* 2012; Tumusiime *et al.,* 2014a). Lack of association was suggested to be due to the restricted proportion of d4T users or other risk factors apart from d4T (Cettomai *et al.,* 2010; Banerjee *et al.,* 2011). Further, these findings suggest that the contradictory results observed in the above-named studies are also due to confounding by factors such as height, alterations in metabolites or genetic influence.

5.4. Potential Biomarkers to Antiretroviral Toxicity

There are limited data on how ethnicities influence the metabolomic profiles of HIV-infected populations in Sub-Saharan Africa. This is the first study that has

investigated variations in plasma metabolites in HIV-infected Kenyan and German patients. Studies conducted in the in the United States observed that the exposure to atenolol (an antihypertensive drug) showed significant changes in the levels of many metabolites including palmitic, oleic, palmitoleic, arachidonic, and linoleic, which were diminished in Caucasians compared to African Americans (Wikoff *et al.*, 2013). Another study recorded few statistically significant changes in metabolite levels in African-Americans, Hispanics, and Caucasians (Lawton *et al.*, 2008). In the current study, HIV-infected Kenyan patients with a history of polyneuropathy (PN-K) had considerably high concentrations of threonine, SM C24:0, PC aa C42:0, LysoPC a C17:0 and C3 compared to HIV-infected German patients. Treatment naïve, HIV-infected German patients (Txn-G) showed considerably higher concentrations of isoleucine, SDMA, creatinine, PC ae C36:2, PC ae C34:0 and LysoPC a C18:2 compared to Kenyan patients with and without a history of polyneuropathy. Whereas the present study supports the role of genetic variability in influencing the observed metabolite variations between the Kenyan and German cohorts, variations in the level of metabolites may also be due to a combination of different factors including differences in viral load, ARV exposure time, and previous exposure to d4T.

Although this study did not measure the viral load, cohorts from very different medical settings can continually have variations in viral loads. There is evidence in the literature that associates some metabolites with higher viral loads (Riddler *et al.*,

2008). Considering that the longer the patient spends on ART presents a different toxicity profile, so variations in duration to ART exposure time may additionally contribute to variations in plasma metabolites. At the time of this study, German patients had a mean exposure time of 8.1 years compared to Kenyan patients who had a mean exposure time of 4.4 years. However, the German patients showed better ART treatment outcome record than the Kenyan patient population. Lastly, previous exposure to d4T like in the case of Kenyan patients was a risk factor for polyneuropathy. Variation in metabolomic profiles was observed between patients who never used d4T (German cohort) and patients who switched from d4T-based ART to TDF-based ART (Kenyan cohort).

Studies involving plasma samples have shown that the plasma metabolome is exquisitely sensitive to a broad range of intrinsic and extrinsic factors such as gender and the way plasma was collected (Lawton *et al.,* 2008; Lewis *et al.,* 2010; Psychogios *et al.,* 2011).

Therefore, any difference in the collection of plasma can result in interindividual variability. However, the role of gender on the metabolite changes in plasma cannot be underestimated. In the current study, HIV-infected Kenyan female patients with a history of polyneuropathy (PN-F) showed considerably high levels of PC aa C42:0, LysoPC a C17:0 and C3 compared to male German patients. The concentration of acylcarnitines between males and females described in this study concur with

previous findings that showed elevated levels of acetylcarnitines in male patients compared to female patients (Slupsky *et al.,* 2007; Reuter *et al.,* 2008; Mittelstrass *et al.,* 2011). HIV-infected ART naïve, male German patients had considerably high levels of SDMA, creatinine, and PC ae C36:2 compared to Kenyan and German female patients. HIV-infected German female patients on ART had considerably elevated levels of PC aa C34:4 compared to German male patients.

These data suggest that metabolite concentration can be predictive biomarkers to indicate the presence or severity of a disease depending on sex. A previous study ascertained a significant increase in 27 metabolites in male compared to female patients, while eight were elevated in females compared to males (Lawton, *et al.,* 2008). This study adds new data on sex-specific variations in cellular regulatory processes that take place during long-term ART.

Although this study supports an earlier study that linked dysregulated glycerophospholipids to ART in HIV-infected patients on long-term treatment, there were variations in species of glycerophospholipids between these studies (Cassol *et al.,* 2013). The observed metabolite variations may result from a number of factors including previous exposure to d4T. The Kenyan study patients with polyneuropathy started d4T as their first-line ART before changing to TDF.

In previous studies, changes in the levels of lysophosphatidylcholines (lysoPC), were linked to atherogenic effects and inflammation (Quinn *et al.,* 1988; Schmitz & Ruebsaamen, 2010), obesity (Heimerl *et al.,* 2014) and a reduced risk of developing a cardiovascular disease (CVD) (Fernandez *et al.,* 2013). Specifically, Fernandez et al. (2013), associated higher baseline levels of lysoPC 16:0 and lysoPC 20:4 with a decreased risk of developing a CVD. The current findings report on elevated levels of different species of lysophosphatidylcholines (LysoPC a C17:0 and PC aa C42:0) in HIV-infected Kenyan patients with polyneuropathy compared to other patient groups. Considering that studies on various species of lysophosphatidylcholines have been less consistent, these findings "contribute to the contradictive discussion on the role of lysophosphatidylcholines in the development of various chronic diseases in older HIV-infected populations" (Heimerl *et al.,* 2014). Therefore, elevated levels of the two metabolites in HIV-infected Kenyan patients previously diagnosed with polyneuropathy could mean that this patient group could have a reduced risk of developing CVD, especially after changing d4T to a well-tolerated option TDF, but long-term use of TDF has yet to be studied.

The findings of the current study showed increased levels of propionylcarnitine in HIV-infected Kenyan patients contradict previous studies that associated L-carnitine deficiency in HIV-infected patients with oxidative stress and mitochondrial dysfunction" (Herzmann *et al.,* 2005; Benedini *et al.,* 2009). Both "mitochondrial dysfunction and oxidative stress play a role in the development of polyneuropathy"

(Areti *et al.,* 2014). In addition, reduced levels of L-carnitine in the plasma of HIV patients diagnosed with polyneuropathy and lipoatrophy were associated with the use of zidovudine, stavudine, and didanosine (Herzmann *et al.,* 2005). Recently, a reduction in plasma levels of C3 was associated with advanced HIV infection and rapid disease progression (Waagsbø *et al.,* 2016). These authors linked rapid disease progression in advanced HIV-infected patients with low levels of propionyl-carnitine and octanoyl-carnitine acylcarnitines. In their explanation on how changes in acylcarnitines lead to mitochondrial dysfunction, Koves et al. (Koves *et al.,* 2008) associated "increased acylcarnitines with the mitochondrial overload, which involves mitochondrial long-chain fatty acid (LCFA) delivery, catabolism, and tricarboxylic acid (TCA) cycle" (Koves *et al.,* 2008). The findings of this study could "reflect selectively disturbed metabolism involving short- and medium-chain acylcarnitines in the peroxisomes and liver microsomes of HIV-infected Kenyan patients previously diagnose with stavudine-related polyneuropathy" Studies by Reuter et al. (2008) and Schooneman et al. (2013) observed similar association between polyneuropathy and selectively disturbed metabolism of short- and medium-chain acylcarnitines.

5.5. STUDY LIMITATIONS

The sample size of this study could have influenced different results of common types of ADRs, their risk factors as well as alterations in plasma metabolites in HIV-infected Kenyan and German patients. However, by relying on a combination of

study designs involving retrospective, nested case-control and exploratory cohorts, the comparison between different groups was possible.

Compared to longitudinal or prospective studies, this study provided a snapshot of the common ADRs, risk factors, and metabolic profiles. All HIV-infected Kenyan patients with a history of polyneuropathy (cases) and patients without previous history of polyneuropathy (controls) were female. The variations in plasma metabolites in HIV-infected female Kenyan patients could have over-emphasized the distinction in plasma metabolites compared to HIV-infected German patients. Moreover, this study did not measure viral load for both German and Kenyan patients. The concentration of plasma metabolites might also vary depending on how the study collected and processed plasma. Even if the collection of plasma samples in this study followed strict procedures, the mere fact that collection of plasma samples took place in two different settings (Kenya and Germany) and performed by different personnel contributed to the limitations of the study.

However, this study in spite of these limitations has provided significant findings have the potential to impact on the understanding of molecular mechanisms concerned with long-term ADRs in HIV-infected patients.

CHAPTER SIX:

CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

The findings of the present study support efforts in expanding global access to ART. Overall, it describes lipodystrophy and polyneuropathy as the most common ADRs that are likely to occur in Kenyan patients compared to German patients. The study identified age as a risk factor for ADRs, particularly polyneuropathy. Despite the withdrawal of d4T as the mainstay first-line ART drug, polyneuropathy remains a challenge for patients infected with HIV including patients with no history of exposure to d4T. Further, these findings suggest differentially regulated glycerophospholipids and fatty acid oxidative metabolism in HIV-infected patients receiving long-term ART. The up-regulation of glycerophospholipids (PC aa C42:0 and LysoPC a C17:0) and short-chain acylcarnitine (C3) were related to HIV-infected Kenyan patients with a history of polyneuropathy.

6.2. RECOMMENDATIONS

Future studies among HIV-infected Kenyan patients could systematically concentrate on other aspects associated with the development of ART-related ADRs as well as concomitant medications, alcohol and drug abuse, other infections such as cancer, tuberculosis, hepatitis C virus, and nutritional deficiencies.

Since the current study is just an initial, attempt to explore factors that relate to long-term ART among HIV-infected Kenyan and German patients, there is a need for large-scale or similar studies to consolidate much needed empirical evidence on risk factors that may influence clinical outcomes of long-term use of ART. Attention must concentrate on longitudinal cohort design studies that aim at establishing causal effects or those that concentrate on validating the findings of this study.

Polyneuropathy remains an enormous public health concern, even in the absence of well-known risk factors like exposure to "d-drugs". This gap demands targeted large-scale investigations to assist clear the controversy encompassing independent risk factors of polyneuropathy including height and gender. Future studies need to focus on confounders like nutritional deficiencies, others infections, alcohol and abuse, genetic factors and metabolites. Targeting metabolites and pathways affected by dysregulated metabolites would facilitate in distinguishing potential biomarkers capable of minimizing the debilitating effects of ART-related polyneuropathy.

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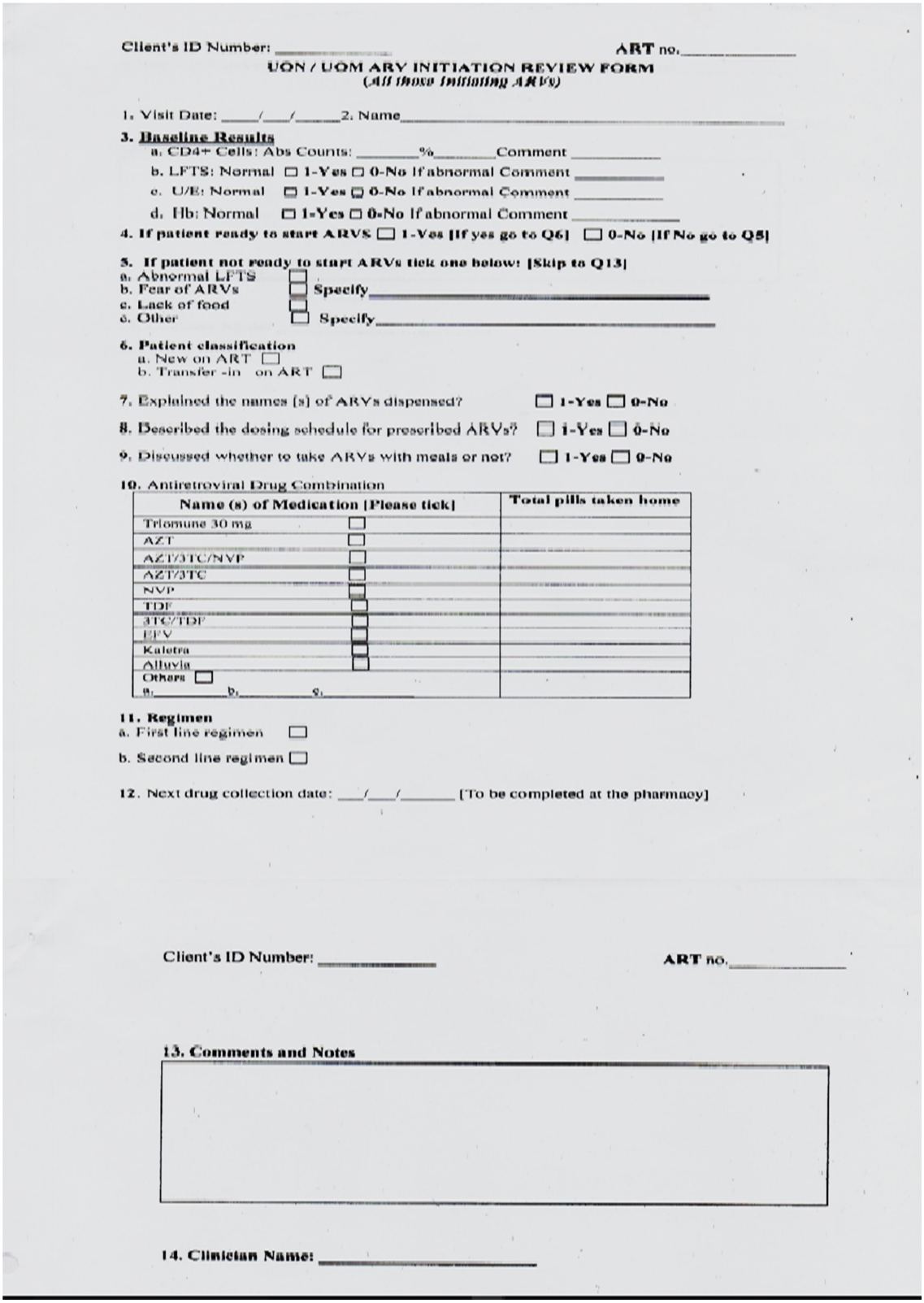
APPENDICES:

Appendix A: Data abstraction instruments



Client's ID Number: _____ ART no._____

UON / UOM ARV INITIATION REVIEW FORM
(All those initiating ARVs)

1. Visit Date: ____/___/_____2. Name_____

3. Baseline Results

a. CD4+ Cells: Abs Counts: _____%_____Comment _____

b. LFTS: Normal ☐ 1-Yes ☐ 0-No If abnormal Comment _____

c. U/E: Normal ☐ 1-Yes ☐ 0-No If abnormal Comment _____

d. Hb: Normal ☐ 1-Yes ☐ 0-No If abnormal Comment _____

4. If patient ready to start ARVS ☐ 1-Yes [If yes go to Q6] ☐ 0-No [If No go to Q5]

5. If patient not ready to start ARVs tick one below: [Skip to Q13]

a. Abnormal LFTS ☐

b. Fear of ARVs ☐ Specify_____

c. Lack of food ☐

d. Other ☐ Specify_____

6. Patient classification

a. New on ART ☐

b. Transfer -in on ART ☐

7. Explained the names [s] of ARVs dispensed? ☐ 1-Yes ☐ 0-No

8. Described the dosing schedule for prescribed ARVs? ☐ 1-Yes ☐ 0-No

9. Discussed whether to take ARVs with meals or not? ☐ 1-Yes ☐ 0-No

10. Antiretroviral Drug Combination

Name (s) of Medication [Please tick]		Total pills taken home
Triomune 30 mg	☐	
AZT	☐	
AZT/3TC/NVP	☐	
AZT/3TC	☐	
NVP	☐	
TDF	☐	
3TC/TDF	☐	
EFV	☐	
Kaletra	☐	
Alluvia	☐	
Others ☐ a._____b._____c._____		

11. Regimen

a. First line regimen ☐

b. Second line regimen ☐

12. Next drug collection date: ___/___/_____ [To be completed at the pharmacy]

Client's ID Number: _____ ART no._____

13. Comments and Notes

14. Clinician Name: _____

UOM/UON Medical Review form

1. Client's ID Number _____ 2. Visit date:...../...../.....

3. Name: _____

4. Current Complaints

5. Vital Signs Assessment

TEMP		HR		BP		RR		Wt (kg)	

6a. LMP___/___/____ (Females) 6b. Menopause ☐ 6c. DUB ☐

6d. Current Family planning: ☐ 0=none ☐ 1=oral ☐ 2-IUCD ☐ 3-depo ☐ 4-TL ☐ 5-Condom ☐

7- Diaphragm ☐ 8. Spermicidal ☐ 9. Norplant 10.Other_____

7. Are you pregnant: Yes ☐ No: ☐ [Females only]

8. Systemic Examination

	Normal	Abnormal	Not Done	Findings
General Appearance				
Mouth				
Skin				
Lymph Nodes				
Throat				
Nose				
Eyes				
Ear				
Lungs				
Cardiovascular				
Abdomen				
Genitourinary				
Musculoskeletal				
Neurologic				
Psychological				
Others Specify				

8a. Are you on TB treatment: Yes ☐ No ☐

8b. If yes, what phase of treatment: Intensive ☐ Continuation phase ☐

9. Are you on ARVS? Yes: ☐ **[If Yes Go to 10]** No: ☐ **[If No go to 13]**

10 Regimen type [Please review the type of ARVs and tick the appropriate box. Client may not know regime level]

a. First line : ☐ b. Second Line : ☐

11. Drug change? ☐ 1-Yes ☐ 0- No [Go to Q13]

12. If yes, reason...

a. Lipodystrophy	☐	e. Tuberculosis Treatment	☐
b. Peripheral neuropathy	☐	f. Treatment failure	☐
c. Hepatitis	☐	g. Poor adherence	☐
d. Pregnancy	☐	h. Other	☐
		1._____ 2._____	

UOM/UON Medical Review form

13. Medical tests requested:

LFTs: ☐ 1-Yes ☐ 0-No RFTs ☐ 1-Yes ☐ 0-No CXR ☐ 1-Yes ☐ 0-No Sputum AFB ☐ 1-Yes ☐ 0-No

Others a._____ b._____ c._____ d._____

14. Clinical Impression: _____

15. Management

	STI Results		
	1. GC	Pos ☐	Neg ☐
	2. Syphilis	Pos ☐	Neg ☐
	3. BV	Pos ☐	Neg ☐
	4. TV	Pos ☐	Neg ☐
	5. Candidiasis	Pos ☐	Neg ☐
	6. LFTs	N ☐	Abn ☐
	7. RFTs	N ☐	Abn ☐

16. Next Appointment_____ **(dd/mm/yyyy) Clinician:** _____ **Signature:** _____

Client's ID Number _____ Interviewer_____

UON/UOM: Adult Clinical Staging

1. First Name: _____ **Mid Name:** _____

Last Name: _____

2. Visit date:…..:*I*……*I*…… Time in:_____

Past Medical Problems

In the past 1-year have you had?

a. Weight loss>10kg	Y	N	
b. Diarrhea > 1 month	Y	N	Duration _____ wks
c. Cough >1-month	Y	N	Duration _____ wks
d. Fever/sweats/chills >1 month	Y	N	Duration _____ wks
e. Itchy skin rash	Y	N	Duration _____ wks
f. Painful skin rash (Zoster)	Y	N	Duration _____ wks

TB treatment history

3. Have you ever been treated for tuberculosis ☐ 1-Yes ☐ 0-No **[If yes go to 4]**

4. If yes please state

a. How many times? Once ☐ Twice ☐ More ☐ _____ **[Please write number]**

b. When_____ [year] If More than once 1._____2._____3._____

c. Duration of treatment: 6 months ☐ 9 months ☐ 12 months ☐ Other _____

d. Are you currently on TB Rx: Yes ☐ No ☐ Is yes date started _____

5. Drug treatment History

	Names	Response[Please respond to all]		Names [if applicable	Duration of use	Place of Treatment	Reason [I f stopped]
a.	ARVs	☐ 1-Yes	☐ 0-No				
b.	Septrin Prophylaxis	☐ 1-Yes	☐ 0-No				
c.	Fluconazole Prophylaxis	☐ 1-Yes	☐ 0-No				
d.	Multivitamins	☐ 1-Yes	☐ 0-No				
e.	Others	☐ 1-Yes	☐ 0-No				

6. Are you allergic to any drugs: **Yes** ☐ **No** ☐

6a.If yes specify_____

7. Current Medical Problems

Fever	N	Y ___ day	Night sweats	N	Y ___ day
Chills	N	Y ___ day	Fatigue	N	Y ___ day
Wt loss	N	Y ___ day	Headaches	N	Y ___ day
Dizziness	N	Y ___ day	Cough*	N	Y ___ day
Swollen glands	N	Y ___ day	Diarrhea	N	Y ___ day
Itchy Rash	N	Y ___ day	Throat pain	N	Y ___ day
Backache	N	Y ___ day	Skin Infection	N	Y ___ day
Vag discharge	N	Y ___ day	Dysuria	N	Y ___ day
Abd pain	N	Y ___ day	Vulva itch	N	Y ___ day
Chest pain	N	Y ___ day	Joints pain	N	Y ___ day
Painful rash	N	Y ___ day	Abscess (es)	N	Y ___ day

Client's ID Number _____ Interviewer_____

Other (s)

** Those with persistent coughs (> 1 wk) despite medications OR suspected to have TB require the TB ICF check list filled out*

8. Vital Signs Assessment

TEMP		HR		BP		RR	

Wt (kg)		**Ht (m)**		**BMI**		**Waist circ**		**HIP circ**		**Waist: Hip Ratio**	

9a. LMP__/__/____ (Females) 9b. Post Menopausal ☐ 9c. DUB ☐ 9d. Gravid _____

10. Systemic Examination

	Normal	**Abnormal**	**Not Done**	**Findings**
General Appearance				
Mouth				
Skin				
Lymph Nodes				
Throat				
Nose				
Eyes				
Ear				
Lungs				
Cardiovascular				
Abdomen				
Genitourinary				
Musculoskeletal				
Neurologic				
Psychological				
Others Specify ___				

11. Baseline Results

a.*CD4+ Cells: Abs Counts: _____ b. %_____ c. Hb:_____ Date: _____

Client's ID Number _____ Interviewer_____

12. Clinical Staging Criteria

WHO Stage 1

☐1 D1Asymptomatic stage
☐1D2Persistent Generalized lymphadenopathy (PGL)

WHO Stage 2

☐2D1 Loss of < 10% of body weight
☐2D2 Minor mucocutaneous manifestations,
☐2D3 Herpes zoster (*within last 5yrs)
☐2D4 Recurrent URTI

WHO Stage 3

☐3D1 Oral hairy leukoplakia
☐3D2 Loss of weight higher than 10%
☐3D3 Unexplained persistent diarrhea (>14 days)
☐3D4 Unexplained persistent fever (> 1 month)
☐3D5 Oral candidiasis (Thrush)
☐3D6 Unexplained prolonged fever
☐3D7 Pulmonary tuberculosis within previous 1yr,
☐3D8 severe bacterial pneumonia (*>2 episodes/12 months)

WHO Stage 4

☐4D1 Salmonella Septicemia, Non-typhoid
☐4D2Mycosis, Disseminated Endemic (I .e., Histoplasmosis, Coccidiomycosis)
☐4D2 HIV Wasting syndrome
☐4D3 Cryptococcal extrapulmonary
☐4D4 Pneumocystis carinii pneumonia
☐4D5 Toxoplasmosis of the brain
☐4D6 Cryptosporidiosis with Diarrhea (> 1 month duration)
☐4D7 HIV Encephalopathy
☐ 4D8 Candidiasis (Esophageal, Bronchi, Trachea, or Lungs)
☐4D9 Isosporidiosis with diarrhea for more than 1 month
☐4D10 Extrapulmonary Tuberculosis
☐4D11 Lymphoma (Including cerebral lymphoma)
☐4D12 Herpes simplex mucocuntaneous for more than1 month,
☐4D13 Atypical mycobacteriosis disseminated
☐4D14 Progressive Multifocal Leucoencephalopathy
☐4D15 Any disseminated endemic mycosis
☐4D16 Kaposis sarcoma
☐4D17 Cytomegalovirus other lymph node, liver or spleen

12b. Clinical Stage: I ☐ II ☐ III ☐ IV ☐

Management

13. Prescribed Drugs

a._____

b._____

c._____

14. Others:

a. Septrin prophylaxis ☐ 1-Yes ☐ 0-No **Dapsone** ☐ 1-Yes ☐ 0-No Fluconazole prophylaxis ☐ 1-Yes ☐ 0-No

b. Nutritional Supplementation : _____

15 Medical tests requested:

Liver FTs ☐ 1-Yes ☐ 0-No Renal FTs ☐ 1-Yes ☐ 0-No CD 4 counts ☐ 1-Yes ☐ 0-No

CXR ☐ 1-Yes ☐ 0-No Sputum AFB ☐ 1-Yes ☐ 0-No Full Hemogram: ☐ 1-Yes ☐ 0-No

Viral Load: ☐ 1-Yes ☐ 0-No HIV PCR: ☐ 1-Yes ☐ 0-No

Others _____

Client's ID Number _____ Interviewer_____

16. Comment:

17. Date of next appointment: ____/____/____

Clinician: _____ **Signature:**_____

18. Time Out: _____

Client's ID Number: _____ ART no._____

UON/UOM ART REVIEW FORM:

(All those on ARVs – Quarterly and all Medical Reviews

1. Visit Date: ____/____/____ **2.** Date of appointment ____/____/____

3. Names: Last: _____Middle_____First_____

4. Vital Signs

TEMP		HR		BP		RR	

Wt (kg)		Ht (Cm)		BMI		Waist circ		HIP circ		Waist: Hip Ratio	

5a. Are you on TB treatment: Yes ☐ No ☐

5b. If yes, what phase of treatment: Intensive ☐ Continuation phase ☐

5c. Current Medical Problems

Fever	N	Y ____ day	Night sweats	N	Y ___ day
Chills	N	Y ____ day	Fatigue	N	Y ___ day
Wt loss	N	Y ____ day	Headaches	N	Y ___ day
Dizziness	N	Y ____ day	Cough*	N	Y ___ day
Swollen glands	N	Y ____ day	Diarrhea	N	Y ___ day
Itchy Rash	N	Y ____ day	Throat pain	N	Y ___ day
Backache	N	Y ____ day	Skin Infection	N	Y ___ day
Vag discharge	N	Y ____ day	Dysuria	N	Y ___ day
Abd pain	N	Y ____ day	Vulva itch	N	Y ___ day
Chest pain	N	Y ____ day	Joints pain	N	Y ___ day
Painful rash	N	Y ____ day	Abscess (es)	N	Y ___ day

Other (s)

*** Those with persistent coughs (> 1 wk) DESPITE MEDICATIONS OR suspected to have TB require the ICF TB check list filled out**

6a. Are you pregnant: Yes ☐ No: ☐ [Females] **[If No skip to 6c]**

6b. LMP___/____/____ (Females) Post Menopausal ☐ D.U.B ☐ N/A ☐

6c. **Current Contraceptive:** ☐0-none ☐ 1-oral ☐ 2-IUCD ☐ 3-depo ☐ 4-TL ☐

5-Condom ☐ 7- Diaphragm ☐ 8.Spermicidal ☐ 9.Norplant ☐ 10.Other_____

7a. Has your partner been tested for HIV? 1-Yes ☐ [Go to **7b**] 0-No ☐ [Go to **8a**]

No spouse ☐ [Go to Q.9] Don't Know ☐ [Go to **8b**]

7b. If Yes, what the Status? +ve ☐ [Go to **7c**] -ve ☐ [Go to **8b**]

7c. **If Positive,**

i. Is your spouse enrolled into a care clinic? ☐ 1-Yes [Go to **ii below**] ☐ 0-No ☐ **[Go to 8b]**

ii. Is yes, are they on ARVs? ☐ 1-Yes ☐ 0-No ☐

Client's ID Number: _____ ART no._____

8a. If No **[in 7a above]** Probe for details

_____

_____

_____

8b. Do you use condoms with spouse: ☐ 1-Yes **[Go to 8d]** ☐ 0-No ☐

8c. If yes, ☐ 1-Always ☐ 0-Sometimes

9. Systemic Examination

	Normal	Abnormal	Not Done	Findings
General Appearance				
Mouth				
Skin				
Lymph Nodes				
Throat				
Nose				
Eyes				
Ear				
Lungs				
Cardiovascular				
Abdomen				
Genitourinary				
Musculoskeletal				
Neurologic				
Psychological				
Others Specify _				

10. Grading Side Effects

Side Effects (Common Culprit drugs)	Yes	No	Tick noted	Grade
Rash				
Hepatotoxicity (e.g. Nevirapine, PIs				
Peripheral Neuropathy (e.g. D4T, DDI etc)				
Lipodystrophy (e.g. D4T, AZT, DDI etc)				
Diarrhoea (e.g. Lopinovir/ritonovir, other PIs etc)				
Anemia (e.g. AZT etc)				
Others				

Grades: 1= Mild, 2=Moderate, 3=Severe, 4= Very Severe

11. Clinical Impression:

_____

_____

Client's ID Number: _____ ART no._____

12 Nutritional Supplementation : ☐ 1-Yes ☐ 0-No

13. Medical tests requested:

Liver FTs ☐ Renal FTs ☐ CD 4 counts ☐ Pregnancy Test ☐

CXR ☐ Sputum AFB ☐ Viral Load ☐

Others ☐ 1)_____ 2)_____ 3)_____

14. Management

a._____

b._____

c._____

d._____

Prescribed or Continuing Antiretroviral

15. Are you on ARV Therapy ☐ 1-Yes ☐ 0-No [Go to Q20]

16 Regimen type [Please review the type of ARVs and tick the appropriate box. Client may not know regime level]

a. First line : ☐ b. Second Line : ☐

17. Switching ARVs? ☐ 1-Yes ☐ 0-No [Go to Q20]	
18. If yes, reason... a. Lipodystrophy ☐ b. Peripheral neuropathy ☐ c. Hepatitis ☐ d. Pregnancy ☐	e. Tuberculosis Treatment ☐ f. Treatment failure ☐ g. Poor adherence ☐ h. Other ☐ 1._____ 2._____
19. If regimen changed please indicate drugs stopped	a._____ b._____ c._____

20. Prescribed or Continuing Prophylaxis

a. Septrin ☐ 1-Yes ☐ 0-No b. Fluconazole ☐ 1-Yes ☐ 0-No c .Dapsone ☐ 1-Yes ☐ 0-No

Additional Notes:

21. Date of next appointment: ____/____/_____

Clinician: _____ **Signature:**_____

Client's ID Number: _____ ART no._____

ART PSYCHOSOCIAL ASSE

22. Sexual contact:

a-Spouse	b-Regular partner
c-Irregular partner	d- Sex worker
e. Abstaining _____ Months/yrs	
f. Alternative sexual expressions_____	

23. Condom use:

1. Always	2.Sometimes	3-Never
4. N/A	5. Abstaining	

24. Alcohol use ***(CAGE SCORE)*** Each Yes answer Scores 1:

1.Have you ever felt you needed to Cut down on your drinking? ☐ 1-Yes ☐ 0-No

2.Have people Annoyed you by criticizing your drinking? ☐ 1-Yes ☐ 0-No

3.Have you ever felt Guilty about drinking? ☐ 1-Yes ☐ 0-No

4.Have you ever felt you needed a drink first thing in the morning (Eye-opener) to steady your nerves or to get rid of a hangover? ☐ 1-Yes ☐ 0-No

25. Who provides you with family support?

_____

_____

25. What is the nature of support?

_____

_____

26. Member of post-test support group ☐ 1-Yes ☐ 0-No

27. Support group meeting:
Does the client attended ART support meetings? ☐ 1-Yes ☐ 0-No

28. Mood assessment:

Low spirits	☐ 1-Yes ☐ 0-No
Loss of interest	☐ 1-Yes ☐ 0-No
Been worrying a lot	☐ 1-Yes ☐ 0-No
Felt hopeless	☐ 1-Yes ☐ 0-No
Felt worthless	☐ 1-Yes ☐ 0-No

Mood assessment (cont'd)

Have you been irritable	☐ 1-Yes ☐ 0-No
Lost confidence in self	☐ 1-Yes ☐ 0-No
Had difficulty relaxing	☐ 1-Yes ☐ 0-No
Lack sleep/early wake	☐ 1-Yes ☐ 0-No
Loss of appetite	☐ 1-Yes ☐ 0-No

29. Side-effects
Have you experienced any of the following?

Nausea	☐ 1-Yes ☐ 0-No
Vomiting	☐ 1-Yes ☐ 0-No
Fatigue/Malaise	☐ 1-Yes ☐ 0-No
Headache	☐ 1-Yes ☐ 0-No
Dizziness	☐ 1-Yes ☐ 0-No
Bad dreams	☐ 1-Yes ☐ 0-No
Swellings of legs	☐ 1-Yes ☐ 0-No
Rash	☐ 1-Yes ☐ 0-No
Hair loss	☐ 1-Yes ☐ 0-No

Others Specify _____

Client's ID Number: _____ **ART** no._____

ART ADHERENCE ASSESSMENT
UOM/UON ART/ CARE EXPERIENCE INDICATORS:
(Pills pick up visits only)

30. Visit Date: ____/____/____ [mm/dd/yyyy]

31. Name: _____

32. Date of appointment: ____/____/____ [mm/dd/yyyy]

33. Date of last drug collection: ____/____/____ [mm/dd/yyyy]

34. Current Medication **Last 72 hour time pill(s) swallow recall [Date Format: dd/mm/yyyy]**

Name of Medication [Please tick]	Date: / /			Date / /			Date / /		
	AM	PM	No:	AM	PM	No.	AM	PM	No.
Triomune 30 mg ☐									
AZT ☐									
AZT/3TC/NVP ☐									
AZT/3TC ☐									
NVP ☐									
TDF ☐									
3TC/TDF ☐									
EFV ☐									
Kaletra ☐									
Alluvia ☐									
Others ☐ 1._____ 2._____ 3._____									

35. Pill count table

Date	Each Drug	Present Count	Pills Expected Taken [PE]	Actual Pills Taken [AP]	Refill Count	Total Pills Taken Hme	% Evaluation

Evaluation percent = **AP /PE** x 100%; [100%-*95%* - *perfect*]; [94% - 90% -*can Improve*]

[89%–81% -*alarm*] ;[< *80%-change/stop*]

36. Current Regimen Level

a. First line regimen: ☐

b. Second line regimen: ☐

37. Adherence Level:

a. Perfect ☐

b. Can Improve ☐ **[Refer to counselor]** ☐

c. Alarm ☐ **[Refer to counselor]** ☐

d. Change/Stop ☐ **[Refer to counselor]** ☐

38. Drug change ? ☐ 1-Yes ☐ 0- No

39. Regimen level change? ☐ 1-Yes ☐ 0- No

*Clinician*_____ **Signature** _____

Client's ID Number: _____ **ART** no._____

UOM/UON ART/ CARE EXPERIENCE INDICATORS:
(Pills pick up visits only)

40. Drugs Dispensed

Name of Medication [Please tick]	
Triomune 30 mg	☐
AZT	☐
AZT/3TC/NVP	☐
AZT/3TC	☐
NVP	☐
TDF	☐
3TC/TDF	☐
EFV	☐
Kaletra	☐
Alluvia	☐
Others ☐ 1._____ 2._____ 3._____	

41. Dispensed Regimen Level

a. First line regimen: ☐

b. Second line regimen: ☐

42. Comments:

43. Prophylaxis given:

a. Septrin ☐ 1=Yes ☐ 0=No

b. Dapsone ☐ 1-Yes ☐ 0-No

c. Fluconazole ☐ 1=Yes ☐ 0=No

d. Multivitamins ☐ 1-Yes ☐ 0-No

44. Name of HCW: _____ **Next Appointment Date:** _____

Appendix B: Ethics Clearance

KENYATTA NATIONAL HOSPITAL APPROVED 06 JUN 2013 ETHICS & RESEARCH COMMITTEE

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/158

6th June 2013

Frank Ndaks Ndakala
Reg.No.W80/84341/2012
UNITID
University of Nairobi

Dear Frank

RESEARCH PROPOSAL: GENETIC ANALYSIS OF ADVERSE DRUG EVENTS ASSOCIATED WITH ANTIRETROVIRAL THERAPY IN KENYA (P68/2/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 6th June 2013 to 5th June 2014.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

KENYATTA NATIONAL HOSPITAL APPROVED 06 JUN 2013 ETHICS & RESEARCH COMMITTEE

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Yours sincerely

PROF. M.O. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N. Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The HOD, Records, KNH
Principal College of Health Sciences, UON
The Director, UNITID, UON
Supervisors: Prof. George M.N. Behrens, University Hospital Hanover, Germany

Appendix C: Informed Consent

Informed consent Form

Project Title: Metabolomic Analysis of Adverse Drug Events associated with Antiretroviral Therapy in Kenya

Ref No: _____

Principal Investigator:

Mr. Frank Ndakala

Phone: 2219420 Ext: 1144 or 0723361283.

INTRODUCTION:

We would like to perform metabolomic analysis (studying metabolites) on a sample of your blood. We will explain to you what this involves so that you can decide if you will consent to genotyping.

WHAT IS THE PURPOSE OF THIS GENETIC RESEARCH?

You are currently taking ARVs as part of SWOP project. There are enzymes in your body (i.e. proteins in the body, usually in the liver and bowel), that help to break down any drug that you take, including ARVs. After reacting with ARVs, enzymes lead to the formation of metabolites. These metabolites differ in different groups of people and there is little understanding of theses metabolites in the Kenyan population. It is important to understand how these metabolites are distributed in our population and how they affect the development of ART-related toxicities.

OBJECTIVES OF THE STUDY:

The aim of the study is to understand the metabolic causes of observed differences in antiretroviral drug response and effectiveness in HIV–positive patients in Kenya. Specifically, the study will investigate the following:

- How variants of Cytochrome P450 (*CYP450*) gene and mitochondrial DNA (mtDNA) gene influence drug levels in your blood and the development of ART side effects

TYPES OF SPECIMEN

- If you decide to participate, we request you to donate 5 ml of blood for genetic analysis and determination of drug plasma levels.

- About 1.5 ml of the donated whole blood will be stored at UNITID at -20 °C for the purposes of extracting DNA.
- About 3 ml of the donated whole blood will be centrifuged to separate plasma, which will be stored at UNITID at -20 °C.
- We hereby declare that no other test will be conducted on your samples.

RISKS AND DISCOMFORTS

- You will experience minimal risk resulting from your participating in this study. You may feel some pain associated with having blood withdrawn from your vein and may experience discomfort, bruising and / or slight bleeding at the site.

BENEFITS:

- This study is mainly for general medical advances, but could also benefit you as an individual if your genetic status is associated with drug response and the risk of side effects of certain drugs that you may be given in the future (not in this study), or may be taking.
- Although there may be no direct benefits to you by participating in this stage, family members and future generations may benefit if the researchers succeed in scientifically delineating certain disease states and drug responses further. The identification of the genes, and their genetic variants, involved in such drug responses, could, in the end, lead to the development of methods for prevention and forms of new treatment aimed at curing or alleviating these conditions.

CONFIDENTIALITY

- As a sign of your willingness to participate in this study, please sign this document below. There are arrangements to release the results of the study to health authorities and publish in journals. When this happens, your identity will remain confidential throughout. Information will not be associated with your name. The research staff will use only a coded number, Access will be limited to authorized scientists and any scientific publications, lectures or reports resulting from the study will not identify you by your name.

SAMPLE STORAGE FOR FUTURE USE

As a sign of your willingness to participate in this study, please sign this document below. we plan to store your whole blood sample and plasma for future studies on genes that influence how individuals respond to treatment. Stored samples will be coded so that they cannot be linked to your identity. You can still participate in this study even if you do not want your

whole blood sample and plasma sample to be stored for future use. If you do not agree, your samples will be destroyed after the study. If you agree, you will not be asked again for your consent in the future. If you choose to withdraw from participating in the study, we will retain any samples collected prior to your withdrawal unless you inform us that you would like them destroyed. If we wish to use the samples for research in the future, we will submit our request to the Kenyatta National Hospital/Nairobi University Ethics Review Committee. You will not be contacted with the results of any future research.

SHIPMENT OF STORED SAMPLES FOR FURTHER ANALYSIS

- While whole blood and plasma samples will be separated and stored in a laboratory at UNITID in Nairobi, Kenya, further studies will need to be performed at the Clinic for Immunology and Rheumatology, University Hospital Hannover, Germany.
- Therefore, if you indicate your willingness to participate in the study, we plan to ship your stored whole blood sample and plasma sample from the UNITID laboratory in Nairobi to an advanced laboratory at the Clinic for Immunology and Rheumatology, University Hospital Hannover, Germany from where we will carry out further analysis

ADDITIONAL COSTS

- All costs are borne by SWOP. There are no additional costs to participating in this study.

IN THE EVENT OF INJURY

Any injury associated with you participation in this stud should be reported to:

Dr. Makobu Kimani on 0722258102 or Mr. Frank Ndakala on 0723361283

VOLUNTARY PARTICIPATION

- Participation in this study is voluntary. If you decide not to participate in this study, your decision will not affect your future relations with the University of Nairobi, nor Sex Work Outreach Program, its personnel, and associated clinics. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without penalty.

DISSEMINATION OF FINDINGS

- As a participant in this research study, you may request a copy of the final report. If you change your mind after feeling that you no long wish to participate, you may withdraw from the research study at any time.

CONTACT NUMBERS

If you have any questions regarding the study or your participation in the study, you can call Mr. Frank Ndakala the Principal Investigator, on phone no: 0723 361 283. If you have a question about your rights as a research volunteer, you should contact Prof. Guantai, the Chairperson of the Ethics Committee at Kenyatta National Hospital, Tel: 726300-9.

STATEMENT OF CONSENT

If you agree to participate in this study, please sign below.

I, _____have read or have had read to me, the consent form for the above study and have discussed with_____. I understand that the following (Please tick if you fully understand and agree with each statement):

? The goal of this research is to study genes influencing drug-related toxicities in my blood.

? Enrolment is voluntary and I can withdraw from the study anytime.

? Blood specimens will be required for this study

? I am aware and give permission for part of the sample taken and stored at UNITID to be shipped to the Clinic for Immunology and Rheumatology, University Hospital Hannover, Germany.

I HEREBY CONSENT VOLUNTARILY TO PARTICIPATE:

_____ _____

Participant's name Signature and date

_____ _____

Study staff conducting consent Signature and date

_____ _____

Witness name Signature and date

SAMPLE STORAGE AND SHIPMENT

Please indicate if you agree to allow your blood sample to be stored long term at the University of Nairobi, Institute of Tropical Infectious Diseases (UNITID) and subsequently be shipped to Hannover Medical School in Germany for further analysis. The stored specimen will be coded so that they cannot be linked to your identity. You can still participate in this study even if you do not want your blood sample to be stored for future analysis. If you do not agree, your samples will be destroyed after the study. If you agree, you will not be asked again for your consent in future. If you choose to withdraw from participating in this study, we will retain any samples collected prior to your withdrawal unless you inform us that you would like them destroyed. Before the samples are shipped to Germany, we will submit our request to the Kenyatta National Hospital Ethics/ University of Nairobi Review Committee. Please indicate your preference by indicating one of the following:

_____I **AGREE** to let my blood sample be stored at the University of Nairobi for future use and later on be shipped to Germany at the Hannover Medical School for further analysis.

_____**I DO NOT WANT** my blood sample to be stored at the University of Nairobi for future use and later on be shipped to Germany at the Hannover Medical School for further analysis.

_____	_____
Participant's name	Signature and date
_____	_____
Study staff conducting consent	Signature and date
_____	_____
Witness name	Signature and date

Appendix D: A list of plasma metabolites measured by Biocrates kit IDQ p180 (Draisma, *et al.,* 2013)

No.	Abbreviation	Full biochemical name	No.	Abbreviation	Full biochemical name
1.	C0	Carnitine	60.	PC aa C36:0	Phosphatidylcholine diacyl C36:0
2.	C2	Acetylcarnitine	61.	PC aa C36:1	Phosphatidylcholine diacyl C36:1
3.	C3	Propionylcarnitine	62.	PC aa C36:2	Phosphatidylcholine diacyl C36:2
4.	C3-OH	Hydroxypropionylcarnitine	63.	PC aa C36:3	Phosphatidylcholine diacyl C36:3
5.	C3:1	Propenonylcarnitine	64.	PC aa C36:4	Phosphatidylcholine diacyl C36:4
6.	C4	Butyrylcarnitine	65.	PC aa C36:5	Phosphatidylcholine diacyl C36:5
7.	C4-OH	Hydroxybutyrylcarnitine	66.	PC aa C36:6	Phosphatidylcholine diacyl C36:6
8.	C4:1	Butenylcarnitine	67.	PC aa C38:0	Phosphatidylcholine diacyl C38:0
9.	C5	Valerylcarnitine	68.	PC aa C38:1	Phosphatidylcholine diacyl C38:1
10.	C5-	DC Glutarylcarnitine	69.	PC aa C38:3	Phosphatidylcholine diacyl C38:3
11.	C5-M-DC	Methylglutarylcarnitine	70.	PC aa C38:4	Phosphatidylcholine diacyl C38:4
12.	C5-OH	Hydroxyvalerylcarnitine	71.	PC aa C38:5	Phosphatidylcholine diacyl C38:5
13.	C5:1	Tiglylcarnitine	72.	PC aa C38:6	Phosphatidylcholine diacyl C38:6
14.	C5:1-DC	Glutaconylcarnitine	73.	PC aa C40:1	Phosphatidylcholine diacyl C40:1
15.	C6	Hexanoylcarnitine	74.	PC aa C40:2	Phosphatidylcholine diacyl C40:2
16.	C6:1	Hexenoylcarnitine	75.	PC aa C40:3	Phosphatidylcholine diacyl C40:3
17.	C7-DC	Pimelylcarnitine	76.	PC aa C40:4	Phosphatidylcholine diacyl C40:4
18.	C8	Octanoylcarnitine	77.	PC aa C40:5	Phosphatidylcholine diacyl C40:5
19.	C8:1	Octenoylcarnitine	78.	PC aa C40:6	Phosphatidylcholine diacyl C40:6
20.	C9	Nonaylcarnitine	79.	PC aa C42:0	Phosphatidylcholine diacyl C42:0
21.	C10	Decanoylcarnitine	80.	PC aa C42:1	Phosphatidylcholine diacyl C42:1
22.	C10:1	Decenoylcarnitine	81.	PC aa C42:2	Phosphatidylcholine diacyl C42:2
23.	C10:2	Decadienylcarnitine	82.	PC aa C42:4	Phosphatidylcholine diacyl C42:4
24.	C12	Dodecanoylcarnitine	83.	PC aa C42:5	Phosphatidylcholine diacyl C42:5
25.	C12-DC	Dodecanedioylcarnitine	84.	PC aa C42:6	Phosphatidylcholine diacyl C42:6
26.	C12:1	Dodecenoylcarnitine	85.	PC ae C30:0	Phosphatidylcholine acyl-akyl C30:0

27.	C14	Tetradecanoylcarnitine	86.	PC ae C30:1	Phosphatidylcholine acyl-akyl C30:1
28.	C14:1	Tetradecenoylcarnitine	87.	PC ae C30:2	Phosphatidylcholine acyl-akyl C30:2
29.	C14:1-OH	Hydroxytetradecenoylcarnitine	88.	PC ae C32:1	Phosphatidylcholine acyl-akyl C32:1
30.	C14:2	Tetradecadienylcarnitine	89.	PC ae C32:2	Phosphatidylcholine acyl-akyl C32:2
31.	C14:2-OH	Hydroxytetradecadienylcarnitine	90.	PC ae C34:0	Phosphatidylcholine acyl-akyl C34:0
32.	C16	Hexadecanoylcarnitine	91.	PC ae C34:1	Phosphatidylcholine acyl-akyl C34:1
33.	C16-OH	Hydroxyhexadecanoylcarnitine	92.	PC ae C34:2	Phosphatidylcholine acyl-akyl C34:2
34	C16:1	Hexadecenoylcarnitine	93.	PC ae C34:3	Phosphatidylcholine acyl-akyl C34:3
35	C16:1-OH	Hydroxyhexadecenoylcarnitine	94.	PC ae C36:0	Phosphatidylcholine acyl-akyl C36:0
36	C16:2	Hexadecadienylcarnitine	95.	PC ae C36:1	Phosphatidylcholine acyl-akyl C36:1
37	C16:2-OH	Hydroxyhexadecadienylcarnitine	96.	PC ae C36:2	Phosphatidylcholine acyl-akyl C36:2
38	C18	Octadecanoylcarnitine	97.	PC ae C36:3	Phosphatidylcholine acyl-akyl C36:3
39.	C18:1	Octadecenoylcarnitine	98.	PC ae C36:4	Phosphatidylcholine acyl-akyl C36:4
40.	C18:1-OH	Hydroxyoctadecenoylcarnitine	99.	PC ae C36:5	Phosphatidylcholine acyl-akyl C36:5
41.	C18:2	Octadecadienylcarnitine	100.	PC ae C38:0	Phosphatidylcholine acyl-akyl C38:0
42.	Ala	Alanine	101.	PC ae C38:1	Phosphatidylcholine acyl-akyl C38:1
43.	Arg	Arginine	102.	PC ae C38:2	Phosphatidylcholine acyl-akyl C38:2
44.	Asn	Asparagine	103.	PC ae C38:3	Phosphatidylcholine acyl-akyl C38:3
45.	Asp	Aspartate	104.	PC ae C38:4	Phosphatidylcholine acyl-akyl C38:4
46.	Cit	Citrulline	105.	PC ae C38:5	Phosphatidylcholine acyl-akyl C38:5
47.	Gln	Glutamine	106.	PC ae C38:6	Phosphatidylcholine acyl-akyl C38:6
48.	Glu	Glutamate	107.	PC ae C40:0	Phosphatidylcholine acyl-akyl C40:0
49.	Gly	Glycine	108.	PC ae C40:1	Phosphatidylcholine acyl-akyl C40:1
50.	His	Histidine	109.	PC ae C40:2	Phosphatidylcholine acyl-akyl C40:2
51.	Ile	Isoleucine	110.	PC ae C40:3	Phosphatidylcholine acyl-akyl C40:3
52.	Leu	Leucine	111.	PC ae C40:4	Phosphatidylcholine acyl-akyl C40:4
53.	Lys	Lysine	112.	PC ae C40:5	Phosphatidylcholine acyl-akyl C40:5
54.	Met	Methionine	113.	PC ae C40:6	Phosphatidylcholine acyl-akyl C40:6
55.	Orn	Ornithine	114.	PC ae C42:0	Phosphatidylcholine acyl-akyl C42:0
56.	Phe	Phenylalanine	115.	PC ae C42:1	Phosphatidylcholine acyl-akyl C42:1

57.	Pro	Proline	116.	PC ae C42:2	Phosphatidylcholine acyl-akyl C42:2
58.	Ser	Serine	117.	PC aeC42:3	Phosphatidylcholine acyl-akyl C42:3
59.	Thr	Threonine	118.	PC ae C42:4	Phosphatidylcholine acyl-akyl C42:4
119.	Trp	Tryptophan	155.	PC ae C42:5	Phosphatidylcholine acyl-akyl C42:5
120.	Tyr	Tyrosine	156.	PC ae C44:3	Phosphatidylcholine acyl-akyl C44:3
121.	Val	Valine	157.	PC aeC44:4	Phosphatidylcholine acyl-akyl C44:4
122.	xLeu	Leucine/Isoleucine	158.	PC ae C44:5	Phosphatidylcholine acyl-akyl C44:5
123.	Ac Orn	Acetylornithine	159.	PC ae C44:6	Phosphatidylcholine acyl-akyl C44:6
124.	ADMA	Asymmetric Dimethylarginine	160.	LPC a C14:0	LysoPhosphatidylcholine acyl C14:0
125.	SDMA	Symmetric Dimethylarginine	161.	LPC a C16:0	LysoPhosphatidylcholine acyl C16:0
126.	total DMA	Sum of ADMA and SDMA	162.	LPC a C16:1	lysoPhosphatidylcholine acyl C16:1
127.	alpha AAA	alpha-Aminoadipic acid	163.	LPC a C17:0	LysoPhosphatidylcholine acyl C17:0
128.	Carnosine	Carnosine	164.	LPC a C18:0	LysoPhosphatidylcholine acyl C18:0
129.	Creatinine	Creatinine	165.	LPC a C18:1	lysoPhosphatidylcholine acyl C18:1
130.	Histamine	Histamine	166.	LPC a C18:2	lysoPhosphatidylcholine acyl C18:2
131.	Kynurenine	Kynurenine	167.	LPC a C6:0	LysoPhosphatidylcholine acyl C6:0
132.	Met SO	Methioninesulfoxide	168.	LPC a C20:3	lysoPhosphatidylcholine acyl C20:3
133.	Nitro-Tyr	Nitrotyrosine	169.	LPC a C20:4	lysoPhosphatidylcholine acyl C20:4
134.	OH-Pro	Hydroxyproline	170.	LPC a C24:0	LysoPhosphatidylcholine acyl C24:0
135.	PEA	Phenylethylamine	171.	LPC a C26:0	LysoPhosphatidylcholine acyl C26:0
136.	Putrescine	Putrescine	172.	LPC a C26:1	lysoPhosphatidylcholine acyl C26:1
137.	Sarcosine	Sarcosine	173.	LPC a C28:0	LysoPhosphatidylcholine acyl C28:0
138.	Serotonin	Serotonin	174.	LPC a C28:1	lysoPhosphatidylcholine acyl C28:1
139.	Spermidine	Spermidine	175.	SM C16:0	Sphingomyeline C16:0
140.	Spermine	Spermine	176.	SM C16:1	Sphingomyeline C16:1
141.	Taurine	Taurine	177.	SM C18:0	Sphingomyeline C18:0
142.	PC aa C24:0	Phosphatidylcholine diacyl C24:0	178.	SM C18:1	Sphingomyeline C18:1
143.	PC aa C26:0	Phosphatidylcholine diacyl C26:0	179.	SM C20:2	Sphingomyeline C20:2
144.	PC aa C28:1	Phosphatidylcholine diacyl C28:1	180.	SM C22:3	Sphingomyeline C22:3
145.	PC aa C30:0	Phosphatidylcholine diacyl C30:0	181.	SM C24:0	Sphingomyeline C24:0
146.	PC aa C30:2	Phosphatidylcholine diacyl C30:2	182.	SM C24:1	Sphingomyeline C24:1
147.	PC aa C32:0	Phosphatidylcholine diacyl C32:0	183.	SM C26:0	Sphingomyeline C26:0
148.	PC aa C32:1	Phosphatidylcholine diacyl C32:1	184.	SM C26:1	Sphingomyeline C26:1
149.	PC aa C32:2	Phosphatidylcholine diacyl C32:2	185.	SM (OH) C14:1	Hydroxysphingomyeline C14:1
150.	PC aa C32:3	Phosphatidylcholine diacyl C32:3	186.	SM (OH) C16:1	Hydroxysphingomyeline C16:1
151.	PC aa C34:1	Phosphatidylcholine diacyl C34:1	187.	SM (OH) C22:1	Hydroxysphingomyeline C22:1
152.	PC aa C34:2	Phosphatidylcholine diacyl C34:2	188.	SM (OH) C22:2	Hydroxysphingomyeline C22:2
153.	PC aa C34:3	Phosphatidylcholine diacyl C34:3	189.	SM (OH) C24:1	Hydroxysphingomyeline C24:1
154.	PC aa C34:4	Phosphatidylcholine diacyl C34:4	190	H1	Hexose

Appendix E: Characteristics of the metabolites measured by Biocrates IDQ p180 kit

Class	Abbreviation	Mean Concentration	Standard Deviation	% > LOD	Application
Acylcarnitines	C0	35.45	9.09	100.00	Used
	C10	0.24	0.06	0.00	Excluded
	C10:1	0.11	0.03	0.00	Excluded
	C10:2	0.03	0.01	0.00	Excluded
	C12	0.09	0.02	0.00	Excluded
	C12-DC	0.04	0.01	0.00	Excluded
	C12:1	0.08	0.02	0.00	Excluded
	C14	0.05	0.01	0.00	Excluded
	C14:1	0.11	0.03	100.00	Used
	C14:1-OH	0.01	0.01	16.46	Excluded
	C14:2	0.02	0.01	46.84	Excluded
	C14:2-OH	0.01	0.00	0.00	Excluded
	C16	0.12	0.05	100.00	Used
	C16:1	0.02	0.01	64.56	Used
	C16:1-OH	0.01	0.00	0.00	Excluded
	C16:2	0.01	0.00	0.00	Excluded
	C16:2-OH	0.01	0.00	0.00	Excluded
	C18	0.04	0.02	82.28	Used
	C18:1	0.14	0.07	100.00	Used
	C18:1-OH	0.01	0.00	1.27	Excluded
	C18:2	0.05	0.03	98.73	Used
	C2	5.35	2.24	100.00	Used
	C3	0.27	0.11	100.00	Used
	C3-DC (C4-OH)	0.04	0.03	74.68	Used
	C3-OH	0.01	0.01	1.27	Excluded
	C3:1	0.02	0.01	3.80	Excluded
	C4	0.14	0.07	82.28	Used
	C4:1	0.01	0.00	1.27	Excluded
	C6 (C4:1-DC)	0.05	0.01	8.86	Excluded
	C5	0.09	0.04	100.00	Used
	C5-M-DC	0.02	0.01	30.38	Excluded
	C5-OH (C3-DC-M)	0.04	0.01	94.94	Used
	C5:1	0.06	0.01	5.06	Excluded
	C5:1-DC	0.01	0.00	7.59	Excluded
	C5-DC (C6-OH)	0.01	0.01	18.99	Excluded
	C6:1	0.01	0.00	3.80	Excluded
	C7-DC	0.03	0.01	65.82	Used
	C8	0.16	0.04	2.53	Excluded
	C9	0.03	0.01	75.95	Used

Amino acids	**Ala**	**1319.90**	**405.86**	**100.00**	**Used**
	Arg	206.80	76.39	100.00	Used
	Asn	52.82	39.86	100.00	Used
	Asp	106.46	163.80	30.38	Excluded
	Cit	73.31	51.50	100.00	Used
	Gln	1967.66	1581.41	89.87	Used
	Glu	1108.95	984.31	100.00	Used
	Gly	783.25	351.80	100.00	Used
	His	285,73	44,85	100,00	Used
	Ile	239.23	81.45	100.00	Used
	Leu	514.73	419.19	59.49	Used
	Lys	659.29	176.32	100.00	Used
	Met	48.28	19.38	100.00	Used
	Orn	289.18	96.49	100.00	Used
	Phe	205.34	52.11	100.00	Used
	Pro	657.35	209.14	100.00	Used
	Ser	355,72	101,03	100.00	Used
	Thr	290.63	29.10	100.00	Used
	Trp	158.07	39.98	100.00	Used
	Tyr	217.37	62.90	100.00	Used
	Val	724.51	210.69	100.00	Used
	Ac-Orn	0.00	0.00	0.00	Excluded
	ADMA	0.25	0.71	20.25	Excluded
	alpha-AAA	0.00	0.00	0.00	Excluded
	c4-OH-Pro	0.00	0.00	0.00	Excluded
	Carnosine	0.00	0.04	0.00	Excluded
	Creatinine	224.56	62.77	100.00	Used
	DOPA	0.00	0.00	0.00	Excluded
	Dopamine	0.00	0.00	0.00	Excluded
	Histamine	0.00	0.00	0.00	Excluded
	Kynurenine	7.01	5.69	94.94	Used
	Met-SO	12.59	11.06	67.09	Used
	Nitro-Tyr	0.00	0.00	0.00	Excluded
	PEA	0.00	0.00	0.00	Excluded
	Putrescine	0.13	0.24	44.30	Excluded
	Sarcosine	6.19	5.38	88.61	Used
	Serotonin	1.54	1.54	87.34	Used
	Spermidine	0.74	0.95	75.95	Used
	Spermine	0.00	0.00	0.00	Excluded
	t4-OH-Pro	34.91	21.58	100.00	Used
	Taurine	224.20	83.38	48.10	Excluded
	SDMA	17.48	4.94	100.00	Used

Glycerophospholipids	**LysoPC a C14:0**	**3.47**	**0.70**	**0.00**	**Excluded**
	LysoPC a C16:0	111.26	33.29	100.00	Used
	lysoPC a C16:1	3.12	1.16	100.00	Used
	LysoPC a C17:0	2.04	0.87	100.00	Used
	LysoPC a C18:0	32.57	11.15	100.00	Used
	lysoPC a C18:1	19.32	5.65	100.00	Used
	lysoPC a C18:2	21.16	7.64	100.00	Used
	lysoPC a C20:3	2.97	1.01	98.73	Used
	lysoPC a C20:4	6.47	1.94	100.00	Used
	LysoPC a C24:0	0.53	0.39	59.49	Used
	LysoPC a C26:0	1.38	1.27	84.81	Used
	lysoPC a C26:1	0.52	0.36	67.09	Used
	LysoPC a C28:0	1.22	0.99	83.54	Used
	lysoPC a C28:1	1.11	0.72	94.94	Used
	PC aa C24:0	0.52	0.42	93.67	Used
	PC aa C26:0	1.60	0.94	59.49	Used
	PC aa C28:1	2.02	0.74	100.00	Used
	PC aa C30:0	3.37	1.38	100.00	Used
	PC aa C32:0	11.00	2.32	100.00	Used
	PC aa C32:1	14.93	7.31	100.00	Used
	PC aa C32:3	0.39	0.13	100.00	Used
	PC aa C34:1	225.62	68.75	100.00	Used
	PC aa C34:2	300.72	97.13	100.00	Used
	PC aa C34:3	13.90	4.18	100.00	Used
	PC aa C34:4	1.48	0.65	100.00	Used
	PC aa C36:0	2.64	1.77	96.20	Used
	PC aa C36:1	50.12	16.85	100.00	Used
	PC aa C36:2	186.39	53.86	100.00	Used
	PC aa C36:3	117.08	28.17	100.00	Used
	PC aa C36:4	166.29	56.50	100.00	Used
	PC aa C36:5	17.81	8.29	100.00	Used
	PC aa C36:6	1.05	0.60	100.00	Used
	PC aa C38:0	3.22	0.88	100.00	Used
	PC aa C38:3	53.61	14.90	100.00	Used
	PC aa C38:4	93.51	26.75	100.00	Used
	PC aa C38:5	43.37	13.65	100.00	Used
	PC aa C38:6	53.69	26.07	100.00	Used
	PC aa C40:1	0.89	0.58	44.30	Excluded
	PC aa C40:2	1.47	1.22	100.00	Used
	PC aa C40:3	1.30	0.94	100.00	Used
	PC aa C40:4	4.73	1.54	100.00	Used
	PC aa C40:5	10.57	3.13	100.00	Used
	PC aa C40:6	21.38	9.32	100.00	Used
	PC aa C42:0	0.71	0.25	100.00	Used

Glycerophospholipids	**PC aa C42:1**	**0.44**	**0.24**	**100.00**	**Used**
	PC aa C42:2	0.49	0.30	100.00	Used
	PC aa C42:4	0.47	0.30	100.00	Used
	PC aa C42:5	0.51	0.19	100.00	Used
	PC aa C42:6	0.63	0.21	94.94	Used
	PC ae C30:0	0.46	0.14	94.94	Used
	PC ae C30:1	0.44	0.17	100.00	Used
	PC ae C30:2	0.32	0.25	98.73	Used
	PC ae C32:1	2.51	0.60	100.00	Used
	PC ae C32:2	0.68	0.22	100.00	Used
	PC ae C34:0	1.33	0.53	100.00	Used
	PC ae C34:1	8.20	2.80	100.00	Used
	PC ae C34:2	8.31	3.26	100.00	Used
	PC ae C34:3	5.52	1.85	100.00	Used
	PC ae C36:0	0.95	0.31	100.00	Used
	PC ae C36:1	12.25	4.01	100.00	Used
	PC ae C36:2	11.19	3.38	100.00	Used
	PC ae C36:3	5.81	1.87	100.00	Used
	PC ae C36:4	18.44	4.26	100.00	Used
	PC ae C36:5	9.57	2.95	100.00	Used
	PC ae C38:0	2.50	1.59	100.00	Used
	PC ae C38:1	4.54	1.79	100.00	Used
	PC ae C38:2	4.85	2.32	100.00	Used
	PC ae C38:3	8.85	4.25	100.00	Used
	PC ae C38:4	11.41	2.74	100.00	Used
	PC ae C38:5	12.97	4.19	100.00	Used
	PC ae C38:6	5.52	2.03	100.00	Used
	PC ae C40:1	2.15	1.05	100.00	Used
	PC ae C40:2	2.66	1.32	100.00	Used
	PC ae C40:3	3.61	2.73	100.00	Used
	PC ae C40:4	3.44	1.61	100.00	Used
	PC ae C40:5	4.51	1.35	100.00	Used
	PC ae C40:6	3.68	1.31	100.00	Used
	PC ae C42:0	1.08	0.32	25.32	Excluded
	PC ae C42:1	1.05	0.79	100.00	Used
	PC ae C42:2	1.04	0.67	100.00	Used
	PC ae C42:3	1.10	0.58	100.00	Used
	PC ae C42:4	1.09	0.43	100.00	Used
	PC ae C42:5	2.13	0.54	100.00	Used
	PC ae C44:3	0.35	0.22	78.48	Used
	PC ae C44:4	0.44	0.16	100.00	Used
	PC ae C44:5	1.32	0.45	100.00	Used
	PC ae C44:6	0.94	0.29	100.00	Used

Sphingolipids	**SM (OH) C14:1**	**3.54**	**1.29**	**100.00**	**Used**
	SM (OH) C16:1	2.20	0.82	100.00	Used
	SM (OH) C22:1	9.01	4.38	100.00	Used
	SM (OH) C22:2	8.37	5.28	96.20	Used
	SM (OH) C24:1	0.04	0.08	24.05	Excluded
	SM C16:0	76.05	17.79	100.00	Used
	SM C16:1	10.23	2.66	100.00	Used
	SM C18:0	19.14	5.76	100.00	Used
	SM C18:1	7.82	2.58	100.00	Used
	SM C20:2	0.05	0.07	29.11	Excluded
	SM C24:0	16.96	4.00	100.00	Used
	SM C24:1	30.77	14.27	97.57	Used
	SM C26:0	0.00	0.00	0.00	Excluded
	SM C26:1	0.03	0.04	18.99	Excluded
Sugars	H1	5943.92	2539.66	100.00	Used

Appendix F: Material Transfer documents

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@unobi.ac.ke
Website: www.unobi.ac.ke
www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/SH/60

21st October 2014

Frank N. Ndakala
UNITID
College of Health Sciences
University of Nairobi

Dear Frank

Re: Approval for shipment of samples – Genetic Analysis of Adverse Drug Reactions Associated with Antiretroviral Therapy in Kenya (P68/2/2013)

Reference is made to your communication of 2nd October, 2014.

The KNH/Uon-ERC has reviewed and approved shipment of the following biological materials

Material	Quantity	Purpose
Cryo vial stored whole blood(DNA)	360	Genotyping
RNA –later stored whole blood (mRNA)	200	mRNA quantification
Cryo vial stored plasma	360	HPLC assay

The samples will be under the custodian of the following:

Prof. Georg Behrens
Clinic for Immunology & Rheumatology
University Hospital Hanover
Carl-Neuberg-Str. 1 30625 Hanover, Germany
Phone: +49 511 532 5713
Email: Behrens.georg@mh-hanover.de

Yours sincerely

PROF. M.L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC

"Protect to Discover"

MINISTRY OF HEALTH
OFFICE OF DIRECTOR OF MEDICAL SERVICES

Telegrams:"MINHEALTH". Nairobi
Telephone; Nairobi 2717077 Fax: 2715239

OFFICE OF DIRECTOR OF
MEDICAL SERVICES
AFYA HOUSE
CATHEDRAL ROAD
P.O. BOX 30016
NAIROBI

MOH/ADM/1/1/81 VOL.1

28th October, 2014

Frank N. Ndakala
Principal Investigator
Ag. Assistant Director, Research & Development
State Department of Science and Technology
NAIROBI

Dear Frank,

RE: AUTHORITY TO SHIP BIOLOGICAL SAMPLES

Your request for specimen export permit dated 28th October, 2014 refers.

The title of your study is noted to be "***Genetic Analysis of Adverse Drug Reactions Associated with Antiretroviral Therapy in Kenya (P68/2/2013)***".

Authority is hereby granted for shipment of biological samples related to this research.

Material	Quantity	Purpose
Cryo vial stored whole blood (DNA)	360	Genotyping
RNA- Later stored whole blood (mRNA)	200	mRNA qualification
Cryo vial stored plasma	360	HPLC assay

The shipment contact details are as follows:

Prof. Georg Behrens
Clinic for Immunology & Rheumatology
University Hospital Hanover
Carl-Neuberg-Str. 1 30625 Hanover, Germany
Phone: +49 511 532 5713
Email: Benrens.georg@mh-hanover.de

Dr. Charles K. Kandie
FOR: DIRECTOR OF MEDICAL SERVICES